Photobiomodulation Effect of 850 nm Near-infrared Diode Laser on the Release of M1-related Cytokines Inmonocytic THP-1 Cells

Ruwaidah A. Mussttaf[1,*], Mayyadah Hasan Rhaif Al-Sahlanee[2], Numan S. Dawood[3], David F. L. Jenkins[4], Awadhesh N. Jha[5]

[1]*Department of Physiology, College of Medicine*
University of Al-Nahrain, Baghdad, Iraq
E-mail: ruwaidahmussttaf16@nahrainuniv.edu.iq

[2]*Department of Physiology, College of Medicine*
Mustansiriyah University, Baghdad, Iraq
E-mail: dr.mayyadahhasan@uomustansiriyah.edu.iq

[3]*Department of Physiology, College of Medicine*
University of Baghdad, Baghdad, Iraq
E-mails: numans@comed.uobaghdad.edu.iq, numan_salman2012@yahoo.com

[4]*School of Computing, Electronics and Mathematics*
University of Plymouth, Plymouth, UK
E-mail: d.f.jenkins@plymouth.ac.uk

[5]*School of Biological and Marine Sciences*
University of Plymouth, Plymouth, UK
E-mail: a.jha@plymouth.ac.uk

[*]*Corresponding author*

Received: November 03, 2021 **Accepted: August 08, 2022**

Published: March 31, 2023

Abstract: *Photobiomodulation (PBM) is a form of the use of visible red and Near-infrared (NIR) light at low power, where a laser light photon is absorbed at the electronic level, without heat production. PBM can be applied in wide range of treatment to help the wound, inflammation, edema, and pain reduction. However, there is a lack of scientific documentation regarding its actual effects. Objectives: This study assesses the impact of PBM on the release of M1-related cytokine in monocyte cells with particular emphasis on interleukin-1β (IL-1β) and Tumour Necrosis Factor α (TNF-α). Methods: Tamm-Horsfall Protein 1 (THP-1) macrophages M1 cells have been exposed to the light from the diode laser of 850 nm at different doses (0, 0.6, 1.2 and 3.6 J/cm^2). The release of cytokines was determined by enzyme-linked immunosorbent assay, after different periods of incubation (0, 12, 24, and 48 hours) post-irradiation. The proliferation of fibroblast cells suspended in irradiated M1-supernatent was evaluated for the same periods of incubation. Results: The results showed that PBM significantly enhanced M1-related cytokine release ($p < 0.05$). Obviously, IL-1β increased post-irradiation at 1.2 J/cm^2 more than other doses for all incubation periods. TNF-α was decreased significantly after two days of irradiation ($p < 0.005$) for all doses. A significant increase in fibroblast proliferation ($p < 0.005$) was observed concomitant with the boost of cytokine release. Conclusion: This in vitro study has demonstrated that the PBM of the 850 nm diode laser therapy can enhance M1-related cytokine release, which in turn increases the proliferation of fibroblast cells. Moreover, PBM at 850 nm plays an anti-inflammatory role, which manifested by decreasing the level of TNF-α. Therefore, this therapy may be able to accelerate the wound healing process.*

Keywords: *Photobiomodulation, Cytokines, Anti-inflammatory effect, Macrophages.*

Introduction

Photobiomodulation (PBM) has seen widespread use for therapeutic purposes. It is a useful therapeutic technique for dealing with a variety of situations that necessitate healing to be energised, for pain relief, to alleviate inflammation, and re-establish function [8]. PBM uses light within the red and infrared region of the electromagnetic spectrum. PBM is a monochromatic or quasi-monochromatic light with low power level emitted from laser or Light Emitting Diode (LED) sources, and which is able to modify or modulate biological functions [4, 12].

Low power lasers have been demonstrated to be a secure, effective in different of medical fields including dentistry and ophthalmology and non-invasive [19]. It appears that PBM can have an impact at the molecular, cellular, and tissue levels [8]. Several mechanisms of action have been proposed, with the most accepted theory being that the absorption of red and Near-infrared Region (NIR) light by certain photoreceptors such as Cytochrome C Oxidase (CCO) is the essential biological effect of lasers. This photoreceptor (chromophore) is the fourth of five proteins found in the mitochondria's respiratory chain and triggers a cascade of reactions take place in the mitochondria, resulting in biological changes to numerous processes [3]. Absorption of light may result in photo dissociation of the restricted nitric oxide from CCO, causing an increase in enzyme activity, enhanced electron transport, oxygen consumption, mitochondrial respiration, or adenosine triphosphate (ATP) synthesis. Thus, through modifying the mitochondria or redox state of the cell, PBM may stimulate several of the known intracellular signalling pathways and alterations to cellular functions, involving proliferation, renovation, and cell survival [2, 21].

