Global Scientific and Academic Research Journal of Multidisciplinary Studies
ISSN: 2583-4088 (Online)
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journals-gsarjms-home/

In Vitro Study of 532 nm Wavelength Laser Irradiation on Packed Erythrocytes Using Uv-Vis Spectroscopy

BY

Silvester Gemanam Jande[1], Ode Samuel Omenka[2]
[1,2]Department of Physics, Benue State University Makurdi

Article History

Received: 19/032024
Accepted: 26/03/2024
Published: 28/03/2024

Vol – 3 Issue –3

PP: - 39-45

Abstract

Low-level laser wavelengths were used in a variant of medical applications on account of blood rheology modulation ability and microcirculation improvement. Human blood response to low-level laser irradiation (LLLI) provides valuable information regarding reaction of laser light with tissues. The research was designed to elucidate whether in vitro irradiation changes packed red blood cells and whole blood. Blood samples were collected from Wellness Center, Universiti Sains Malaysia. Samples were each split into two equal aliquots recognized as control (non-irradiated) and irradiated groups. Irradiated sample was exposed to LLLI output powers of 60, 80, and 100 mW at wavelength 532 nm with various irradiation time ranged from 30, 60, 90, 120, and 150 s. The findings showed substantial difference in light absorption with different irradiation exposure duration. The absorption spectrum for packed red blood cells (RBCs) for the irradiated samples before mixed with plasma in return is lower than irradiated samples after mixed with plasma. When compared the absorption spectrum between RBCs is higher than whole blood. Blood samples smeared showed variations in the packed RBCs morphology between the control samples. Blood samples smeared showed no changes in the red blood cell morphology between the control and irradiated samples. The optimum laser dose obtained from the result for RBC packed cell is at 100 mW and 150 s while for whole blood is at 80 mW and 60s exposure time which suggested……..

Keywords: Low level laser; Blood; UV-vis spectroscopy, Irradiation

1.0.Introduction

Laser as an acronym is a powerful light beam that produced intense heat when focused at short distance [1, 2]. Lasers are instruments producing or amplifying coherent radiation at frequencies in the electromagnetic spectrum's infrared, visible, or ultraviolet regions [3, 4]. Many lasers are used in medicine for microchirurgy, cauterisation, medical uses, etc. For example, these lasers are used for microchirurgical cutting and debulking, soft tissues [5, 6]. LLLI's contributions to therapeutic measures of different pathological situations like healing of wounds and certain inflammations as well as pain treatment were initiated by Mester and colleagues' efforts, whom applied a low-energy (1 J/cm2) ruby laser in wound healing enhancement therapy [7, 8].

Several researchers have reported that laser biostimulation effects depends on several factors and certain laser irradiation parameters like wavelength, beam range, dosage, laser intensity, irradiance, specific absorption rate (SAR), polarization, and most importantly considering the irradiated cells [9, 10 & 11]. Researches indicated that lasers emissions within powers output of 1 – 500 mW and energy densities between 0.04 – 50 J/cm^2 at a monolayer of cells or target tissue are considered to be LLLI. This irradiation prevents apoptosis, stimulates cell proliferation improvement, control cell migration, and adhesion at this low levels of visible light exposure. It was also researched that laser irradiation causes conformation transformations of the red blood cells membranes and is due to changes in the structural states of both erythrocytes membrane proteins and lipid bi-layers, resulting in changes in the activity of membrane ion pumps [13]. LLLI is not thermal or an ablative mechanism although considered as photochemical effect similar to photosynthesis process in plants where the absorbed light causes a chemical change [12].

In the literature, experimental analysis of laser effects between visible and near-infrared wavelengths displayed significant variations with better results achieved with a visible wavelength [13]. Further investigation is required to elucidate the effects of low-level laser irradiation on human blood cells therefore the study is very pertinent in understanding the interaction mechanisms of laser irradiation of the biological

