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Investigating the Effects of 70 mW Laser Irradiation at 808 and 635 nm Wavelengths on Proliferation and Apoptotic Mechanisms in Colon Cancer Cells

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Abstract

Recent research has shown that light-laser diode radiation is an effective therapy for wound healing, inflammation reduction, and cancer prevention. This study aimed to assess the biological response of human colon cancer cells subjected to a single laser irradiation dosage (2.5, 5, 7.5, and 10 J/cm2). After seeding human colon cancer cells onto laboratory plates, various dosages of an 808 and 635 nm laser were used to irradiate their exposure at 70 milliwatts, cells exposed to radiation were then incubated for one day. Changes in cell proliferation have been examined using MTT (3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assays. The viability of cells was decreased in nm-irradiated cultures than in control cultures. The results showed that doses of 2.5, 5, 7.5, and 10 J/cm2 were sufficient to cause visible alterations in colon cancer cells.

Keywords: Colon Cancer. Low-level laser. MTT. Human cells. Biological response.

دراسة تأثير أشعة الليزر بقدرة 70 ملي واط عند أطوال موجية 808 و635 نانومتر على آليات الانتشار والموت الخلوي المبرمج في خلايا سرطان القولون

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الخلاصة

أظهرت الأبحاث الحديثة أن إشعاع الليزر هو علاج فعال لشفاء الجروح وتقليل الالتهاب والوقاية من السرطان . تهدف هذه الدراسة إلى تقييم الاستجابة البيولوجية لخلايا سرطان القولون البشري المعرضة لجرعة واحدة من إشعاع الليزر (2.5 و 5 و 7.5 و 10 جول / سم ^{2}). بعد زرع خلايا سرطان القولون البشري على أطباق في المختبر، تم تشعيع هذه الخلايا باستخدام جرعات ليزر مختلفة وبأ طوال موجية 808 و 635 نانومتر وبقدرة 70 ملي واط ثم حضنت الخلايا المشععه ليوم واحد . تم فحص التغيرات في تكاثر الخلايا باستخدام اختبارات ام تي تي (3-(4,5-ثنائي ميثيل ثيازول-2-يل)-2,5- ثنائي فينيل-2اتش تيترازوليوم بروميد). انخفضت قابلية الخلايا للبقاء في المزارع المشعة مقارنة بالمزارع غير المشعة. وأظهرت النتائج أن جرعات 2.5 و5 و7.5 و10 جول/سم^2 كانت كافية لإحداث تغييرات مرئية في خلايا سرطان القولون.

1. Introduction

Low-level laser therapy (LLLT) is an important treatment method for many diseases[1]. The laser applications can be classified into surgical high-power lasers and lasers with low-intensity or therapy with cold lasers. In addition to treating both acute and chronic pain, soft laser treatment is thought to be incredibly safe and employed in all of the body's organs and tissues to promote healthy cellular function, such as boosting blood vessel growth and halting the flow of fluids to tissues [2] [3]. Cold laser therapy utilizes the visible red area and the near-IR portion of the electromagnetic wavelengths because research has shown that these regions of the spectrum when greatly absorbed by biological systems, have positive therapeutic effects on living tissues [4]. Low-power laser treatment is believed to have a bio-stimulatory effect, converting laser rays into metabolic energy and thereby triggering several mechanisms related to cell growth[5-6] . The treatment of injuries and lesions can be achieved through cold laser therapy, also known as photobiology or bio-stimulation, which uses low-power "monochromatic" wavelength. Soft laser therapy is a treatment by light in which cannot be sound, heat, or vibration is generated. Lower-level laser treatment acts in cells by methods other than thermal impacts, such as non-thermal or photochemical reactions [6]. It is imperative to bear in mind that the outcomes are contingent upon an ideal therapeutic window that is delineated via a distinct response for a given tissue and/or cell type. Also, the literature in this regard, exhibits a notable disparity in parameterization, leading to divergent views regarding the therapeutic outcomes of the technique and an inadequate understanding of the molecular pathways presently implicated in accomplishing the documented advantages [7]. It has long been recognized that low-level treatments have both stimulating and inhibiting impacts. Given at varying dosages with the same wavelength, various effects are generated. Researchers call this phenomenon a biphasic dosage, or hormesis [8]. Cold laser therapy experiments have seen the biphasic response multiple times [9][10].