At present, the suggested basic action includes eclectic absorption of NIR photons by constructed water layers (also known as interfacial water) [15] or water clumps [17] at power levels that are inadequate to trigger any noticeable rise in the heat of the tissue. A slight elevation in vibrational energy by water clumps formed in or on a certain protein, such as a heat-gated ion channel, may be enough to disturb the structure of tertiary protein, enabling the channel to open and intracellular calcium levels to be modulated [28]. When visible or near-infrared light is absorbed, interfacial water can undergo charge separation, according to Pollack et al. [28]. This charge separation (corresponding to localized pH variations) may have an impact on protein structure [7, 10].

Irradiation via PBM has shown considerable promise in terms of stimulating wound healing. The healing process, after injury, starts with the recruitment of immune cells at the injured site [25]. Neutrophils, monocytes, and lymphocytes are inflammatory cells that reach the inflammatory site via prostaglandins and persist there, and are associated with increasing levels of the major inflammatory cytokines, such as Tumour Necrosis Factor α (TNF-α) and interleukin (IL-1β and IL-6) during the repair and regeneration process [9, 22]. PBM, as a therapeutic strategy, is predicted to have the ability to modify the secretion of pro-inflammatory cytokines. However, the impact of PBM on these occurrences is unclear, particularly in terms of which set of parameters is most effective in promoting them [1]. Therefore, the aim of the current study is to verify the influence of PBM on IL-1β and TNF-α in macrophage-likeTHP-1 cells.

Materials and methods

Cell culture

The THP-1 cell line is the human monocytic leukaemia (permanent) cell line, obtained from the peripheral blood of a one-year-old male with acute monocytic leukaemia.THP-1 were preserved according to [9], in RPMI-1640 (Roswell Park Memorial Institute medium) complemented with 10% foetal bovine serum (FBS), 5 ml of L-glutamine and 5 ml

penicillin/streptomycin. The cells were grown in a humidified incubator at 37 °C with 5% CO_2 in a 75 ml culture flask containing 20 ml medium plus cells (more information in [24]).

THP-1 cells differentiation with phorbol-12-myristate-13-acetate

THP-1 cells were plated (at a density of 1×10^6 cells/ml) in six well tissue culture plates in complete medium (as stated in the previous section) and treated with phorbol-12-myristate-13-acetate (PMA) (25 ng/ml, three days; for differentiation into M1-like macrophages).

Fibroblast cells culture

Human skin fibroblast cells (WS1; Adcock, ATCC CRL 1502, Adcock Ingram S.A., Bryanston, SA) were grown as described by [1]. Cells were continually seeded in 75 cm^2 culture flasks for many passages in complete medium containing an additional 17 mmol/l D-glucose until demanded. The cultures were incubated at 37 °C with 5% CO_2 and 85% humidity. To evaluate the influence of laser-induced cytokines, cells were confluent, with each culture flask containing approximately $3\text{-}4\times10^6$ cells. The excess medium decanted from each flask, and the cells were rinsed with phosphate-buffered saline (PBS). Cells were detached by trypsinization, where 5 ml 0.25% trypsin was added to each flask and left for 30 s. The excess trypsin was removed, and after 10 min the cells were detached. 0.03% ethylenediaminetetraacetic acid (EDTA), and 6×10^5 cells in 3 ml complete culture media were seeded in the flask. 1000 cells were pipetted in 50 µl volumes into a 96 well culture plate. 50 µl of fresh culture media was then added to each well to give a final volume of 100 µl per well.

