

Different Effects of Low-Level Laser Therapy on the Proliferation of HT29 Cells in Culture and Xenograft Models



Najmeh Malekzadeh Gonabadi¹ , Mehdi Shamsara^{2*} , Dor Mohammad Kordi Tamandani^{1*} , Sahar Shojaei³ , Seyed Mehdi Alavi⁴

¹Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran

²Department of Animal Biotechnology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran

³Middle East Gene and Cell Therapy Company, Tehran, Iran

⁴Department of Plant Biotechnology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran

*Correspondence to

Mehdi Shamsara,
Email: shamsa@nigeb.ac.ir
and Dor Mohammad Kordi
Tamandani, Email: dor_kordi@science.usb.ac.ir

Received: May 15, 2023

Accepted: July 9, 2023

Published online August 30, 2023

Abstract

Introduction: Different kinds of treatments have been developed to fight cancers. Low-level laser therapy (LLLT), also known as photobiomodulation therapy (PBMT), is a low-power monochromatic and coherent light that has been used successfully for healing injuries and combating malignancies. However, there are concerns about the application of LLLT to cancers due to the increased proliferation of some cancer cells after LLLT.

Methods: This study investigated the effects of 650 nm and 870 nm lasers on the proliferation of HT29 colorectal cancer cell lines *in vitro* and *in vivo*.

Results: The results showed that the laser with a wavelength of 870 nm did not meaningfully alter the proliferation of cultured cells. However, cell proliferation was promoted when the laser was applied within a wavelength of 650 nm. Treatment of HT29-derived tumors in nude mice with the 650 nm laser resulted in the decline of the tumor progression rate compared to controls. This result was inconsistent with the proliferative effects of the laser on the cultured cells.

Conclusion: Cell behavior in response to LLLT might be different between cell culture and xenograft models.

Keywords: Colorectal cancer; Cell proliferation; Low-level laser therapy; Photobiomodulation.



Introduction

Different kinds of treatments have been developed to fight cancers. The types of treatments that patients receive will be dependent on the cancer type and how advanced it is. Some patients are treated in only one way, but most patients receive combination therapies, such as surgery followed by chemotherapy, immunotherapy, and/or radiation therapy.¹

A laser (mild amplification through stimulated emission of radiation) is a tool that generates a quite uniform wavelength, phase and polarization electromagnetic radiation.² Different kinds of lasers have been developed with applications in industry and medicine. Low-level laser therapy (LLLT), also named photobiomodulation therapy (PBMT), is used to treat wounds, stimulate the immune system, reduce swelling, and relieve acute or chronic pain. It has also been applied to reduce acute and chronic symptoms caused by cancer or cancer treatment.^{3,4} A wide range of biomodulatory effects, from cell proliferation to programmed cell death,

has been reported for LLLT. Each of these effects has the potential to be therapeutic. For example, cell proliferation can describe the application of lasers in wound healing, while programmed cell death can describe the application of lasers in cancer treatment. The biostimulatory effect is thought to be related to an increase in ATP production, while the bioinhibitory effect is thought to be related to oxidative stress caused by an excess load of reactive oxygen species (ROS).⁵

The safety and efficacy of low-power laser irradiation in cancer remain unknown. Investigating the effects of lasers on cultured tumor cell lines has generated conflicting results, and a few of them are consistent with the behavior of tumor cells *in vivo*.⁶ Lasers can stimulate the immune system or generate excessive amounts of ROS to attack tumors under certain conditions.⁷ Ottaviani et al showed that laser irradiation inhibits tumor progression, induces tumor angiogenesis, and stimulates the immune system to produce type I interferons. Their study proved the safety of laser-based therapies in cancers.⁸ Another study by Xia et

al revealed that the blue laser dose-dependently decreased the cell viability in bladder cancer. Moreover, reduced cell migration and invasion were observed possibly due to the suppression of the MAPK/MEK/ERK pathway.⁹ However, there are several reports on the promotion of cell proliferation after laser irradiation. For instance, in a study by Marchesini, the HT-29 cell proliferation was enhanced under laser radiation.¹⁰ Also, a study by Rhee et al showed increased cell proliferation and angiogenesis following PBM in the thyroid cell line.¹¹ Therefore, it is of great importance to study the relationships between laser radiation at different wavelengths and cancer progression or regression.

