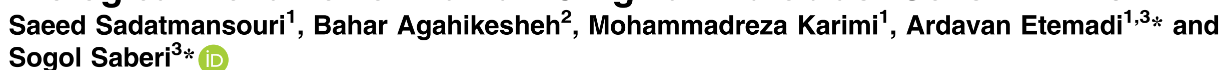
Photochemistry and Photobiology, 20**, **: *–*

Research Article

Effect of Different Energy Densities of 915 nm Low Power Laser on the Biological Behavior of Human Gingival Fibroblast Cells *In Vitro*



Saeed Sadatmansouri[1], Bahar Agahikesheh[2], Mohammadreza Karimi[1], Ardavan Etemadi[1,3]* and Sogol Saberi[3]*

[1]Department of Periodontics, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2]Private Dentist, Tehran, Iran
[3]Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Received 26 July 2021, accepted 22 November 2021, DOI: 10.1111/php.13577

ABSTRACT

Photobiomodulation is recognized as an effective method for adjunct therapy in periodontal treatments. Our purpose in this study was to investigate the effects of different energy densities of 915 nm diode laser on the viability and viability capacity of human gingival fibroblast cells. Cell samples were examined in five groups, including four irradiation groups with low-level diode laser 915 nm, 1, 2, 3, 4 J cm^{-2} and a control group (no Laser irradiation). Cell viability and viability were measured 1, 3 and 5 days after irradiation by MTT and DAPI assay. Statistical differences between groups at any time were analyzed by one-way ANOVA and a *post hoc* Turkey's test. The cell viability and viability capacity increased on the third day at an energy density of 3 J cm^{-2}; (*P*-value = 0.007) and the fifth day at energy densities of 2, 3 and 4 J cm^{-2} was recorded compared with the control group (*P*-value = 0.000). Also, a significant decrease in the viability and viability of irradiated cells with an energy density of 1 J cm^{-2} was found (*P*-value = 0.033). According to our results, Photobiomodulation with 915 nm diode laser has a positive stimulating effect on the viability and viability capacity of human gingival fibroblast cells.

INTRODUCTION

Photobiomodulation is based on the use of light energy and its conversion into biochemical energy to modify or stimulate normal cellular processes without causing thermal effects within the treated tissue and refers to the application of both low-level lasers (LLL) and light-emitting diode (LED) (1). Low-level lasers with a power output less than 500 mW at red to near-infrared wavelengths are absorbed primarily by tissue pigment and hemoglobin. Among the low-level lasers, diode lasers are becoming more admitted in dental procedures due to their convenience to use and portability (2). A 915 nm diode laser is one of the favorable lasers for clinical usages on bone due to high penetration depth and also is one of the newest lasers that are still not fully understood as a PBM tool (3).

The mechanism of action of low-level diode lasers is described by the absorption of photons via cellular chromophores and light receptors followed by changes in the mitochondrial respiratory chain and enhancing the intracellular calcium concentrations (4,5). These changes conduct the cellular positive impacts such as stimulating the secretion of growth factors, an enhancement in gene expression, cell growth, viability and differentiation and an increase in cell chemotaxis (6–8). Consequently, the clinical effects of low-level diode lasers are manifested by reducing inflammation, relieving pain, improving tissue regeneration and accelerating the wound healing process (9–11).

Shortening the time of tissue repair and regeneration of periodontal tissue have always been one of the fundamental therapeutic purposes in periodontal treatment. The reconstruction of periodontal tissue during the healing process briefly is achieved through the migration and viability of various cells following the release of growth factors and the process of angiogenesis. One of the most essential cells during the process of wound healing is fibroblasts, which have a major function through providing growth factors during re-epithelialization, organizing granulation tissue, rebuilding the collagen network to deposit the new extracellular matrix, differentiating into myofibroblasts and helping angiogenesis (12,13). The bio stimulatory effect of low-level diode lasers on cell function is specified through laser parameters, including wavelength, power density, energy density, exposure time and irradiation mode (1).

The effect of diode lasers on human gingival fibroblast has been reported in various *in vitro* experiments (14). However, most of them have centered on the red light spectrum and there is no study about the effect of 915 nm diode lasers on human gingival fibroblast; therefore, in this study, we examined the effect of 915 nm diode laser with different energy densities on the viability and viability of cultured human gingival fibroblasts cells.