*Corresponding Author: **Ode Omenka Samuel**

tissues. In a more specific order, the research is meant to investigate in vitro effects of different low-level 532 nm wavelength laser doses on normal red blood cells (RBCs) in human blood. Light's biological effects differ in correspondence to wavelength, time of light exposure, light intensity (dose or fluence) recieved. For light-induced biological processes to occur, the photo acceptor molecules in the cells will absorb light. While high-output power lasers ablate tissues, low-power lasers are proposed to activate tissue and promote processing of cells. This low irradiation is incorporated with conventional medicine with ongoing work to determine whether a demonstrable impact occurs [15 and 16]. For wound healing, phototherapy from various light sources (i.e., illumination, ultra-violet irradiation, lasers, and light-emitting diodes) was used. Sunlight was used by Ancient Greeks to cure numerous skin disorders. Irradiation therapy with low-power output lasers or light emitting diodes within the red to near-infrared region (630-1000 nm) has been used in the soft tissue injury treatment over the past 40 years and shown to facilitate tissue rejuvenation in both in vivo and in vitro [17, 18, 19].

2.0. Materials and method

I. Blood sample collection and preparation

Blood samples from healthy individuals who have no previous antiquity of any major diseases or treatment were obtained. The research was carried out using 40 fresh human blood samples obtained from 17 males and 23 females within the age bracket of 21 to 60 years. These blood samples were provided by the Hematology Laboratory, Wellness Center, Universiti Sains Malaysia. From the 40 individuals involved, 5 mL blood samples each were collected into laboratory test tubes with EDTA (1.3 mg/mL blood anti-coagulation substance). The collected samples were immediately analyzed after collection by dividing each sample into two aliquots for used as control (non-radiated) and irradiated samples respectively.

II. Power and wavelength measurement setup

The 532 nm wavelength green laser is turned on for 10 minutes before irradiation to achieve stable beam output. The separation between the sample and laser is 6 cm. The sample is placed vertically under the laser with upright position. The plain tube is adjusted so that the laser beam is irradiated at the center of the tube. The power output was varied from 60, 80, and 100 mW with different exposure times of 30, 60, 90, 120, and 150 seconds.

III. Blood smear preparation

The morphology of blood components was examined using blood slides prepared before and after laser irradiation for light microscopy. A droplet of well-stirred blood was pipetted at the end of a microscopic slide at 1 cm from the edge. With the aid of a spreader slide carrying a chipped edged placed in front of the blood droplet inclined at an angle about 30^{o} - 45^{o} to the blood. It was moved backwards therefore spread the blood over the width of the slide forming a thin layer smear for microscopic examination. The smeared blood on slide was then left to air dry for about 3 min.

IV. UV-Vis spectrophotometer

Shimadzu UV-Vis spectrophotometer used was calibrated for each experiment carried to ensure accuracy and precision of the instrument. The spectral bandwidth of the instrument is 1 nm with the scanning range of 200 to 1000 nm. The instrument was used to determine the diluted blood light absorption capability for the control (non-irradiated) and irradiated samples (after laser irradiation). The reference cell used contained 4 mL normal saline solvent in polystyrene cuvette of volume 4.5 mL with 10 mm length. The 4 µL of RBCs and whole blood pipetted using micropipette into the same type of polystyrene cuvette then mixed thoroughly with 4mL of normal saline. Both samples are place together in the UV Spectrophotometer to get the absorbance reading of the diluted packed RBCs. The spectrum obtained is saved and analyzed using UVProbe version 2.3 software. A graph of wavelength versus absorbance is plotted in the Microsoft Excel and analyzed.

3.0. Result and discussion

I. Absorption spectrum of packed RBC irradiation

The research results in Table-1 shows the first peak of absorption spectrum of the packed RBC irradiated observed to occur at 340 nm wavelength at different time durations. There is a significant variation in level of absorbance which shows the irradiated samples higher comparable to the non-irradiated samples. The absorption spectrum values for irradiated samples after plasma mixture are also measured to be higher than irradiated samples before plasma (packed RBCs) mixture. For irradiated samples before mixed with plasma, the highest absorption is at 60s irradiation time and the lowest absorption is at 30s. While the irradiated samples after mixed with plasma has the highest value occurred within 150s irradiation time and the lowest absorption is at 90s irradiations. Table 2 shows the value of absorption at 419nm which represented the highest peak. As in Table 2 the absorption spectrum of irradiated samples is higher than the non-irradiated samples within the time of irradiation for the third peak as in Table 3. The fourth peak is at 583nm wavelength and the value of absorption spectrum shown in Table 4 according to irradiation time.

Table-1: The first peak of absorption spectrum at 340 nm wavelength within different time variations.