Based on the Arndt-Schulz law, mild stimuli have a minor acceleration on important jobs and are strengthened by stronger stimuli; however, Once the climax is reached, larger stimuli suppress it until a negative reaction is reached [11]. This behavior is often cited as a convenient model to describe the dose-dependent effects of cold laser treatment [12][13]. A biphasic trajectory (as Figure 1 illustrates) can be used to characterize the expected dosage response to light at the subcellular, cellular, or tissue level. It illustrates the amount of energy required to either stimulate, inhibit, or eliminate the reaction to the dosage. Researchers [14] used the trypan blue exclusion test and the MTT colorimetric assay to assess cell survival and growth.

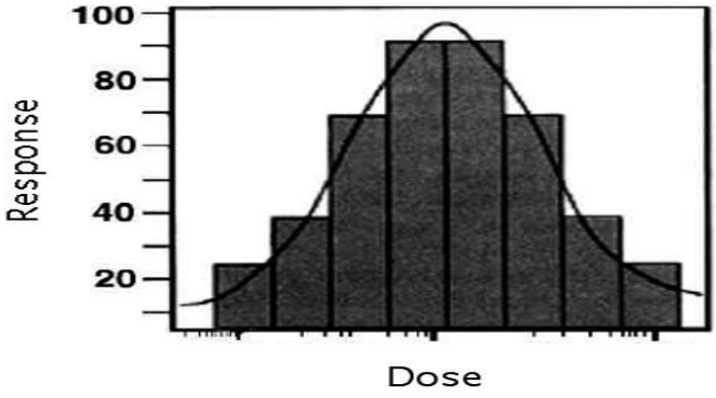

Figure -1 The biphasic response's idealized dose-response curve [14] **.**

2. Materials and Techniques Employed

2.1. Devices

1- Diode laser (808 nm), key characteristics: The model MGL_III_808 has a variable output power with a maximum power of 2 Watts, an operating mode of CW, a transverse mode close to TEM00, an estimated lifetime of 10,000 hours, and power stability lasting more than 4 hours. China is the manufacturing or producing nation.
2- Diode laser (635nm), the model MRI_III_635 has variable output power, a maximum power of 340 mW, operates in CW mode, transverse mode close to TEM00, and a projected lifespan of 10,000 hours. These are its most significant features. Stability of power for more than 4 hours China is the manufacturing or producing nation. In addition to the incubator, pH meter, magnetic stirrer, ELISA reader, inverted microscope, cell culture plate, and CCD camera for laser spot size measurement.

2.2. Cell Culture

Cells may be coaxed to rip in vitro or their tissue in a suitable environment when given a medium having growth hormones and nutrients. Therefore, cell culture is one of the most extensively used procedures in the research of biomedical. In a lab culture setting, cells are paired with the Vivo and allowed to grow in the same way as they do in the body. At 37°C, the cell cultures were being brooded. Inverse microscopy was employed to examine the cells[15]. In handy plastic containers, the complicated medium is employed to promote the development of these cells.

2.3. Basic idea behind the MTT test

The colorimetric MTT test was first reported in 1983 [16]. It is used to gauge a live cell's metabolic activity. The basic idea behind this test is that by adding metabolically active cells, the yellow-colored, water-soluble tetrazolium salt MTT is reduced to non-water-soluble purple formazan crystals. This conversion is carried out by the active mitochondria's succinate dehydrogenase system[17]. Using a spectrophotometer or a plate reader, the optical density (OD) of the resultant-colored solution is determined at a certain wavelength between 500 and 600 nm [18]. It is possible to measure the viability of different cell types by measuring the intensity of formazan color, which is related to the amount of viable, metabolically active cells [19].