MATERIALS AND METHODS

Cell culture. Human gingival fibroblasts (C-10459 hugu-PI1) were obtained from the Iranian Biological Resource Center. The cells were cultured in Dulbecco's modified Eagle medium (DMEM; Cegrogen Biotech, Germany) supplemented with 10% fetal bovine serum (FBS), 100 μg ml^{-1} streptomycin, 100 IU ml^{-1} penicillin, 2.5 μg ml^{-1} amphotericin B in a 95% humidified incubator with 5% CO_2 at 37°C.

*Corresponding authors email: S-saberi@razi.tums.ac.ir (Sogol Saberi) and a-etemadi@farabi.tums.ac.ir (Ardavan Etemadi)
© 2021 American Society for Photobiology

The cells were detached using 0.25% sterile trypsin-EDTA solution followed by centrifugation 1200RPM for 5 min to obtain the required number of cells. Cells (1×10^4 cell ml^{-1}) were seeded in 96-well plates with adequate distance between wells in DMEM supplemented with 10% FBS.

Laser irradiation. A gallium-aluminum-arsenide (GaAlAs) diode laser source (915 nm Pocket laser, 88 Dent Company, Italy) was used in this study. Using 96-well plates, laser irradiation was performed 1 cm above the cell culture, and the culture dishes were uncovered under the laser source. All laser treatments were administered in a dark room by the same operator using a hand piece with a beam diameter of 8 mm (spot size (πr^2): 0.5 cm^2) in a continuous irradiation mode. Cells were irradiated at different energy densities (1, 2, 3, 4 J cm^{-2}) corresponding to irradiation time 2.5, 5, 7.5, 10 s. The control group received no laser irradiation. 1, 3 and 5 days after the irradiation, the viability of the cells were evaluated using the MTT assay and DAPI staining was performed to determine the nucleus morphological changes (Table 1).

Cell viability assay. Cell viability was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay at 1, 3 and 5 days after the irradiation. This method is based on mitochondrial activity in which the activity of the succinic dehydrogenase enzyme is demonstrated by the reduction of a yellow tetrazolium salt to purple formazan crystals. For this purpose, each well received 50 μL of MTT (Sigma, Germany) solution (5 mg ml^{-1}), and the cells were incubated at 37°C for 4 h. Then, the culture medium and the MTT solution were aspirated and replaced by 60 μL of DMSO solution to dissolve the violet formazan crystals. Eventually, absorbance was measured at 570 nm with a spectrophotometer (Bio Tek).

Cell nucleus morphology analysis. Nuclear damage of cells was determined by DAPI staining at 1, 3 and 5 days after the irradiation. To detect the morphological changes in the cell nucleus related to apoptosis, the cells were fixed whit 70% ethanol. Then, they were incubated with 4′-6-diamidino-2-phenylindole (DAPI; Santa Cruz Biotechnology) diluted (1 μg mL^{-1}) for 30 min. Unbound cells were washed with PBS solution, then cells were examined under a fluorescence microscope (Olympus; IX-71) and the pyknotic and fragmented nuclei were considered as apoptotic cells.

Statistical analysis. Data were expressed as mean ± SD and were analyzed by using one-way ANOVA with Tukey's post hoc test by SPSS version 16. At least four experiments were carried out for each treatment. The difference between experimental groups was considered statistically significant at $P < 0.05$.

RESULTS

Cell viability and viability of human gingival fibroblasts were obtained by the MTT method, and results are shown in Table 2. On the first day after irradiation, no significant difference in cell viability and viability was observed in any of the irradiated groups compared with the control group. While a significant increment in energy density of 2, 3 J cm^{-2} was observed compared with the energy density of 1 J cm^{-2} ($P = 0.019$). A significant increase in cell viability and viability was recorded at energy density of 3 J cm^{-2} compared with the control group ($P = 0.007$) and irradiation dose of 1, 2 J cm^{-2} on the third day ($P < 0.05$). Five days after irradiation, all irradiated groups revealed a significant difference in viability and viability of cells compared with the control group ($P < 0.05$). Also, a significant increase in energy density 2, 3 and 4 J cm^{-2} compared with 1 J cm^{-2} was recognized ($P = 0.000$). The difference observed in the cell viability in all groups under laser irradiation on day 5 was recognized as significant compared with days 1 and 3 (Fig. 1).

The results of DAPI staining demonstrated that only cells were irradiated with the dose of 1 J cm^{-2} exhibited significant morphological nuclear damage compared with the control group (P = 0.016) and other irradiated groups ($P < 0.05$). Also, no significant difference was found between any of the studied days ($P > 0.05$). Microscopic image of HGF cells with DAPI staining on day five is shown in Fig. 2.