Time Irradiation (second)	Absorption spectrum		
	Non-irradiated	Irradiated before plasma	Irradiated after plasma
30s	1.156	1.337	1.708
60s	1.468	1.881	2.139
90s	1.212	1.419	1.684
120s	1.302	1.425	1.787

*Corresponding Author: **Ode Omenka Samuel**

150s	1.312	1.592	2.223

Table 2: The second peak of absorption spectrum at 419 nm wavelength within different time variations.

Time Irradiation (second)	Absorption spectrum		
	Non-irradiated	Irradiated before plasma	Irradiated after plasma
30s	1.666	1.902	2.18
60s	1.946	2.349	2.804
90s	1.677	1.866	2.185
120s	1.684	1.927	2.304
150s	1.786	2.029	2.589

Table-3: The third peak of absorption spectrum at 544 nm wavelength within different time variations.

Time Irradiation (second)	Absorption spectrum		
	Non-irradiated	Irradiated before plasma	Irradiated after plasma
30s	0.924	0.995	1.205
60s	1.155	1.361	1.425
90s	0.979	1.055	1.225
120s	1.014	1.13	1.264
150s	1.072	1.167	1.428

Table 4: The fourth peak of absorption spectrum at 583 nm wavelength within different time variations.

Time Irradiation (second)	Absorption spectrum		
	Non-irradiated	Irradiated before plasma	Irradiated after plasma
30s	0.9	0.965	1.152
60s	1.12	1.331	1.371
90s	0.942	1.021	1.172
120s	0.992	1.093	1.225
150s	1.041	1.127	1.371

The presented results in Figures1, 2, 3, 4, 5, 6, and 7 show difference between non-irradiated and irradiated samples before and after mixed plasma. The first value found is about 340 nm where the metabolism of the blood carbohydrate is considered to be at its highest peak due to structural variations in Nicotinamide Adenine Dinucsleotide (NAD) to NADH and Nicotinamide Adenine Dinucleotide Phosphate (NADP) to NADPH by reduction mechanism. This reduced coenzyme form results in an increase in high absorption. Further peaks were observed at 414, 542, and 576 nm where co-oxyhemoglobin d-f is represented [19]. The highest absorption at this 340 nm wavelength for irradiated samples before mixed plasma is at 60 s and after mixed with plasma again at 150 s irradiation time. When time is increase from 30 s to 60 s, the absorption light is increased. Then, as the time increased from 60s to 90s the absorption is decreasing. But when the time irradiation is increase to 120s and 150s the absorption light is decreasing. The light absorption fluctuations are recognized and known as biphasic responses.

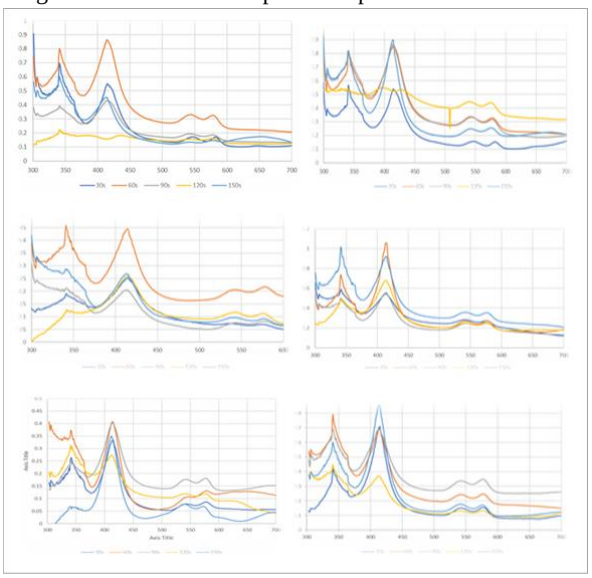

Figure 1. A. Difference of absorption spectrum before mixed with plasma at (100 mW), B. after mixed with plasma at (100 mW), C. before mixed with plasma at (80 mW), D. after mixed with plasma at (80 mW), E. before mixed with plasma at (60 mW) and F. after mixed with plasma at (60 mW).