Colorectal cancer is the third most common cancer type worldwide; in 2020, almost 2 million cases were diagnosed. Colorectal cancer is the second most common cause of cancer death, leading to almost 1 million deaths per year. In Iran, the number of prevalent cases is 319 740 among 80 000 000 people.^{12,13}

In this study, we investigated the effects of LLLT on the proliferation of colorectal cancer cells in culture and animal models. Human colorectal cancer HT-29 cells were treated with 650 nm (200 W) or 870 nm (5 MW) lasers in both culture and xenograft models, and the consensus on cell proliferation and tumor progression was evaluated. The result showed that the laser at the wavelength of 650 nm stimulated the proliferation of HT29 cells in culture but suppressed tumor growth in the mouse model.

Materials and Methods

Cell Line and Cell culture

HT29 cells (human colorectal cancer cell line) were purchased from the National Cell Bank of Iran and cultured in the DMEM medium supplemented with 10% fetal bovine serum (GIBCO, Thermo Fisher Scientific, US), 100 U/mL penicillin, and 100 mg/mL streptomycin (Merck, US). The cells were incubated in a 37 °C humidified atmosphere containing 5% CO₂.

MTT Assay

HT29 cells were seeded at the density of 3×10^4 cells per well in 96-well culture plates. The following day, the cells were divided into untreated (control) and treated groups, and they were exposed to the laser within the wavelengths of 650 nm for 40 seconds and 870 nm for 180 seconds. The cells were incubated for an additional 24 hours. The MTT reagent (Sigma Aldrich, US) was added to wells and incubated for 4 hours at 37 °C and 5% CO₂. Dimethyl sulfoxide (Merck, US) was added to dissolve the formed formazan crystals. The plates were slowly rotated and the absorbance was measured at 570 and 630 nm (ELISA reader, labs system, Multi scan MS serial RS_232C).

In Vivo Study

For the *in vivo* study, ectopic colorectal tumors were

induced to the dorsal hip of nude mice by subcutaneous injection of 4×10^6 HT29 cells. The xenotransplant mice were divided into two groups, and the effects of the laser were tested with a 650 nm laser. Laser irradiation was applied to the back of the neck to reduce stress and also to the tumor, liver, spleen, and bone marrow three times (40 seconds per each time) at a one-day interval. The size of the tumors was measured to determine the effects of PBMT on tumor growth. The mice were dissected at the end of the experiments and the tumors were subjected to histology using Hematoxylin and eosin (H&E) staining. Moreover, for immunohistochemistry (IHC), the biopsies were stained against the Ki67 protein as a cell proliferation marker.

Statistical Analysis

The data were reported as mean $\pm$ standard deviation (SD), and statistical analysis was performed by a two-sided student *t* test. The error bars in the results came from three experimental repetitions. Results with a *P* value equal to or less than 0.05 ($P \leq 0.05$) were considered to be statistically significant. The curves were depicted using GraphPad prism version 5 software.

Results

Laser Treatment Affected Cell Viability

The MTT assay was accomplished to investigate the cytotoxic effects of the low-level laser on HT29 cells. The cells were exposed to 870 nm or 650 nm lasers for 180 seconds, and the MTT was performed after 24 hours. As shown in [Figures 1A and 1B](#), treatment with the 870 nm laser had no significant effect on cell proliferation, while the laser at the 650 nm wavelength caused significantly increased viability of HT29 cells ($p = 0.0002$).

Laser Treatment Increased In Vivo Progression Of Tumors

Ectopic colorectal tumors were grown in nude mice by the injection of HT29 cells. Two weeks later, the mice were exposed to 650 nm laser irradiation three times every other day. The size of the tumors was measured 10 days after LLLT in both test and control groups. The data showed that the tumor progression in the laser-received group was significantly less than in the controls ([Figures 2A and 2B](#)) (P value = 0.0254).

Histology and Immunostaining Confirmed the Visual Observations of Tumor Growth

For histology, H&E staining revealed mitotic bodies. As shown in [Figures 3A and 3B](#), the number of mitotic bodies was more in the control sample than in the treatment (laser) sample. The mitotic counts in the control group and in the laser group were 38% and 25% respectively.