The laser irradiation

The laser device used in this study was a diode laser, model APMT25 (850-40)/5342 (Power Technology Incorporated, Alexander, AR, USA) with a power of 9.5 mW, a power density of 29.6 mW/cm^2 and a wavelength of 850 nm. The laser operates in continuous wave (CW) mode, giving light spot with a diameter of 8.7 mm. For counting and testing purposes, trypsinisation with 0.5% (v/v) trypsin and 0.25% (v/v) EDTA was used to detach adherent cells (M1-like), and trypsinisation was halted with fresh medium. M1-like macrophage cells with a density of 10,000 cells/200 µl were conveyed to 96 well plate to be exposed to the diode laser for periods of 20, 40, and 120 seconds for varied doses of 0.6, 1.2, and 3.6 J/cm^2, respectively, with leaving a group unexposed as control. Determination of cytokine levels (IL-1β and TNF-α) by enzyme-linked immunosorbent assay (ELISA) assay was achieved after 0, 12, 24 and 48 h incubation post-irradiation (more information in [24]).

Quantitative analysis of cytokine levels

Quantifying of IL-1β and TNF-α Levels was achieved by ELISA using commercial kits (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. The reading was carried out using an OPTI Max tuneable microplate reader set to 450 nm and the results analysed via the Soft Max Pro version 2.4.1 software (Versa Max, Molecular Devices, Sunny Vale, CA, USA).

Proliferation of fibroblast cells

The proliferation of fibroblast cells was determined microscopically using the Trypan blue exclusion test. 20 µl cell suspensions were transferred into eppendorfs and mixed with 20 µl of 0.4 M trypan blue solution, and 10 µl of the mixture was taken to be counted in a Neubauer haemocytometer chamber. The haemocytometer consists of nine 1 mm^2 squares, each square representing a volume of 1×10^{-4} ml. An inverted microscope was used to count cells in the four corner squares, and by taking the average the number of cells per 1 mm^2 can be determined.

Statistical analysis

All data were initially summarized in the form of mean and SE. Analysis was under taken using a Tukey's pairwise comparisons as post hoc tests and two-way ANOVA with Holm-Sidak for pairwise comparisons and comparisons versus a control group. Statistics were calculated using Minitab (v. 15.1.0; Minitab Inc., State College, PA, USA) and SigmaPlot (v. 13). The significance for all tests was set at $p \leq 0.05$.

Results

The release of inflammatory cytokines and the proliferation of fibroblasts were both studied as essential events in the healing process. A range of doses (0.6, 1.2, and 3.6 J/cm^2) were used for the irradiation of the M1-likemacrophages, and all results obtained were measured immediately, and after 12, 24 and 48 h incubation following irradiation: (i) effect of PBM on M1 macrophage cytokines, and (ii) IL-1β of PBM-induced M1 macrophage.

The present study was conducted to develop an investigational strategy to ensure that PBM or low level-near infrared laser radiation was capable of modulating the mediators of healing process of wounds that have not healed properly. The concentration of IL-1β was measured via ELISA at 0, 12, 24, and 48 h after the irradiation of the M1-likemacrophage. The data showed a significant increase ($p < 0.005$) in IL-1β at 1.2 J/cm^2 and 3.6 J/cm^2 doses, while there was a nonsignificant increase ($p > 0.05$) at 0.6 J/cm^2 irradiation for all incubation times following irradiation compared to the non-incubated cells (0 h). There is a remarkable increase showed in IL-1β after exposure to all doses compared to the control (0 J/m^2), and statistically significant difference ($p < 0.005$) between 0.6 J/cm^2, 1.2 J/cm^2 and 3.6 J/cm^2 for the same incubation time. This indicates that the effect is dose and time post-irradiation-dependent (Fig. 1). The data are represented in the form of mean ± SE.