II. Absorption spectrum for whole blood irradiation

Table 5 shows the first peak of absorption at 340 nm wavelength according to various times. The value absorption spectrums for irradiated samples are higher than non-irradiated samples. For irradiated samples, the highest value absorption spectrum is at 30s irradiation time and the lowest absorption is at 120s irradiations. Tables 6, 7, and 8 show the absorption spectrum at 419, 544 and 583 nm. As in tables 6, 7, and 8 the absorption spectrum of irradiated samples is higher than non-irradiated samples according to time irradiation. At 340 nm when time is increase from 30s to 60s and 90s the absorption light is decreasing. But when the irradiation time was increased from 120 s to 150 s, the absorption increases.

Table-5. Absorption spectrum for whole blood at 340 nm

Time irradiation (second)	Absorption spectrum	
	Non-irradiated	irradiated

*Corresponding Author: **Ode Omenka Samuel**

30s	0.889	1.196
60s	0.805	1.042
90s	0.805	0.981
120s	0.656	0.624
150s	0.491	0.951

Table-6. Absorption spectrum for whole blood at 419 nm

Time Irradiation (second)	Absorption spectrum	
	Non-irradiated	irradiated
30s	1.157	1.506
60s	1.069	1.442
90s	1.069	1.206
120s	0.851	0.904
150s	0.771	1.454

Table-7. Absorption spectrum for whole blood at 544 nm

Time Irradiation (second)	Absorption spectrum	
	Non-irradiated	irradiated
30s	0.709	0.975
60s	0.664	0.875
90s	0.664	0.811
120s	0.523	0.526
150s	0.415	0.751

Table-8. Absorption spectrum for whole blood at 583 nm

Time Irradiation (second)	Absorption spectrum	
	Non-irradiated	irradiated
30	0.703	0.957
60s	0.65	0.851
90s	0.651	0.796
120s	0.513	0.521
150s	0.408	0.743

A look at the plotted graphs shows, there are four observable peaks recorded. The absorption of spectrum from the UV-V in spectrophotometer is shown in form of plotted graphs. It can be observed that the first peak is around 340 nm, which represent the maximum carbohydrate metabolism of red blood cell (RBC). The second peak around 419 nm represents the transition of carbon monoxide oxyhaemoglobin. The transition of oxyhaemoglobin of alpha band particle represents at the third peak which is around 544nm. The fourth peak which is around 583 nm represents the transition of oxyhaemoglobin of beta band particle.

Tissues penetration by the ultra violet light (UV) and the absorbed wavelength by the photoacceptor are the essential factors in laser therapy. The absorption has to do with the biphasic responses of the laser therapy which show the two types of reactive oxygen species (ROS), that is the great ROS and bad ROS [21]. The purpose for the good ROS production is to be linked with the enhancement of the mitochondrial electrons transportation, as shown by the rise in the ATP production. The good ROS can activate beneficial cells signaling pathways that lead to the redox-sensitive transcription factors activation. Though, at increased exposure, the beneficial ROS production in mitochondria decreased due to reduced output of ATP. The one considered as bad ROS can harm the mitochondria that lead to apoptosis [20]. Figures 7, 8, and 9 compared the difference absorption between non-irradiated and irradiated samples for different irradiation time. The UV-light that hits the biological tissues is been absorbed. This phenomenon is the gateway for desired effect on the tissues.

Absorption of solute is linearly dependent on its concentration since absorption is ideal for quantitative. The Uv-light absorption parameters of molecules depend on the molecular environment and that of the chromophores mobility. Multiwavelength UV-visible spectroscopy is relatively simple technique that can provide considerable quantity of information. The UV-visible spectrum of blood contains information on the light absorption and scattering parameters of suspended particles in it [22]. The method of laser irradiation at cellular level has been affiliated with the uptake of monochromatic UV-visible and near-infrared radiation. Efficient tissues penetration is maximized in a limited optical range [23].

As the light hits the sample, energy light facilitates energy from bonding or non-bonding orbitals to one of the vacant anti-bonding orbitals. The electron excites from full orbital to zero anti-bonding orbitals. If the energy is the same as the

*Corresponding Author: **Ode Omenka Samuel**

wavelength energy it will absorb the wavelength energy. The high-energy transition absorbs high-wavelength light. In the UV-Visible spectrophotometer, at a wavelength of between 200 and 1000 nm, the energy transition would be between i) π bonding and π anti bonding ii) n-non-bonding to π anti bonding iii) n-non-bonding to antibonding. The molecules must then contain either π bond or anti-bonding orbital atoms [17]. Low-level laser radiation alters the ATBase activity of the membrane ion pumps in the dose and fluence rate-dependent manner. At the same time alters have been observed in integral parameters such as cell stability, membrane lipid peroxidation levels, intracellular reduced glutathione levels. The fractionation of the light dose significantly changed the membrane to laser radiation. Change in tryptophan fluorescent parameters of erythrocyte membrane proteins and the raise in lipid bilayer fluidity measured by pyrene monomer/excimer fluorescence proportion were observed [7].