The proliferation activity of tumors in both laser and control groups was assayed by determining the

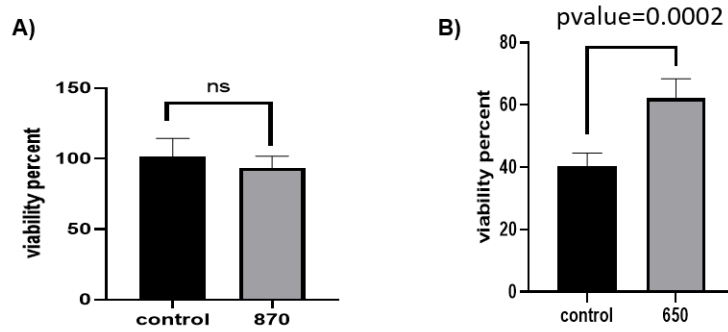


Figure 1. Effect of LLLT on the Viability of HT29 Cells. (A) 870 nm laser; (B) 650 nm laser. ns=not significant

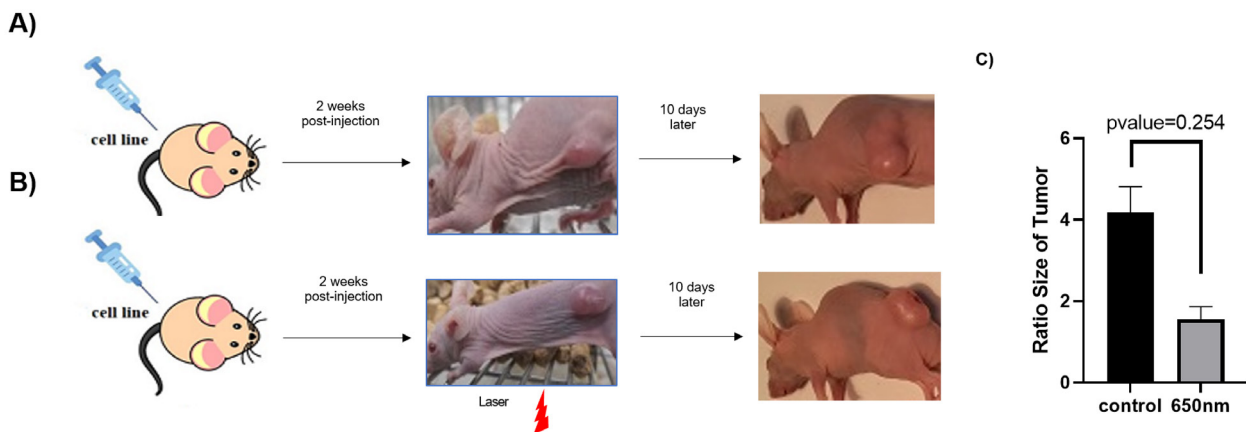


Figure 2. Effect of Laser Irradiation on Tumor Progression. (A) tumor growth in the control animals; (B) tumor growth in the laser group; (C) Comparing the ration of tumor size in the control and laser groups

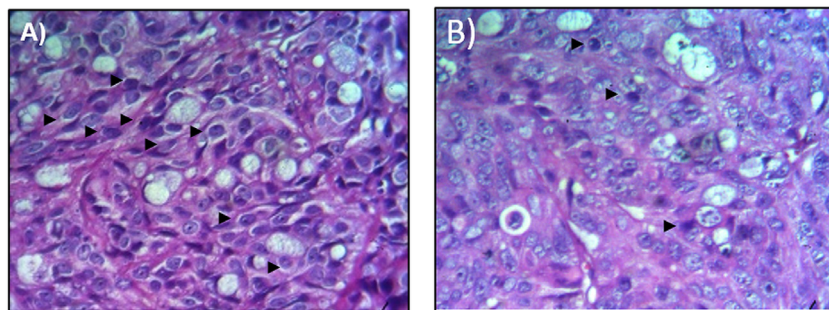


Figure 3. Histology of Tumors. H&E staining showed mitotic bodies. (A) control group; (B) laser group. The arrowheads indicate mitotic bodies

expression of the Ki67 marker (Figures 4A and 4B). The result showed that the Ki67 marker was expressed in 63% of tumor cells in the laser group, while this number was 90% in the control group (P value=0.028) (Figure 4C).