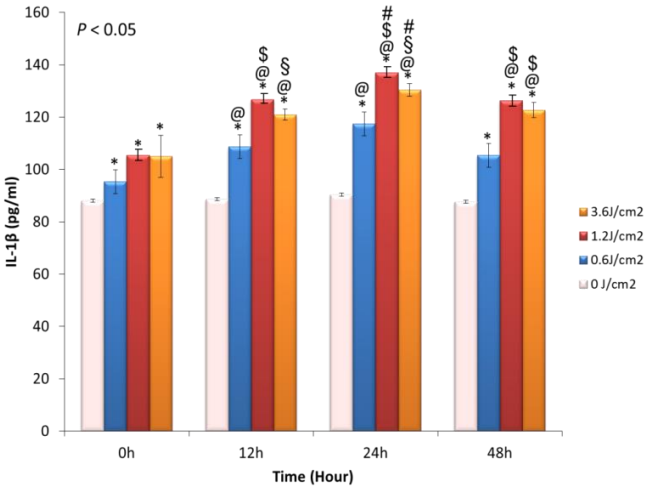


Fig. 1 Concentrations of IL-1β following exposure to various dosages of PBM for 0, 12, 24, and 48 h incubation times post-irradiation.
*) $p < 0.05$ represents significant differences compared to the control (0 J/cm^2);
@) $p < 0.05$ refers to the significant differences compared to the non-incubated cells (0 h);
$) and §) $p < 0.05$ indicate the differences between the doses for the same incubation time;
#) $p < 0.05$ represents the differences between doses at 24 h from the equivalent incubation times; (note: each experiment was repeated three times, $n = 3$).

TNF-α of PBM-induced M1 macrophage

In Fig. 2, the concentration of TNF-α induced by PBM irradiation compared to the control is illustrated. The data are represented in the form of mean ± SE and the same notation as in Fig. 1 are used. Statistically, a significant increase ($p < 0.001$) was found at 12 h and the first day for the 1.2 J/cm^2 and 3.6 J/cm^2 doses compared to the control (non-irradiated group) for the same incubation time and compared to the non-incubated cells (0 h), with a nonsignificant increase ($p > 0.05$) for 0.6 J/cm^2 irradiation compared to the non-incubated cells (0 h) and 48 h incubation. There is a significant difference ($p < 0.05$) between 0.6 J/cm^2 and 1.2 J/cm^2 and 3.6 J/cm^2 after 12, 24 and 48 hour. However, a considerable decrease ($p < 0.0001$) by 50% after laser irradiation was observed for the 1.2 J/cm^2 and 3.6 J/cm^2 doses 48 h after irradiation. This indicates that the effect is dose and time post-irradiation-dependent.

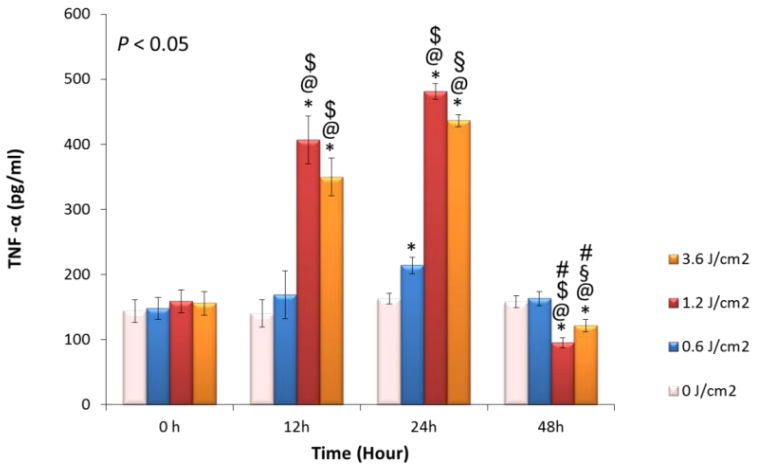


Fig. 2 Concentrations of TNF-α following exposure to various dosages of PBM for 0, 12, 24, and 48 h incubation times post-irradiation

Proliferation of fibroblast cells

The proliferation of fibroblast cells was determined using the microscope and haemocytometer. M1supernatant irradiated with the 850 nm diode laser at fluencies of 0.6, 1.2, and 3.6 J/cm^2 induced a marked alteration in the count of fibroblast cells, and thus proliferation, as compared with the non-irradiated group. The data in Fig. 3 (represented in the form mean ± SE) indicates that the proliferation of fibroblast cells was dose and time-dependent. Statistically, a significant increase ($p < 0.05$) was observed at all incubation times for each dose compared to control (non-incubated) cells. There was a significant increase ($p = 0.034$) in the proliferation at 1.2 J/cm^2 and 3.6 J/cm^2 doses after 24, and 48 h post-irradiation, while there was no significant elevation ($p > 0.05$) in proliferation at 12 h post-irradiation for all doses compared to the control (non-irradiated group) (Fig. 3).

Relation analysis

Fig. 4 illustrates the correlations obtained at times of 12 h and 24 h, respectively, between those biomarkers IL-1β and TNF-α sampled at all doses and the count of fibroblast cells. There was a clear temporal trend ($p < 0.05$) that indicated a significant correlation between the levels of IL-1β and TNF-α cytokines and fibroblast count ($R^2 = 0.9034$ and $R^2 = 0.8585$), whereas at 48 h there was no correlation between the biomarkers and cell count (data not shown).