Laser has an impact on biological tissues by bio-stimulation. Light absorption fluctuation shows the biphasic dosage response curve. When the blood sample is irradiated, the enzymatic vigor of the sodium membrane (Na+) and potassium (K+) ion pumps modify in dose and fluence-dependent way. As a consequence, the biological work of the cells is to stimulate and the absorption of light raises. But further increase in irradiation time inhibits enzymatic activity due to suppression of Na+ and K+. Because of the membrane cutout, the MCV decreases due to ion fluxes, motion of ions causes the cell to lose its shape and become peeler. Therefore, light absorption decreases [24].

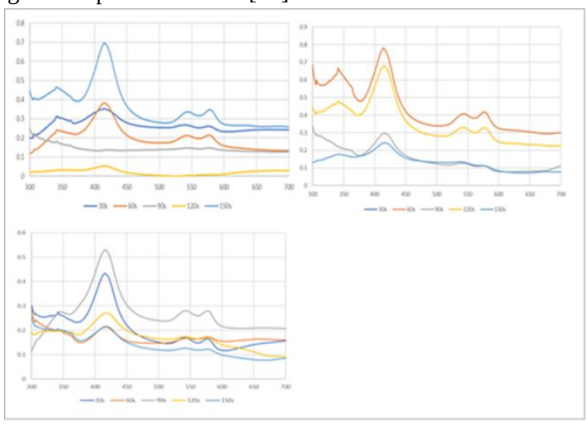

Figure-7: A. Difference between whole blood at 100 mW, B. difference between whole blood at 80 mW, C. difference between whole blood at 60 mW.

III. LLLI-induced changes in RBCs morphology

To observe the effects of laser irradiation on the RBC shape, 40 whole blood seamers were prepared after irradiation by green laser 532nm and different output powers. No hemolysis or morphological changes of the erythrocytes were observed. The RBCs were found to maintain their shape after irradiation with effective laser wavelength 532nm at powers 60, 80, and 100 mW, as shown in Figure 10 compared with non-irradiated RBCs. Based on the Figure, the morphology for all the irradiated blood does not get affected by all laser parameter.

Previous studies have concentrated on the effects of LLLI on blood behavior as a whole (cells, plasma proteins, microcirculation, and other rheological properties), whereas the current research concentrated for the most part on the effects of LLLI on certain blood parameters separately. This part of the present study attempted to evaluate the in vitro effects of LLLI on RBC. For this experiment, all the cells are normal compared to previous study that showed abnormal cells commonly known as echinocytes.