2.4. Exposure to laser radiation

Low energy densities of red and near-infrared light were applied to the cells during Irradiation to promote healing. To ascertain the impact of various power densities on cells, three irradiation tests were conducted in triplicate, and the average value of the outcomes was taken. To enable adhesion, the cells are incubated for the whole night after seeding. Up until they were exposed to radiation, the plates were incubated at 37°C [15]. Moreover, the cells were exposed to laser light while being maintained at vitro temperature to observe any notable alterations in the cells.

3. Action Mechanism of Low Laser Level Treatment

Similar to how plants do photosynthesis, laser radiation to the tissues causes photobiological and photochemical reactions. Low-energy visible light cannot impact any live biological system unless its photons are absorbed by electrons that are part of a photoreceptor or chromophore [20] . According to several theories, mitochondria are stimulated by red or near-infrared light [21]. The molecules known as chromophores, or photoreceptors, provide the molecules or substances they attach to a certain hue. Systems for transporting electrons are called chromophores[22]. Changes in cellular metabolism, including protein signaling, can result from a sequence of photochemical processes that are triggered by the photoreceptor molecules of mitochondria, on which laser light appears to operate as a photo-stimulant. Cytochrome Oxidase C (COX), the last enzyme in the mitochondrial electron transport chain, is the essential photoreceptor molecule (FIG. 2) [23]. It is reported that applying LLLT to cell cultures or experimental models raises the concentration of nitric oxide (NO). This is due to the fact that COX and mitochondria produce NO when exposed to laser radiation [14]. Because oxygen flow is restored, the respiratory interference that had led to the link between NO and COX is eliminated, and ROS are generated after breathing has resumed. Because they are free radicals, oxygen radicals (ROS) are crucial to the synthesis of adenosine triphosphate (ATP), the cell's energy store [24]. Through the release of NO by iron ions and copper COX, oxygen recapture, the generation of ROS, and an increase in ATP synthesis, LLLT causes a respiratory re-start[25]. Transcription factors are synthesized under the influence of LLLT [26] (FIG. 3). In addition to their involvement in intracellular signaling, free radicals also facilitate the synthesis of nucleic acids in the nucleus, the production of proteins, the activation of enzymes, and the advancement of the cell cycle [9]. Lastly, because of the brief heating of the chromophore molecules, LLLT alters the biochemical cell activity [9]. The above-discussed systems are highly intricate and have been suggested as a potential explanation for the effects of radiation on tissues. It has been postulated that these cellular alterations account for the clinically observed effect benefits of LLLT, including enhanced cell proliferation, quicker healing, tissue regeneration and prevention of cell death.

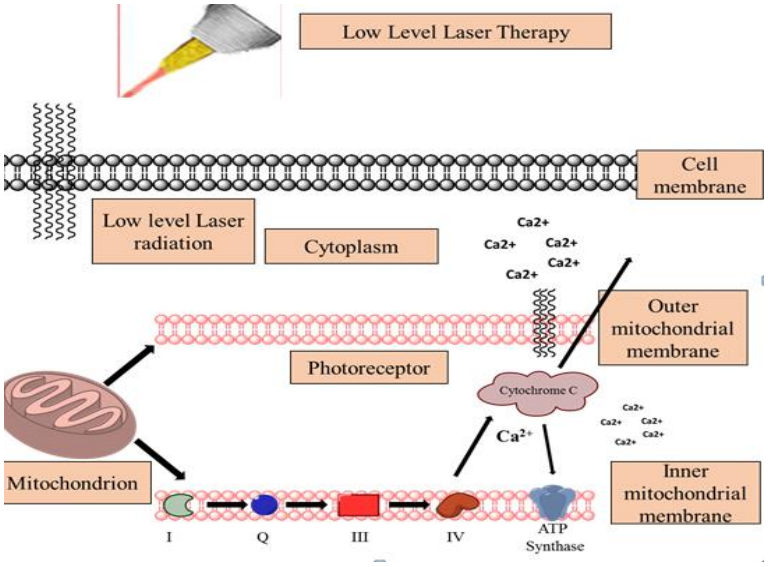

Figure -2 Diagrammatic representation showing how the mitochondrial respiratory chain's photoreceptors absorb laser light[23].