Table 1. Study groups different laser energy densities (irradiation mode: continuous, spot size: 0.5 cm^2, power: 200 mW, power density: 400 mW cm^{-2} for all groups)

Groups	Time (s)	Power (W)	Energy (J)	Energy density (J cm^{-2})
Control	–	–		–
1	2.5	0.2	0.5	1
2	5	0.2	1	2
3	7.5	0.2	1.5	3
4	10	0.2	2	4

DISCUSSION

Cell viability is one of the essential physiological effects of low-level laser therapy, which is mainly mediated by activation of the mitochondrial respiratory chain, resulting in the increase of reactive oxygen species (ROS) and adenosine triphosphate (ATP), followed by protein synthesis, expression of growth factors and cytokines increase and eventually lead to cell viability (5). The results of the present study demonstrated the positive cellular stimulatory response related to an increment in mitochondrial activity at the studied radiation doses (2, 3, 4 J cm^{-2}). Increased mitochondrial metabolic activity after using a 915nm diode laser on the human skin fibroblast cells has also been reported by Belletti *et al.* at the low irradiation doses (15).

One of the most effective factors in stimulating cell viability is the radiation dose of the laser. Therefore, the use of insufficient energy, due to not providing the minimum threshold required for the physiological response, causes a lack of cellular response or in some cases a negative cellular response (16). The decreased metabolic activity of irradiated cells at the energy density of 1 J cm^{-2} that we observed in the present study, is similar to the results of Basso *et al.*'s study on the same cell line with different radiation parameters (780 nm, 40 mW), which the most effective doses in increasing cell viability and viability were reported to be 0.5 and 3 J cm^{-2}, and a significant decrease was observed in the irradiated groups at doses of 1.5 and 7 J cm^{-2} (17). Also, in the study of Marques *et al.*, the viability of stem cells derived from exfoliated human deciduous teeth was

Table 2. Descriptive statistics of MTT assay

Groups	Day	Mean	Std. deviation	Sig groups with control
Control	1	100	5.289	
	3	100	7.221	
	5	100	3.822	
1 J cm^{-2}	1	94.673	2.367	0.627
	3	99.849	3.620	1.000
	5	81.081	3.355	0.033
2 J cm^{-2}	1	107.747	16.843	0.229
	3	102.002	5.034	0.978
	5	129.594	10.284	0.000
3 J cm^{-2}	1	107.747	6.919	0.229
	3	113.813	6.904	0.007
	5	140.945	5.418	0.000
4 J cm^{-2}	1	103.261	4.789	0.886
	3	109.734	1.889	0.108
	5	141.891	13.320	0.000