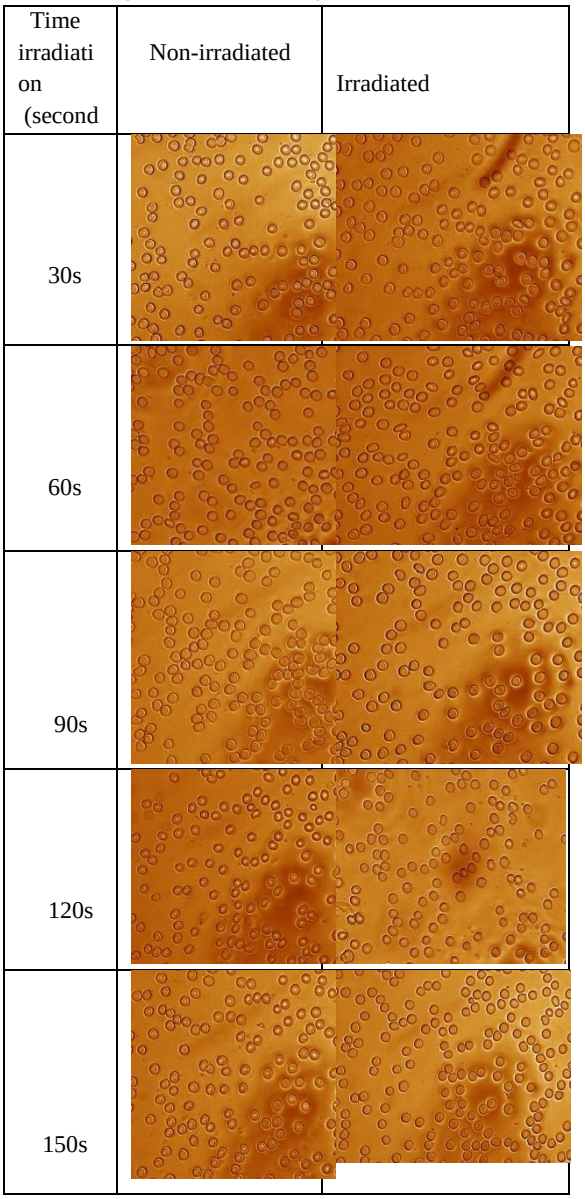

Figure10. Microscopic appearance of a blood smear at ×40 magnification before and after

LLLI of blood samples at 532nm wavelength.

The RBCs consists of proteins, therefore as the irradiation becomes excessive, then increases the local heating. The enormous local heat resulted in denaturation and precipitate stress to the membrane. This triggers the membrane to be under shear stress that changes cells morphology. Echinocytes produced from water and potassium loss as a result of a decline in the production of ATP. Echinocytes can become

*Corresponding Author: **Ode Omenka Samuel**

spherocytes as they lose the vesicle of the membrane. Further loss of surface area and volume leads to haemolysis [14]. However, from the observation for this study, laser parameters used are at low intensity and only caused photochemistry effect instead of photothermal.

4.0 Conclusion

The research studies of the effects of LLLI on blood are considered to be of great importance to elucidate the mechanisms of action of LLLI in tissues. Different methods of therapy by blood irradiation have been developed and used in clinical practice with beneficial effects. The fact that the response of pathological cells to LLLI differs from the response of healthy cells suggests that LLLI could also be diagnostic method for cellular membrane alterations. Low-level laser is proven to have effect on red blood cell. By using different irradiations time, the absorption light toward blood is different. This showed the statement is proven. Analysis of the spectrum from UV-Vis spectrophotometer showed different absorption level when irradiated at different exposure time. The highest absorption time is 150 second, but the maximum absorption for irradiated samples before mixed plasma is at 60 second of irradiation to laser and less absorption is at 30 second irradiation time. For irradiated samples after mixed plasma again, the maximum absorption at 150 second irradiation time and less absorption is at 90 second irradiation time. For the whole blood the maximum absorption is at 30 second and less absorption is at 120 second irradiation time. From the experiment, it is established that LLLI at green laser 532 nm wavelength at different laser parameter does not adversely affect human blood cells. Considering the experimental conditions, the highest absorption peak obtained without affecting the blood for RBC packed cell is at 100 mW with 150 s exposure times and 80 mW at 60 s exposure time for whole blood irradiation is therefore considered that this dose value is the optimum for beneficial effects. The conclusion is made as the radiation maintains the shape of cells without leading to negative phenomena such as spherocytosis and hemolysis that are normal in non-irradiated blood.