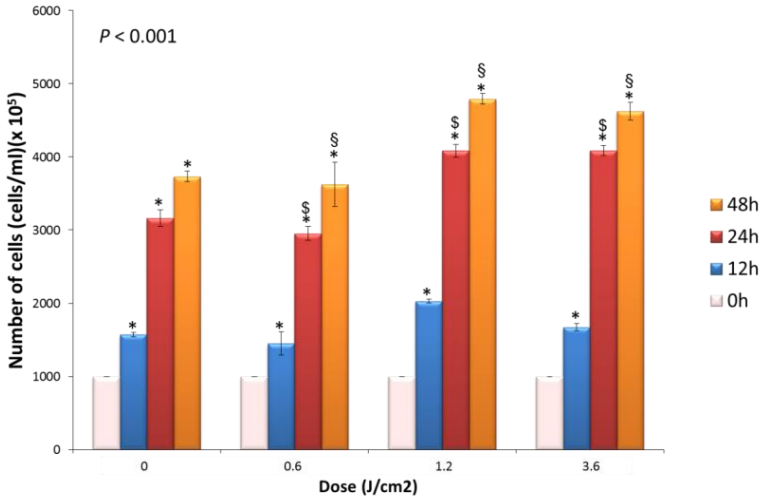
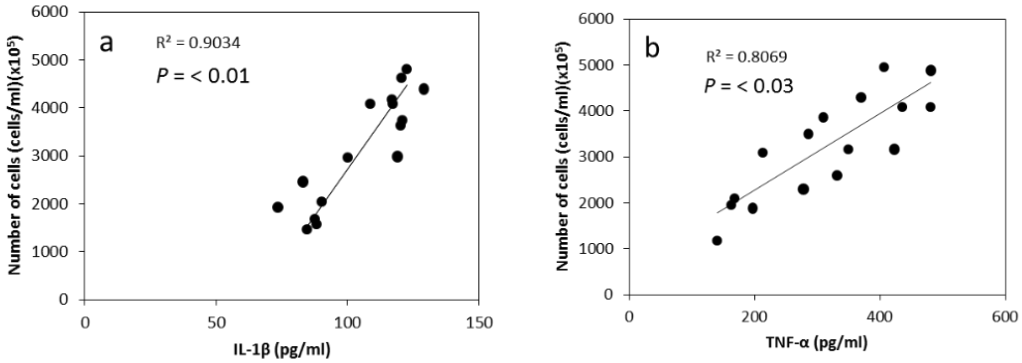


Fig. 3 Proliferation of fibroblast cells grown in the media of M1-like macrophages irradiated with various doses (0, 0.6, 1.2, and 3.6 J/cm^2) at 0, 12, 24, and 48 h post-irradiation.
*) $p < 0.05$ represents significant differences compared to the control (0 J/cm^2) and non-incubated cells (0 h);
$) $p < 0.05$ in comparison with that of the 12 h for the same dose;
§) $p < 0.05$ in comparison between 48 h and 24 h for the same dose;
(note: each experiment was repeated three times, $n = 3$).



a) b)

Fig. 4 Linear regression analyses for the number of fibroblast cells and biomarkers of M1-like macrophages: a) IL-1β and b) TNF-α using Pearson's correlation analysis.

Discussion

Medical concern in PBM has risen progressively over the last decade [1]. PBM has been investigated in various areas of medicine. Wound healing is a biological technique that is widely used to assess the impact of PBM, and the cytokine measurement is the most significant to explore the influence of PBM as a wound-healing accelerator [16].

The result of the present study clearly confirms that PBM irradiation significantly promotes the release of IL-1β and TNF-α.The observation of a boost inIL-1β and TNF-α concentration for all doses after the first day was more than that at the other incubation periods. These findings back up prior research, which confirmed the role of therapeutic PBM in enhancing the release of macrophage cytokines at 24 h post-laser application.

Vivian et al. [32] reported the effects of PBM in regulating the release of cytokine during recovery of oral wounds. Increasing the release of IL-1β was associated with PBM, as compared to a sham control group [32]. Oton-Leite et al. [27] assessed the level of salivary IL-1β in cancer patients following irradiation with PBM, in order to treat oral mucositis caused by radiotherapy. A boost in IL-1β levels was found at 24 h following the starting of radiotherapy [11, 27].