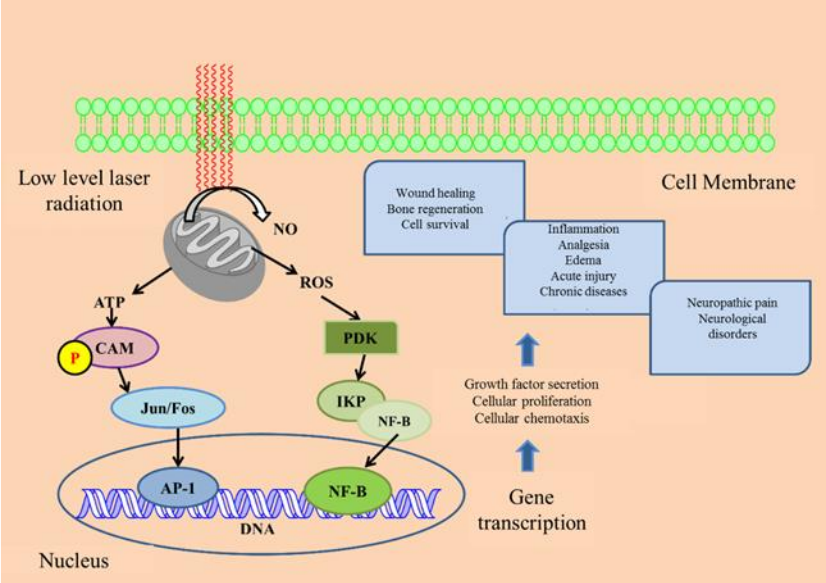

Figure - 3 A figure showing one of the mechanisms of laser action in tissues through the absorption of photons of red and NIR light by mitochondria[26]

4. Results and Discussion

LLLT is a safe and noninvasive procedure. The purpose of this paper was to explore the effect of various laser light dosages (808 and 635nm) on colon cancer cells. Three runs of the tests were conducted, and the average result was used to summarize the results. After irradiation, the cells were incubated under appropriate conditions for 24 hours, after which the MTT assay was performed. At dosages of 2.5, 5, 7.5, and 10 J/cm^2, the ratio of viability was different from the control. The 808nm laser used in this experiment showed inhibition in the cells at 2.5, 5,7.5, and 10 J/cm^2 dosages (Figure 4), indicating a reduction in the viability and mitochondrial function of irradiated cells in comparison to the untreated group. After the first dose of irradiation, (2.5 J/cm^2 at 42s) and as well as after the second dose of irradiation (5 J/cm^2 at 1m 21s), observed an increase in cell Inhibition in comparison to the control group. In addition, inhibition maximum at (7.5 J/cm^2 at 2m 1s) and minimum at (10 J/cm^2 at 2m 42s) cell was also noticed compared with a first and second dose. In contrast to the controls, the inhibition of cell viability was evident at all dosages.

For the treated groups, the maximum inhibition rate measured was 7.5 J/cm^2, whereas the lowest was 10 J/cm^2. In comparison to the dose (5 J/cm^2) and a control group of low-level red laser therapy (Figure 5), the data show that the doses (2.5, 7.5, and 10 J/cm^2) cause lower viability, and therefore suppress the growth of colon cancer cells. The MTT test results of cells showing inhibition at dosages of 2.5, 5, 7.5, and 10 J/cm^2 may indicate that cold laser treatment in these parameters has altered the properties of the cells. The types of lasers, their dosages, and their wavelengths vary greatly, and each change produces a distinct set of outcomes; some studies report increased cell proliferation, while others report decreased cell proliferation. Depending on the parameters employed, it may be possible to propose that LLLT can be used in cancerous lesions to reduce the proliferation of these cells; however, the lack of standardization in laser irradiation protocols for in vitro research prevents the establishment of optimal parameters for this purpose.

Thus, until further research is done, cancer patients should utilize soft laser therapy with caution. Many positive effects, including anticancer, angiogenic, and antioxidative effects, have been discovered through studies of laser-based phototherapy over the last decades [27].