Reference

1. Maiman, T.H. (1960). Nature, 187, 493-494.
2. Jensen, D.M. and Brunetaud J. (1990). Medical Laser Endoscopy, by Kluwer Academic Publishers, Netherlands.
3. Narducci, L.M. and Abraham N.B. (1988). Laser Physics and Laser Instabilities, World Scientific, 1988.
4. Meschede, D. (2007). Optics, light, and lasers: The Practical Approach to Modern Aspects of Photonics and Laser Physics, Physics Textbook, 2nd revised and enlarged edition. Weinheim: Wiley-VCH.
5. Kallard, T. (1977). Exploring Laser Light, Optosonic Press, New York, USA.
6. Haken, H. (1984). Laser Theory, Springer, New York.
7. Mester, E., Spiry, T., Szende, B., & Tota, J. G. (1971). Effect of laser rays on wound healing. The American Journal of Surgery, 122(4), 523-535.
8. Mester, E., Mester, A. F., & Mester, A. (1985). The biomedical effects of laser application. Lasers in Surgery and Medicine, 5(1), 31-39.
9. Almeida- lopes, L., Rigau, J., Amaro Zangaro, R., Guidugi-Note, J., & Marques Jaeger, M. M. (2001). Comparison of the low-level laser therapy effects on cultured human gingival fibroblast proliferation using different irradiance and same fluence. Lasers in Surgery and Medicine, 29(2), 179-184.
10. Moore, P., Ridgway, T. D., Higbee, R. G., Howard, E. W., & Lucroy, M. D. (2005). Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. Lasers in Surgery and medicine, 36(1), 8-12
11. Gemanam, S. J., Suardi, N., Mokmeli, S., & Mustafa, I. S. (2020). Evaluation of the proper level of specific absorption rate of human blood for 532 nm laser in blood low-level laser therapy. *Laser Physics*, *30*(3), 035601.
12. Huang, Y. Y., Chen, A. C. H., Carroll, J. D., & Hamblin, M. R. (2009). Biphasic dose response in low-level light therapy. Dose-Response, 7(4), 358-383.
13. Loevschall H and Arenholt-Bindslev D. 1994. Effect of low-level diode laser irradiation of human oral mucosa fibroblasts in vitro. Lasers Surg Med 14:347-54.
14. Kujawa, Jolanta et al. 2004. “Effect of low-intensity (3.75-25 J/Cm2) near-infrared (810Nm) Laser Radiation on Red Blood Cell ATPase Activities and membrane Structure” Journal of clinical laser medicine & surgery 22(2): 111-17.
15. Karu, T., I. (2003). Low-power laser therapy. Biomedical photonics Handbook ed T Vo-Dinh (Boca Raton, FL: CRC Press).
16. Schindl, A., Schindl, N., & Schindl, L. (1997). Successful treatment of a persistent radiation ulcer by low-power laser therapy. Journal of the American Academy of Dermatology, 37(4), 646-648.
17. Anders, J. J., Geuna, S., & Rochkind, S. (2004). Phototherapy promotes regeneration and functional recovery of injured peripheral nerve. Neurological Research, 26(2), 233-9.
18. Eells, J. T., wong-Riley, M. T., VerHoeve, J., Henary, M., Buchman, E. V., Kane, M. P., & Margolis, D. (2004). Mitochondrial single transduction in accelerated wound and retinal healing by near-infrared light therapy. Mitochondrion, 4(5), 559-567
19. Wong-Riley, M. T. T., Liang, H. L., Eells, J. T., Chance, B., Henry, M. M., Buchmann, E., & Whelan, H. T. (2005). Photobiomodulation directly benefits primary neurons functionally inactivated by

*Corresponding Author: **Ode Omenka Samuel**

toxins: Role of cytochrome c oxidase. Journal of Biological Chemistry, 280(6), 4761-4771.8 40.

20. Gunasekaran, S., R. K. Natarajan, and V. Renganayaki. 2008. "UV Visible Spectrophotometric Approach and Absorption Model for the Discrimination of Diseased blood." Asian Journal of Chemistry 20(1): 48-54.
21. Elblbesy, M. a. (2014). Plasma viscosity and whole blood viscosity as diagnostic tools of blood abnormalities by using simple syringe method. Medical Instrumentation, 2(1), 5. http://doi.org/10.7243/2052-6962-2-5
22. Huang, Y. Y., Sharma, S. K., Carroll, J., & Hamblin, M. R. (2011). Biphasic dose response in low-level light therapy-an update. Dose-Response, 9(4), 602-618. http://dio.org/10.2203/dose-response.11-009.Hamblin
23. Gunasekaran, S., R. K. Natarajan, and V. Renganayaki. 2008. "UV Visible Spectrophotometric Approach and Absorption Model for the Discrimination of Diseased Blood." Asian Journal of Chemistry 20(1): 48-54.
24. Robert Splinter, B. A. H. (2006). An Introduction to Biomedical Optics. Taylor & Francis.
25. Sakinah. 2018. "In Vitro UV-Visible Spectroscopy Study of Yellow Laser Irradiation on Human Blood In Vitro UV-Visible Spectroscopy Study of Yellow Laser Irradiation on Human Blood."

*Corresponding Author: **Ode Omenka Samuel**