Moreover, a study on the effects of low-level laser therapy on the release of cytokines of macrofages in monocytes indicated that a Gallium-Aluminum-Arsenide (GaA1As) laser operating in the infrared at a 1 J/cm^2 dose enhanced TNF-α release 24 h after irradiation [9, 29, 30], which is consistent with our results. The elevation in IL-1β levels induced by PBM after the first day, which could be associated with increased TNF-α, is required for progression to the proliferative stage of healing [32]. This is supported by raising the number of fibroblast cells, incubated with a supernatant of irradiated macrophage suspension, for 12, 24, and 48 h at 1.2 J/cm^2 and 3.6 J/cm^2, respectively.

TNF-α is a critical-phase protein responsible for motivating a chain of other cytokines. This pro-inflammatory cytokine enhances vascular permeability and inhibits the process of inflammation via recruiting inflammatory cells to the infection site [26]. Nonetheless, TNF-α overexpression can result in chronic and slow-healing acute wounds, as well as host-damaging consequences [13]. Therefore, the remarkable decrease in the level of TNF-α at the second day for all doses could reduce the incidence of infection [14, 18, 10, 31]. In addition, the stimulated increase in IL-1β release emphasised the role of PBM at NIR region in accelerating the healing process. This has been proven by the strong relationship between the two markers, IL-1β and TNF-α, and the proliferation of fibroblast skin cells [5, 6, 23].

Conclusions

The present study showed PBM with an 850 nm diode laser has a simulative effect on the secretion of IL-1β and TNF-α in M1 macrophages differentiated from THP-1 cells. Although there is disparity in the previous studies compared to these results, the majority of existing evidence supports these results suggest that PBM may speed wound healing by inducing the production of certain cytokines. PBM can has essential role in wound healing by increasing the release of cytokines that necessary to stimulate the proliferation of fibroblast cells, which is important for optimal tissue healing. Moreover, the decrease in the level of TNF-α cytokine two days post-irradiation is an excellent indicator of the anti-inflammatory effect of PBM.

As recommendations for further work, additional studies on how to use the effect of PBM to stimulate another cytokines and growth factors, as mediators of the wound healing process, are required using the specific properties of this therapy to standardise the method used to apply this effect clinically.

Acknowledgements

The authors would like to thank the University of Al-Nahrain (College of Medicine), the Mustansiriyah University (College of Medicine) and the University of Baghdad (College of Medicine). The author would like to offer special thanks to Dr. Abbas Al-Shabany (Biochemistry in Al-Qadisiyah University), Dr. Sahar Al-Abady (Biology in Al-Qadisiyah University) and to all friends who supported this work.