Discussion

The purpose of this research was to investigate the stimulatory or inhibitory effect of LLLT on the proliferation of human colorectal cancer HT29 cells. The effective wavelength spectrum used in previous studies is 600-800, So far, no definitive treatment protocol has been provided. To reach a definitive protocol we use wavelengths in this range.¹⁴ The effect of the laser on cell proliferation was evaluated in both *in vitro* and *in*

vivo models. First, the laser was applied at two different wavelengths, 650 and 870 nm, to cell culture. The laser at the 870 nm wavelength did not significantly change the growth of cultured HT29 cells, while the 650 nm laser promoted cell proliferation. Since the 870 nm laser was ineffective, the *in vivo* study was continued with the 650 nm laser. Interestingly, the growth of tumors after the treatment with the 650 nm laser showed a significant difference from the controls. Exposure of xenograft tumors with the 650 nm laser was associated with a decrease in tumor growth. This result was inconsistent with the *in vitro* findings. Evaluation of Ki67 expression in the tumor biopsies was concordant with the *in vivo* results, and immunostaining revealed less expression of

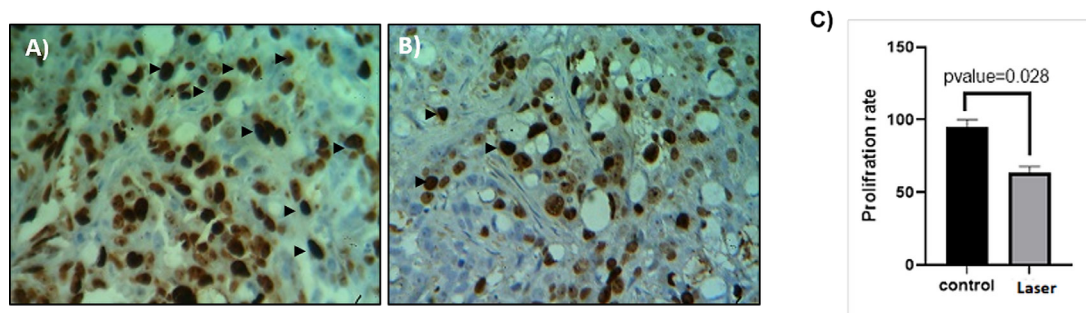


Figure 4. Immunostaining of the Ki67 Marker. (A) control group; (B) laser group; the arrowheads indicate some of the nuclei that are positive for the Ki67 marker. Active cells in terms of proliferation are displayed in brown nuclei

Ki67 in the laser group compared to the controls.

Studies report complex responses of cells to different wavelengths or energy densities of LLLT. For example, wound healing can be accelerated by LLLT likely due to the stimulation of cellular processes such as cell migration and cell differentiation.¹⁵⁻¹⁸ Moreover, LLLT stimulates the respiratory chain in mitochondria, which results in increasing ATP production and heightening DNA, RNA, and protein synthesis.^{19,20} LLLT is known to stimulate or activate many cytokines that contribute to cell proliferation, tumor angiogenesis, tumor migration, invasion and metastasis.²¹⁻²³ This is consistent with our cell culture results, in which LLLT at the wavelength of 650 nm, but not at the wavelength of 870 nm, induced the proliferation of the HT29 cell. This showed that cells behave differently in response to different wavelengths of LLLT.

In contrast to *in vitro* cell culture, our tumor xenograft models showed that laser irradiation reduced tumor progression. Exposure duration to the laser beam was different between the cell culture and the animal study. Cell cultures were exposed to laser irradiation one time, while the xenograft models were treated by the laser three times. Exposure duration is one of the important issues that determine the biological impact of lasers. A previous study conducted by Fernandes et al showed that both time and number of exposure to lasers can be effective in the laser effect. They reported choosing the best parameters for LLLT studies is a difficult task. Many different parameters (wavelength, fluence, power density, mode of delivery, time of application, pulse) can vary, resulting in a large number of possible combinations.^{24,25} We measured the Ki67 protein level in tumor biopsies as a biomarker of cell proliferation and tumor progression. Immunostaining of Ki67 indicated a direct proportion between the Ki67 level and tumor progression. Ki67 is a cell cycle-associated nuclear protein, synthesized by all proliferating cells and deficient in resting cells.^{26,27}

Validation of Ki67 expression in tissues through IHC is a rapid solution to distinguish dividing cells from non-dividing cells.²⁷ According to a study made by Prabhu et al, photobiomodulation caused the upregulation of the cell proliferation marker, Ki67, during dermal wound healing.²⁸

Conclusion

These findings suggest that laser effects on *in vivo* systems might be different from *in vitro* systems. *In vivo* models utilize a lot of determining elements that might influence laser effects, but only a part of these elements is present in *in vitro* models.

Acknowledgements

The authors express their thanks to the Middle East Gene Therapy Company for its support.