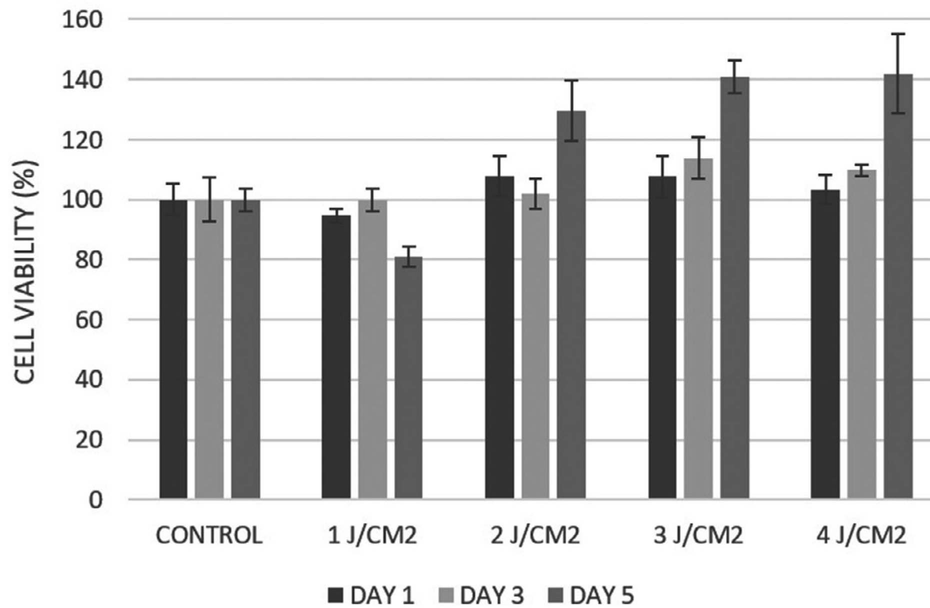


Fig. 1. Cell viability comparison between different days in the study groups

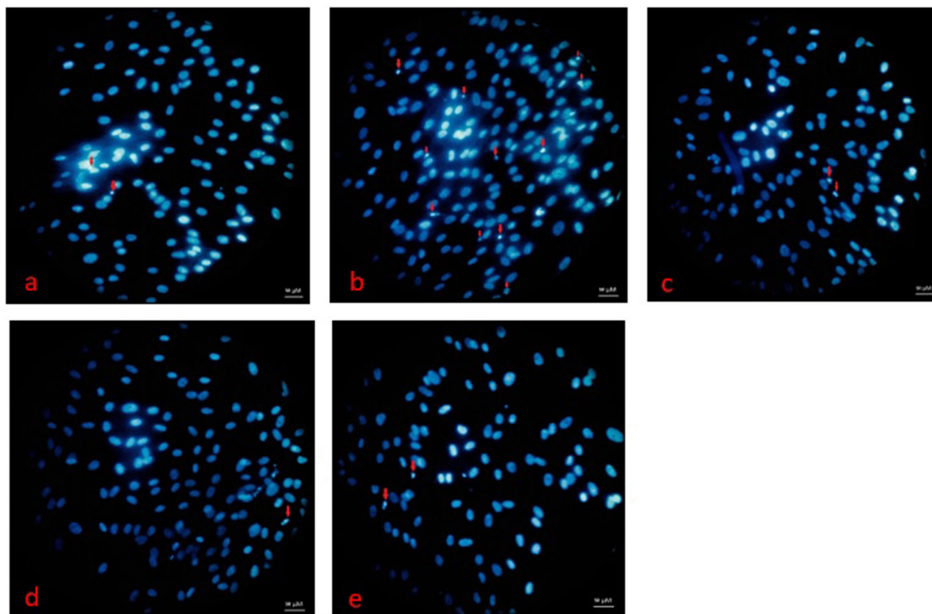


Fig. 2. Microscopic image of HGF cells with DAPI staining on day five. (a) Control; (b) radiation group of 1 J cm^{-2}; (c) radiation group of 2 J cm^{-2}; (d) radiation group of 3 J cm^{-2}; (e) radiation group of 4 J cm^{-2}

decreased in the 780 nm laser-irradiated group with the energy density of 1.2 J cm^{-2}, which is similar to the significant reduction in cell viability with nucleus morphological damage of under irradiated cells of 1 J cm^{-2} in our study (18). The use of high-energy doses inhibits physiological activities in cell and tissue structure. Therefore, low energy densities were applied in the current study and resulted in a significant increase in cell viability along with their survival without damage to the nucleus morphology after irradiation at energy densities of 2, 3 and 4 J cm^{-2}.

In the present study, the effect of 915 nm diode laser radiation on the metabolic activity of HGF cells can be noticed over time. As Forouzanfar *et al*. have found no significant change in the viability of similar cells 24 h after 810 nm diode laser irradiation, then after 48 and 72 h a significant increase happened in cells viability (19). In the present study, no significant changes were detected one day after irradiation compared with the control group, whereas cell viability and viability, on the third day in energy density of 3 J cm^{-2} and then on the fifth day in energy densities of 2, 3 and 4 J cm^{-2} were significantly increased compared with the control group. Similarly, in the clinical study of Madi *et al*., in the first 24 h after 660 nm laser irradiation with energy density 4 J cm^{-2} on the wound area after gingivectomy, no significant difference was observed, while a significant

improvement in wound healing was found on days 5 and 7 after surgery (20). Although in this clinical study patients were irradiated on the day of surgery and 3 and 5 days after surgery, similar results have been justifiable due to the difference between the tissue absorbance level and the level of cellular absorbance.

Our results are in contrast to the study of Mirzaei *et al* that demonstrated an increase in viability and viability of human gingival fibroblast cells and periodontal ligament stem cells 24 h after 980 nm laser irradiation at doses of 0.5 and 2.5 J cm^{-2}, while a significant reduction in cells viability has appeared after 48 h (21). Therefore, it seems that despite the similarity of the cellular parameters and the energy densities investigated, the 915nm laser has shown longer effectiveness in our study. Also, the increase in HGF cell viability 24 h after 809nm laser irradiation in the Kreisler et.al study was considerable due to three times irradiation at a 24-h interval (22).