The effectiveness of the laser is determined by its wavelength, dosage, and intensity, in addition to the conditions of the cell culture dictate, according to Karu [28]. There are biological limitations to LLLT; it cannot stimulate the growth of rapidly proliferating cells or activate every cell function. For different types of cells, radiation has different impacts. Although it has which has no clinical importance, the average value may be reflected in the overall cell response rather than the real, which is crucial for cell analysis. Numerous in vitro tests were conducted, and the behavior shown in the in-test sample—or not—shows how laser therapy affects a particular individual cell [29]. The purpose of this study was to evaluate the cellular activity of colon cancer cells in response to different radiation dosages. Few writers have looked at the biphasic reaction or dose/response curve regarding cellular activity when different dosages of energy are delivered at the same wavelength, despite the vast array of studies and articles that are now accessible. The red and near-infrared light spectrums are what we mean when we discuss the medical applications of low-power laser lighting. When applying low-level laser treatment (LLLT), a low-power milliwatt pulse is focused on the intended tissue and has a comparatively low energy density [30].

These results suggest that the impact of various dosages on the kinds, growth, and behavior of cells may vary. However, it is still unknown how laser-based phototherapy promotes cell division or death. We investigated the effects of various dosages on cell lines because we hypothesized that a certain wavelength of LLLT radiation would suppress colon cancer cell growth, encourage the manufacture of pro-apoptotic molecules, and induce cancer cell death based on prior studies. The autophagy and mitogen-activated protein kinases (MAPKs) pathways are strongly linked to the caspase-dependent apoptosis pathway [31]. Major signal transduction molecules, or MAPKs, play a key role in controlling a range of cellular responses, including as apoptosis, survival, differentiation, and proliferation. It has been demonstrated that MAPKs aid in the growth, migration, and invasion of several cancer cell types [32]. There is mounting data that supports the growth, survival, and invasion of cancer cells through the MAPK pathway[33]. In summary, 808 and 635 nm laser light caused colon cancer cells to become inhibited.

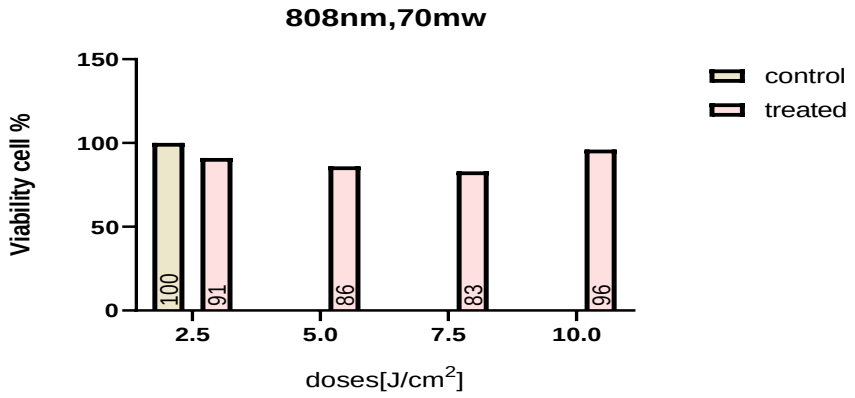

Figure - 4 The MTT assay was used to assess cell growth at various doses

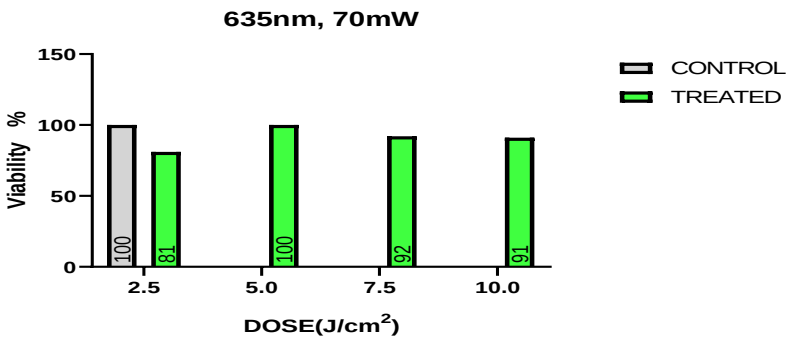

Figure - 5 Evaluate the impact of different exposure times for low-power laser treatment on cell viability.