References

1. Abrahamse H., E. Evans (2007). Effect of Multiple Exposures of Low-level Laser Therapy on the Cellular Responses of Wounded Human Skin Fibroblasts, Photomedicine and Laser Surgery, 24(6), 705-714.
2. Adamskaya N., P. Dungel, R. Mittermayr, et al. (2011). Light Therapy by Blue LED Improves Wound Healing in an Excision Model in Rats, Injury, 42(9), 917-921.
3. AlGhamdi K. M., A. Kumar, N. A. Moussa (2012). Low-level Laser Therapy: A Useful Technique for Enhancing the Proliferation of Various Cultured Cells, Lasers in Medical Science, 27(1), 237-249.
4. Al-Khazragi R., T. Al-Wajidi, F. S. Al-Ani (2004). Effect of Macromolecules on Erythrocytes Aggregation and Sedimentation Kinetics by He-Ne Laser Scattering, Iraqi Journal of Medical Science, 3(3), 235-240.
5. Brochetti R. A., M. P. Leal, R. Rodrigues, et al. (2017). Photobiomodulation Therapy Improves Both Inflammatory and Fibrotic Parameters in Experimental Model of Lung Fibrosis in Mice, Lasers in Medical Science, 32(8), 1825-1834.
6. Cardoso L. M., T. N. Pansani, J. Hebling (2020). Photobiomodulation of Inflammatory-cytokine-related Effects in a 3-D Culture Model with Gingival Fibroblasts, Lasers in Medical Science, 35(5), 1205-1212.
7. Chai B., H. Yoo, G. H. Pollack (2009). Effect of Radiant Energy on Near-surface Water, Journal of Physical Chemistry B, 113, 13953-13958.
8. Chaves M. E., A. R. Araújo, A. C. Piancastelli, M. Pinotti (2014). Effects of Low-power Light Therapy on Wound Healing: LASER × LED, An Bras Dermatol, 89(4), 616-623.
9. Chen C.-H., C. Z. Wang, Y. H. Wang, et al. (2014). Effects of Low-level Lasertherapy on M1-related Cytokine Expression in Monocytes via Histonemodification, Mediators Inflammation, 1-13.
10. Damodaran S. (2015). Water at Biological Phase Boundaries: Its Role in Interfacial Activation of Enzymes and Metabolic Pathways, Subcell Biochemistry, 71, 233-261.
11. Ebrahimi P., M. Hadilou, F. Naserneysari, et al. (2021). Effect of Photobiomodulation in Secondary Intention Gingival Wound Healing – A Systematic Review and Meta-analysis, BMC Oral Health, 21, 258.
12. Enengla J., M. R. Hamblin, P. Dungele (2020). Photobiomodulation for Alzheimer's Disease: Translating Basic Research to Clinical Application, Journal of Alzheimer's Disease, 75(4), 1073-1082.
13. Esposito E., S. Cuzzocrea (2009). TNF-alpha as a Therapeutic Target in Inflammatory Diseases, Ischemia-reperfusion Injury and Trauma, Current Medicinal Chemistry, 16(24), 3152-3167.
14. Fernandes K. P., A. N. Alves, F. D. Nunes, et al. (2013). Effect of Photobiomodulation on Expression of IL-1β in Skeletal Muscle Following Acute Injury, Lasers in Medical Science, 28(3), 1043-1046.
15. Ho M.-W. (2014). Illuminating Water and Life, Entropy, 16(9), 4874-4891.
16. Hong N. (2019). Photobiomodulation as a Treatment for Neurodegenerative Disorders: Current and Future Trends, Biomedical Engineering Letters, 9(3), 359-366.
17. Inoue S., M. Kabaya (1989). Biological Activities Caused by Far-infrared Radiation, International Journal of Biometeorology, 33,145-150.
18. Karkada G., G. A. Maiya, N. N. Houreld, et al. (2020). Effect of Photobiomodulation Therapy on Inflammatory Cytokines in Healing Dynamics of Diabetic Wounds: A Systematic Review of Preclinical Studies, Archives of Physiology and Biochemistry, 1861025.
19. Kneebone W. J. (2006). Practical Applications of Low Level Laser Therapy, Practical Pain Manage, 6(8), 34-40.

20. Langella L. G., H. L. Casalechi, S. S. Tomazoni, et al. (2018). Photobiomodulation Therapy (PBMT) on Acute Pain and Inflammation in Patients Who Underwent Total Hip Arthroplasty – A Randomized, Triple-blind, Placebo-controlled Clinical Trial, Lasers in Medical Science, 33(9), 1933-1940.
21. Lins R. D. A. U., E. M. Dantas, K. C. R. Lucena, et al. (2010). Biostimulation Effects of Low-power Laser in the Repair Process, Anais Brasileiros de Dermatologia, 85(6), 849-855.
22. Michael R. H. (2017). Mechanisms and Applications of the Anti-inflammatory Effects of Photobiomodulation, AIMS Biophysics, 4(3), 337-361.
23. Moradi A., Y. Kheirollahkhani, P. Fatahi (2019). An Improvement in Acute Wound Healing in Mice by the Combined Application of Photobiomodulation and Curcumin-loaded Iron Particles, Lasers in Medical Science, 34, 779-791.
24. Mussttaf R. A. (2018). Laser Light Interaction and Their Influence on DNA and Cell under *in vitro* Conditions, PhD Thesis, University of Plymouth, UK.
25. Nicolette H. (2019). Healing Effects of Photobiomodulation on Diabetic Wounds, Applied Sciences, 9(23), 5114.
26. Olczyk P., L. Mencner, K. Komosinska-Vassev (2014). The Role of the Extracellular Matrix Components in Cutaneous Wound Healing, Biomedical Research International, 2014, 747584.
27. Oton-Leite A. F., G. B. Silva, M. O. Morais, et al. (2015). Effect of Low-level Laser Therapy on Chemoradiotherapy-induced Oral Mucositis and Salivary Inflammatory Mediators in Head and Neck Cancer Patients, Lasers in Surgery Medicine, 47(4), 296-305.
28. Pollack G. H., X. Figueroa, Q. Zhao (2009). Molecules, Water, and Radiant Energy: New Clues for the Origin of Life, International Journal of Molecular Sciences, 10, 1419-1429.
29. Ramos L. R., R. L. Marcos, R. T. Silva, et al. (2018). Characterization of Skeletal Muscle Strain Lesion Induced by Stretching in Rats: Effects of Laser Photobiomodulation, Photomedicine and Laser Surgery, 36(9), 460-467.
30. Souza N. H. C., R. A. M. Ferrari, D. F. T. Silva (2014). Effect of Low-level Laser Therapy on the Modulation of the Mitochondrial Activity of Macrophages, Brazilian Journal of Physical Therapy, 18(4), 308-314.
31. Souza N. H. C., R. A. Mesquita-Ferrari, M. F. Rodrigues, et al. (2018). Photobiomodulation and Different Macrophages Phenotypes during Muscle Tissue Repair, Journal of Cell and Molecular Medicine, 10, 4922-4934.
32. Vivian P. W., C. Marina, P. W. Liana (2016). Photobiomodulation Regulates Cytokine Release and New Blood Vessel Formation during Oral Wound Healing in Rats, Lasers in Medical Science, 41(8), 577-583.