In the recent study, the increment in cell viability did not occur in an energy-dependent manner in any of the studied days. So that, on the first day after irradiation, the cell viability was the same in the 2 and 3 J cm^{-2} radiation groups and significantly increased compared with the 1 J cm^{-2} energy dose. While the highest cell viability rate was discovered after 72 h in the energy density of 3 J cm^{-2} and cell viability increased respectively in groups 2, 3 and 4 J cm^{-2} without significant differences on day five. However, in a similar study on a 940 nm diode laser, the increase in cell viability within 72 h was dose-dependent (1–4 J cm^{-2}), which could because of differences in in vitro conditions such as cell type and wavelength (23).

This study represented the positive optical efficiency of a 915 nm diode laser on the metabolic activity of fibroblasts cells. The effects of this diode laser have been evaluated on another cell line that participates in the repair process. However, in the study of Parenti *et al*. on human osteoblast-like cells with 915 nm laser and energy density of 2 J cm^{-2} after irradiation for 17, 31 and 157 s, no difference was seen in the viability and viability between irradiated and nonirradiated cells (24). Also, in another study on the same cell line with energy densities of 5, 10, 15 J cm^{-2} for 48, 96 and 144 s, respectively, no significant change in cell viability was obtained compared to the control group, while wound healing occurred completely in all irradiated groups (3). So, in addition to wavelength and radiation dose, the duration of radiation therapy can change the cell and tissue responses.

Due to the complexity of laser–tissue interaction and also the different responses in both in vivo and in vitro environments, even with similar parameters, it is necessary to accurately identify radiation parameters in clinical studies. Choung *et al*. did not observe a significant difference in intraoral mucosal wound healing by using a 915 nm laser with a density of 187.5 J cm^{-2}, even after 14 days (25). The effect of repeated radiation due to the cumulative effect of laser to enhance the present cellular results, should also be considered.

According to the results, it seems that 915 nm diode laser irradiation in a single dose can ameliorate the wound healing process by increasing the viability and viability of gingival fibroblast cells.

CONCLUSION

In conclusion, our findings suggest that a 915 nm low-level diode laser application with expressed parameters can stimulate the viability and viability of human gingival fibroblasts cells for an extended period.

Acknowledgements—The authors received no financial support for the study.

REFERENCES

1. Parker, S., M. Cronshaw, E. Anagnostaki, S. R. Bordin-Aykroyd and E. Lynch (2019) Systematic review of delivery parameters used in dental photobiomodulation therapy. photobiomodulation, photomedicine, and laser. *Surgery* **37**(12), 784–797.
2. Suresh, S., Sivasankari, S. Merugu and N. Mithradas (2015) Low-level laser therapy: A biostimulation therapy in periodontics. *SRM J. Res. Dental Sci.* **6**(1), 53.
3. Tschon, M., S. Incerti-Parenti, S. Cepollaro, L. Checchi and M. Fini (2015) Photobiomodulation with low-level diode laser promotes osteoblast migration in an in vitro micro wound model. *J. Biomed. Optics* **20**(7), 078002.
4. Karoussis, I. K., K. Kyriakidou, C. Psarros, M. Koutsilieris and J. A. Vrotsos (2018) Effects and action mechanism of low level laser therapy (LLLT): Applications in periodontology. *Dentistry* **08**(09), https://doi.org/10.4172/2161-1122.1000514.
5. Freitas, L. and M. Hamblin (2016) Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE J. Sel. Top. Quantum Electron.* **22**(3), 348–364.
6. Szezerbaty, S., R. Oliveira, D. Pires Oliveira, C. Pacheco Soares, D. Sartori and R. Poli-Frederico (2018) The effect of low-level laser therapy (660 nm) on the gene expression involved in tissue repair. *Lasers Med. Sci.* **33**.
7. Hakki, S. S. and S. B. Bozkurt (2012) Effects of the different settings of diode laser on the mRNA expression of growth factors and type I collagen of human gingival fibroblasts. *Lasers Med Sci.* **27**(2), 325–331.
8. Etemadi, A., S. Taghavi Namin, M. Hodjat, E. Kosarieh and N. Hakimiha (2020) Assessment of the photobiomodulation effect of a blue diode laser on the viability and migration of cultured human gingival fibroblast cells: a preliminary in vitro study. *J. Lasers Med. Sci.* **11**(4), 491–496.
9. Zhao, H., J. Hu and L. Zhao (2020) 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.* **36**(1), 175–187.
10. Pejcic, A. and D. Mirkovic (2011) Anti-inflammatory effect of low-level laser treatment on chronic periodontitis. *Med. Laser Appl.* **26** (1), 27–34.
11. Lingamaneni, S., L. R. Mandadi and K. R. Pathakota (2019) Assessment of healing following low-level laser irradiation after gingivectomy operations using a novel soft tissue healing index: A randomized, double-blind, split-mouth clinical pilot study. *J. Indian Soc. Periodontol.* **23**(1), 53–57.
12. Martin, P. and R. Nunan (2015) Cellular and molecular mechanisms of repair in acute and chronic wound healing. *Brit. J. Dermatol.* **173** (2), 370–378.
13. Smith, P., C. Martinez, J. Martínez and C. McCulloch (2019) Role of fibroblast populations in periodontal wound healing and tissue remodeling. *Front. Physiol.* **10**.
14. Ren, C., C. McGrath, L. Jin, C. Zhang and Y. Yang (2016) Effect of diode low-level lasers on fibroblasts derived from human periodontal tissue: A systematic review of *in vitro* studies. *Lasers Med. Sci.* **31**(7), 1493–1510.
15. Belletti, S., J. Uggeri, G. Mergoni, P. Vescovi, E. Merigo, C. Fornaini, S. Nammour, M. Manfredi and R. Gatti *et al*. (2014) Effects of 915 nm GaAs diode laser on mitochondria of human dermal fibroblasts: Analysis with confocal microscopy. *Lasers Med. Sci.* **30** (1), 375–381.
16. Alghamdi, K. M., A. Kumar and N. A. Moussa (2012) Low-level laser therapy: A useful technique for enhancing the viability of various cultured cells. *Lasers Med Sci* **27**(1), 237–249.
17. Basso, F., T. Pansani, A. Turrioni, V. Bagnato, J. Hebling and C. Costa (2012) In vitro wound healing improvement by low-level laser