5. Conclusion

The findings of this investigation demonstrate that LLLT suppresses colon cancer cells. These results advance our knowledge of the possible processes that underlie the effects of laser therapy. To fully understand the precise mechanisms of action of laser cold, more investigation is necessary. It is difficult to conclude that LLLT is safe and better than another active or negative intervention due to a lack of evidence and inadequate data.

References

[1] Y. Wang *et al.*, "The effectiveness and safety of low-level laser therapy on breast cancer–related lymphedema: An overview and update of systematic reviews," *Lasers Med. Sci.*, vol. 37, no. 3, pp. 1389–1413, 2022, doi: 10.1007/s10103-021-03446-3.

[2] D. Hawkins and H. Abrahamse, "Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts," *Photomed. Laser Ther.*, vol. 24, no. 6, pp. 705–714, 2006.

[3] M. L. Pascu, "Laser physics elements to consider for low level laser therapy," *Laser Ther.*, vol. 13, no. 1, pp. 114–125, 2000.

[4] D. Hawkins, N. Houreld, and H. Abrahamse, "Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing," *Ann. N. Y. Acad. Sci.*, vol. 1056, no. 1, pp. 486–493, 2005.

[5] M. R. Hamblin, "Mechanisms and mitochondrial redox signaling in photobiomodulation," *Photochem. Photobiol.*, vol. 94, no. 2, pp. 199–212, 2018.

[6] P. J. Pontinen, "Low level laser therapy as a medical treatment modality," *Tampera*, pp. 11–211, 1992.

[7] Y.-Y. Huang, S. K. Sharma, J. Carroll, and M. R. Hamblin, "Biphasic dose response in low level light therapy–an update," *Dose-Response*, vol. 9, no. 4, p. dose-response, 2011.

[8] E. J. Calabrese, "The future of hormesis: where do we go from here?," *Crit. Rev. Toxicol.*, vol. 31, no. 4–5, pp. 637–648, 2001.

[9] R. J. Lanzafame, I. Stadler, A. F. Kurtz, R. Connelly, P. Brondon, and D. Olson, "Reciprocity of exposure time and irradiance on energy density during photoradiation on wound healing in a murine pressure ulcer model," *Lasers Surg. Med. Off. J. Am. Soc. Laser Med. Surg.*, vol. 39, no. 6, pp. 534–542, 2007.

[10] U. Oron *et al.*, "Attenuation of infarct size in rats and dogs after myocardial infarction by low-energy