Ruwaidah Abdulameer Mussttaf, Ph.D.
E-mail: ruwaidahmussttaf16@ced.nahrainuniv.edu.iq

Ruwaidah Abdulameer Mussttaf has B.Sc. in Physics Science from College of Science, Mustansiriyah University, Iraq and M.Sc. in Medical Physics from Physiology Department, College of Medicine, Mustansiriyah University, Iraq, and Ph.D. in Medical Physics from School of Computing, Electronics and Mathematics, University of Plymouth, UK. Dr. Ruwaidah is currently a faculty member in the Department of Physiology/Medical Physics, College of Medicine, Al-Nahrain University, Iraq.

Mayyadah Hasan Rhaif Al-Sahlanee, Ph.D.
E-mail: dr.mayyadahhasan@uomustansiriyah.edu.iq

Mayyadah Hasan Rhaif Al-Sahlanee had B.Sc. in Physics Science from College of Science, Mustansiriyah University, Iraq and M.Sc. in Medical Physics from Physiology Department, College of Medicine, Mustansiriyah University, Iraq, and Ph.D. in Medical Physics from Universiti Sains Malaysia, School of Physics, Malaysia. Dr. Mayyadah is currently a faculty member in the Department of Physiology/Medical Physics, College of Medicine, Mustansiriyah University.

Assist. Prof. Numan Salman Dawood, Ph.D.
E-mails: numan_salman2012@yahoo.com
numans@comed.uobaghdad.edu.iq

Assistant Professor Numan Salman Dawood had B.Sc. in Physics Science from College of Science, University of Baghdad, Iraq and M.Sc. in Medical Physics from College of Medicine, Physiology Department, Mustansiriyah University, Iraq, and Ph.D. in Medical Physics from Department of Physics and Astronomy, University of Leicester, England. Assist. Prof. Numan is currently a faculty member in the Department of Physiology/Medical Physics, College of Medicine, University of Baghdad.

Assist. Prof. David F. L. Jenkins, Ph.D.
E-mail: d.f.jenkins@plymouth.ac.uk

Assistant Professor David Jenkins had B.Sc. Degree in Physics Science, M.Sc. Degree in Laser Physics, and Ph.D. Degree in Applied Physics from School of Engineering, Computing and Mathematics, Plymouth University, UK. Assist. Prof. David is currently a faculty member in the School of Engineering, Computing and Mathematics (Faculty of Science and Engineering), Plymouth University, UK.

Prof. Awadhesh N. Jha, Ph.D.
E-mail: a.jha@plymouth.ac.uk

Professor Awadhesh Jha had B.Sc. and M.Sc. Degrees in Bioscience from Centre of Advanced Study in Zoology, Banaras Hindu University, India, and Ph.D. Degree from Banaras Hindu University, Varanasi, India. Prof. Awadhesh is currently a faculty member in the School of Biological and Marine Sciences (Faculty of Science and Engineering), Plymouth University, UK.