therapy application in cultured gingival fibroblasts. *Int. J. Dent.* **2012**, 1–6.
18. Marques, N., C. Lopes, N. C. T. Marques, L. Cosme-Silva, T. Oliveira, C. Duque *et al*. (2019) A preliminary comparison between the effects of red and infrared laser irradiation on viability and viability of SHED. *Lasers Med. Sci.* **34**, 465–471.
19. Frozanfar, A., M. Ramezani, A. Rahpeyma, S. Khajehahmadi and H. Arbab (2013) The effects of low-level laser therapy on the expression of collagen Type I gene and viability of human gingival fibroblasts (Hgf3-Pi 53): In vitro study. *Iranian J. Basic Med. Sci.* **16**, 1071–1074.
20. Madi, M. and M. Mahmoud (2020) The evaluation of the healing effect of low-level laser treatment following gingivectomy. *Beni-Suef University J. Basic Appl. Sci.* **9**.
21. Mirzaei, A., M. Rigi Ladiz, F. Javani-Jouni, S. Hendi, R. Najafi-Vosough and H. Hashemzehi (2020) Effects of 980 nm diode laser irradiation on gingival fibroblasts and periodontal ligament stem cells. *Acta Sci. Microbiol.* **3**(1), 50–54.
22. Kreisler, M., A. Christoffers, H. Al-Haj, B. Willershausen and B. d'Hoedt (2002) Low-level 809-nm diode laser-induced in vitro stimulation of the viability of human gingival fibroblasts. *Lasers Surg. Med.* **30**, 365–369.
23. Illescas-Montes, R., L. Melguizo-Rodríguez, O. García-Martínez, E. de Luna-Bertos, F. J. Manzano-Moreno, C. Ruiz and J. Ramos-Torrecillas (2019) Human fibroblast gene expression modulation using 940 NM diode laser. *Sci. Rep.* **9**. https://doi.org/10.1038/s41598-019-48595-2
24. Parenti, S. I., S. Panseri, A. Gracco, M. Sandri, A. Tampieri and G. A. Bonetti (2013) Effect of low-level laser irradiation on osteoblast-like cells cultured on porous hydroxyapatite scaffolds. *Annali Dell'istituto Superiore Di Sanità* **49**, 255–260.
25. Choung, H.-W., S.-H. Lee, A. R. Ham, N. R. Lee, B. Kim, K.-M. Pang, J. W. Jahng and J.-H. Lee (2019) Effectiveness of low-level laser therapy with a 915 nm wavelength diode laser on the healing of intraoral mucosal wound: An animal study and a double-blind randomized clinical trial. *Medicina* **55**, 405.