laser irradiation," *Lasers Surg. Med. Off. J. Am. Soc. Laser Med. Surg.*, vol. 28, no. 3, pp. 204–211, 2001.
[11] F. Martius, "Das amdt-schulz grandgesetz," *Munch Med Wschr*, vol. 70, pp. 1005–1006, 1923.
[12] R. T. Chow, G. Z. Heller, and L. Barnsley, "The effect of 300 mW, 830 nm laser on chronic neck pain: a double-blind, randomized, placebo-controlled study," *Pain*, vol. 124, no. 1–2, pp. 201–210, 2006.
[13] D. H. Hawkins and H. Abrahamse, "The role of laser fluence in cell viability, proliferation, and membrane integrity of wounded human skin fibroblasts following helium-neon laser irradiation," *Lasers Surg. Med. Off. J. Am. Soc. Laser Med. Surg.*, vol. 38, no. 1, pp. 74–83, 2006.
[14] Y.-Y. Huang, A. C.-H. Chen, J. D. Carroll, and M. R. Hamblin, "Biphasic dose response in low level light therapy," *Dose-response*, vol. 7, no. 4, p. dose-response, 2009.
[15] R. Ausubel *et al.*, "Short protocols in molecular cloning," *Wiley Sons, Inc. New York Brisbane*, pp. 1–14, 1994.
[16] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *J. Immunol. Methods*, vol. 65, no. 1–2, pp. 55–63, 1983.
[17] F. P. Altman, "Tetrazolium salts and formazans," *Prog. Histochem. Cytochem.*, vol. 9, no. 3, pp. III–51, 1976.
[18] D. M. L. Morgan, "Tetrazolium (MTT) assay for cellular viability and activity," *Polyam. Protoc.*, pp. 179–184, 1998.
[19] J. W. Byun, S. H. Choo, H. H. Kim, Y. J. Kim, Y. J. Hwang, and D. Y. Kim, "Evaluation of boar sperm viability by mtt reduction assay in beltsville thawing solution extender," *Asian-Australasian J. Anim. Sci.*, vol. 21, no. 4, pp. 494–498, 2008.
[20] V. G. Zilov, A. A. Khadartsev, and V. D. Bitsoev, "Effects of polychromatic visible and infrared light on biological liquid media," *Bull. Exp. Biol. Med.*, vol. 157, pp. 470–472, 2014.
[21] V. S. Hentschke *et al.*, "Maximal oxygen uptake and exercise tolerance are improved in rats with heart failure subjected to low-level laser therapy associated with resistance training," *Lasers Med. Sci.*, vol. 32, pp. 73–85, 2017.
[22] S.-R. Tsai and M. R. Hamblin, "Biological effects and medical applications of infrared radiation," *J. Photochem. Photobiol. B Biol.*, vol. 170, pp. 197–207, 2017.
[23] M. Z. Khalid, "Mechanism of laser/light beam interaction at cellular and tissue level and study of the influential factors for the application of low level laser therapy," *arXiv Prepr. arXiv1606.04800*, 2016.
[24] B. J. Quirk, K. Sannagowdara, E. V Buchmann, E. S. Jensen, D. C. Gregg, and H. T. Whelan, "Effect of near-infrared light on in vitro cellular ATP production of osteoblasts and fibroblasts and on fracture healing with intramedullary fixation," *J. Clin. Orthop. Trauma*, vol. 7, no. 4, pp. 234–241, 2016.
[25] S. Shiva and M. T. Gladwin, "Shining a light on tissue NO stores: near infrared release of NO from nitrite and nitrosylated hemes," *Journal of molecular and cellular cardiology*, vol. 46, no. 1. pp. 1–3, 2009.
[26] H. Eslami, P. Motahari, E. Safari, and M. Seyyedi, "Evaluation effect of low level Helium-Neon laser and Iranian propolis extract on Collagen Type I gene expression by human gingival fibroblasts: an in vitro study," *Laser Ther.*, vol. 26, no. 2, pp. 105–112, 2017.
[27] R. D. Almeida, B. J. Manadas, A. P. Carvalho, and C. B. Duarte, "Intracellular signaling mechanisms in photodynamic therapy," *Biochim. Biophys. Acta (BBA)-Reviews Cancer*, vol. 1704, no. 2, pp. 59–86, 2004.
[28] T. I. Karu, "Primary and secondary mechanisms of the action of monochromatic visible and near infrared radiation on cells," *Sci. low-power laser Ther.*, pp. 53–83, 1998.
[29] J. Tuner and L. Hode, *Laser therapy: clinical practice and scientific background: a guide for research scientists, doctors, dentists, veterinarians and other interested parties within the medical field*. Prima books, 2002.
[30] T. Ohshiro and R. G. Calderhead, *Low level laser therapy: a practical introduction*. John Wiley &

Sons, 1988.
[31] D. R. Green, "Apoptotic pathways: ten minutes to dead," *Cell*, vol. 121, no. 5, pp. 671–674, 2005.
[32] E. K. Kim and E.-J. Choi, "Pathological roles of MAPK signaling pathways in human diseases," *Biochim. Biophys. Acta (BBA)-Molecular Basis Dis.*, vol. 1802, no. 4, pp. 396–405, 2010.
[33] A. S. Dhillon, S. Hagan, O. Rath, and W. Kolch, "MAP kinase signalling pathways in cancer," *Oncogene*, vol. 26, no. 22, pp. 3279–3290, 2007.