Authors' Contribution

Conceptualization: Mehdi Shamsara, Dor Mohammad Kordi Tamandani, Sahar Shojaei.

Data curation: Mehdi Shamsara.

Formal analysis: Najmeh Malekzadeh Gonabadi.

Funding acquisition: Sahar Shojaei, Mehdi Shamsara.

Investigation: Najmeh Malekzadeh Gonabadi.

Methodology: Mehdi Shamsara, Sahar Shojaei, Dor Mohammad Kordi Tamandani.

Project administration: Sahar Shojaei, Mehdi Shamsara.

Resources: Sahar Shojaei, Mehdi Shamsara.

Software: Najmeh Malekzadeh Gonabadi.

Supervision: Mehdi Shamsara, Dor Mohammad Kordi Tamandani.

Validation: Mehdi Shamsara, Dor Mohammad Kordi Tamandani.

Visualization: Mehdi Shamsara, Najmeh Malekzadeh Gonabadi.

Writing—original draft: Seyed Mehdi Alavi, Najmeh Malekzadeh Gonabadi.

Writing—review & editing: Seyed Mehdi Alavi, Najmeh Malekzadeh Gonabadi.

Competing Interests

The authors state no conflicts of interest regarding this study.

Ethical Approval

This study was approved by Sistan and Baluchestan university (IR. USB.REC.1399.031).

References

1. Yu S, Wang Y, He P, Shao B, Liu F, Xiang Z, et al. Effective combinations of immunotherapy and radiotherapy for cancer treatment. *Front Oncol.* 2022;12:809304. doi: [10.3389/fonc.2022.809304](https://doi.org/10.3389/fonc.2022.809304).
2. Maiman TH. Stimulated optical radiation in ruby. *Nature.* 1960;187(4736):493-4. doi: [10.1038/187493a0](https://doi.org/10.1038/187493a0).
3. Rocha Júnior AM, Vieira BJ, de Andrade LC, Aarestrup FM. Effects of low-level laser therapy on the progress of wound healing in humans: the contribution of *in vitro* and *in vivo* experimental studies. *J Vasc Bras.* 2007;6(3):258-66. doi:

- 10.1590/s1677-54492007000300009.
4. Azadgoli B, Baker RY. Laser applications in surgery. *Ann Transl Med.* 2016;4(23):452. doi: [10.21037/atm.2016.11.51](#).
5. de Freitas LF, Hamblin MR. Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE J Sel Top Quantum Electron.* 2016;22(3):7000417. doi: [10.1109/jstqe.2016.2561201](#).
6. Frigo L, Luppi JS, Favero GM, Maria DA, Penna SC, Bjordal JM, et al. The effect of low-level laser irradiation (In-Ga-Al-AsP - 660 nm) on melanoma in vitro and in vivo. *BMC Cancer.* 2009;9:404. doi: [10.1186/1471-2407-9-404](#).
7. Kumar Rajendran N, George BP, Chandran R, Tynga IM, Houreld N, Abrahamse H. The influence of light on reactive oxygen species and NF- κ B in disease progression. *Antioxidants (Basel).* 2019;8(12):640. doi: [10.3390/antiox8120640](#).
8. Ottaviani G, Martinelli V, Rupel K, Caronni N, Naseem A, Zandonà L, et al. Laser therapy inhibits tumor growth in mice by promoting immune surveillance and vessel normalization. *EBioMedicine.* 2016;11:165-72. doi: [10.1016/j.ebiom.2016.07.028](#).
9. Xia Y, Yu W, Cheng F, Rao T, Ruan Y, Yuan R, et al. Photobiomodulation with blue laser inhibits bladder cancer progression. *Front Oncol.* 2021;11:701122. doi: [10.3389/fonc.2021.701122](#).
10. Marchesini R, Dasdia T, Melloni E, Rocca E. Effect of low-energy laser irradiation on colony formation capability in different human tumor cells in vitro. *Lasers Surg Med.* 1989;9(1):59-62. doi: [10.1002/lsm.1900090112](#).
11. Rhee YH, Moon JH, Choi SH, Ahn JC. Low-level laser therapy promoted aggressive proliferation and angiogenesis through decreasing of transforming growth factor- β 1 and increasing of Akt/hypoxia inducible factor-1 α in anaplastic thyroid cancer. *Photomed Laser Surg.* 2016;34(6):229-35. doi: [10.1089/pho.2015.3968](#).
12. Roshandel G, Ferlay J, Ghanbari-Motlagh A, Partovipour E, Salavati F, Aryan K, et al. Cancer in Iran 2008 to 2025: recent incidence trends and short-term predictions of the future burden. *Int J Cancer.* 2021;149(3):594-605. doi: [10.1002/ijc.33574](#).
13. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin.* 2023;73(3):233-54. doi: [10.3322/caac.21772](#).
14. Bensadoun RJ, Epstein JB, Nair RG, Barasch A, Raber-Durlacher JE, Migliorati C, et al. Safety and efficacy of photobiomodulation therapy in oncology: a systematic review. *Cancer Med.* 2020;9(22):8279-300. doi: [10.1002/cam4.3582](#).
15. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. *Lasers Med Sci* 2012;27(1):237-249.
16. Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: mechanism and efficacy. *Dermatol Surg.* 2005;31(3):334-40. doi: [10.1111/j.1524-4725.2005.31086](#).
17. Basso FG, Pansani TN, Cardoso LM, Citta M, Soares DG, Scheffel DS, et al. Epithelial cell-enhanced metabolism by low-level laser therapy and epidermal growth factor. *Lasers Med Sci.* 2018;33(2):445-9. doi: [10.1007/s10103-017-2176-z](#).
18. Esmaeeli Djavid G, Bigdeli B, Goliaei B, Nikoofar A, Hamblin MR. Photobiomodulation leads to enhanced radiosensitivity through induction of apoptosis and autophagy in human cervical cancer cells. *J Biophotonics.* 2017;10(12):1732-42. doi: [10.1002/jbio.201700004](#).
19. Gomes Henriques Á C, Ginani F, Oliveira RM, Keesen TS, Galvão Barboza CA, Oliveira Rocha HA, et al. Low-level laser therapy promotes proliferation and invasion of oral squamous cell carcinoma cells. *Lasers Med Sci.* 2014;29(4):1385-95. doi: [10.1007/s10103-014-1535-2](#).
20. Zecha JA, Raber-Durlacher JE, Nair RG, Epstein JB, Sonis ST, Elad S, et al. Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations. *Support Care Cancer.* 2016;24(6):2781-92. doi: [10.1007/s00520-016-3152-z](#).
21. AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. *Lasers Med Sci.* 2012;27(1):237-49. doi: [10.1007/s10103-011-0885-2](#).
22. Scharfetter VH, Galvan O, Riechelmann H, Dudás J. Differential responses of fibroblasts, non-neoplastic epithelial cells, and oral carcinoma cells to low-level laser therapy. *Support Care Cancer.* 2012;20(3):523-9. doi: [10.1007/s00520-011-1113-0](#).
23. Shirazian S, Keykha E, Pourshahidi S, Ebrahimi H. Effects of 660 nm and 810 nm Low-Power Diode Laser on Proliferation and Invasion of Oral Cancer Cells in Cell Culture Media. *Photochem Photobiol.* 2021;97(3):618-626. doi: [10.1111/php.13351](#).
24. Hanna R, Dalvi S, Benedicenti S, Amaroli A, Sălăgean T, Pop ID, et al. Photobiomodulation therapy in oral mucositis and potentially malignant oral lesions: a therapy towards the future. *Cancers (Basel).* 2020;12(7):1949. doi: [10.3390/cancers12071949](#).
25. Fernandes KP, Souza NH, Mesquita-Ferrari RA, de Fatima Teixeira da Silva D, Rocha LA, Alves AN, et al. Photobiomodulation with 660-nm and 780-nm laser on activated J774 macrophage-like cells: Effect on M1 inflammatory markers. *J Photochem Photobiol B.* 2015;153:344-51. doi: [10.1016/j.jphotobiol.2015.10.015](#).
26. Jonat W, Arnold N. Is the Ki-67 labelling index ready for clinical use? *Ann Oncol.* 2011;22(3):500-2. doi: [10.1093/annonc/mdq732](#).
27. Brown DC, Gatter KC. Ki67 protein: the immaculate deception? *Histopathology.* 2002;40(1):2-11. doi: [10.1046/j.1365-2559.2002.01343.x](#).
28. Prabhu V, Rao BSS, Rao ACK, Prasad K, Mahato KK. Photobiomodulation invigorating collagen deposition, proliferating cell nuclear antigen and Ki67 expression during dermal wound repair in mice. *Lasers Med Sci.* 2022;37(1):171-80. doi: [10.1007/s10103-020-03202-z](#).