

In vitro comparison of low-power diode pumping solid-state laser radiation at 589 and 650 nm on red blood cells suspension viscosity, stability, and deformability

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Abstract

Objective: To investigate the effect of diode-pumped solid-state laser irradiation with yellow 589nm and red 650nm wavelengths on rheological parameters in vitro.

Method: The comparative study was conducted between November 2021 and April 2022 at the at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy adult volunteers. The samples were divided into 4 aliquots. The first was preserved as a control sample, while the other 3 were exposed to a diode-pumped solid-state laser, delivering the beam for 589nm and 650nm wavelengths at radiation dose 15, 22.5, 33.5J/cm² for 20, 30 and 45 minutes, and with each laser having a 50mW output power and a 4 mm² spot diameter. Data was analysed using SPSS (version 24)

Results: There were 6 subjects aged 18-60 years. There was no significant difference in the viscosity values between laser wavelengths ($p>0.05$). Compared to the laser wavelength of 650nm, irradiation with a 589nm wavelength at the same dose resulted in a significant reduction ($p<0.009$) in deformability. The rigidity index of red blood cell suspension was reduced the most at a radiation dose of 22.5J/cm² for both lasers compared to the control samples. No significant variations were found in suspended erythrocyte viscosity ($p>0.05$).

Conclusion: The wavelength with 589nm lasers showed more effective influence than its 650nm counterpart in improving the rheological parameters of blood viscosity and erythrocytes deformability.

Key Word: Lasers, Viscosity, Erythrocytes, Radiation,

(JPMA 74: S322 (Supple-8); 2024) DOI: <https://doi.org/10.47391/JPMA-BAGH-16-74>

Introduction

Recent years have witnessed rapid development in the use of low-level lasers (LLS) to treat various diseases¹. However, more studies are needed to explore the dynamics of LLL radiation and the effect on whole blood, in general, and the rheological parameters, in particular². Several studies have found LLL therapy (LLLT) to have a beneficial impact in terms of improving microcirculation by enhancing the rheological factors of the blood^{3,4}, leading to positive results in the recovery of patients with microangiopathy and the treatment of acute cerebral infarction after exposure to intravenous (IV) irradiation clinically⁵. Because blood is composed of biological tissues, studies tend to choose it to see what effect laser irradiation has on rheological properties to understand its behaviour^{6,7}. Knowing the properties of red blood cells (RBCs) is essential, as they are the major blood components⁸. One of the most important characteristics of RBCs is the viscosity of blood⁹. To determine the viscosity, it is necessary to know the deformability of

RBCs, as they are important determinants of blood viscosity¹⁰.

The current study was planned to investigate the effect of diode-pumped solid-state laser (DPSS) irradiation with yellow 589nm and red 650nm wavelengths on rheological parameters in vitro.

Materials and Methods

The comparative study was conducted between November 2021 and April 2022 at the at the Postgraduate Medical Physics Laboratory, College of Medicine, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy adult volunteers who furnished informed consent. After approval from the institutional ethics review committee, 10ml blood was drawn from each subject through venipuncture in tubes containing ethylenediaminetetraacetic acid (EDTA) 1.3mg per ml of blood. The processing started immediately by expelling the anticoagulant from the blood samples at 1,200xg for 10 minutes. Packed RBC was washed centrifugally twice with phosphate buffer saline (PBS). The suspension of RBC was then started with 20% nominal haematocrit (Hct) in a PBS solution, then 20% Hct of the value of the suspended RBC. The sample was

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divided into 4 aliquots. The first was left as a control sample, while the remaining 3 were exposed to LLL irradiation using DPSS at wavelengths 589nm and 650nm (Models F series and HLPS-831, Changchun Dragon Lasers Co., China) as the source of irradiation in the continuous wave mode with an output power of 50mW and doses 15J/cm², 22.5J/cm² and 33.5J/cm² for 20, 30 and 45 minutes. The diameter of the beam was 7mm and the area of the beam was about 4mm². The laser was 5cm away from the blood sample vertically. An optical power meter was used to measure the resulting laser energy (type Gentec-E, Maestro, Canada). The procedure was carried out at room temperature of 25±2°C.

For blood viscosity measurement, erythrocyte suspension was measured by a 325mm graduated capillary tube with a diameter of 0.3µm. The capillary tube was connected to the tank tube vertically to fill until it reached the mark at the top so that the RBC suspension could flow through the graduated capillary tube to the bottom mark. The result of 1.34mL RBC suspension was recorded by a video camera from the start of its descent from the top mark to the bottom mark. The viscosity of the RBC suspension was calculated using the camera (Model MPC-HC version 1.7.9, China) of the multimedia player over which time is required to flow 1.34mL of PBS solution whose viscosity was 0.089CP (Centipoise) at temperature 25 °C.

The capillary tube used to measure viscosity was replaced by a capillary tube with an attached filter. Further, 1.5mL of 10% Hct RBC solution was filtered using a polycarbonate filter with a 5-pore diameter. The time taken for 1.5mL of RBC suspension to flow through the capillary was noted. The RBC suspension flow time of 1.5 mL was divided by the PBS solution flow time of the same filter, accounting for the elimination of air bubbles that occurred in the filter holder.

In the next step, 8ml of 25% Hct of washed RBC suspension was prepared and divided into 4 equal aliquots. The first aliquot was left without irradiation which served as a control sample. The other 3 aliquots were exposed to a radiation dose of 15J/cm², 22.5J/cm² and 33.5J/cm², respectively, with a wavelength of 589nm or 650nm for which a low-power DPSS laser was used in continuous wave. At the end of exposure time to achieve the target radiation dose, 0.5ml of RBC suspension from the control sample was taken and added to 9.5ml of distilled water to achieve 100% haemolysis. Another 0.5ml of RBC suspension from the control sample was taken and added to 9.5ml of PBS (final Hct of 1.25%), which served as

a control sample for stability test of the non-irradiated sample. From each of the 3 irradiated samples, 0.5ml of RBC suspension was taken and added to 10ml PBS (final Hct of 1.25%). All the 5 aliquots were kept in a refrigerator at 4°C for 24 hours. After the end of this time, all the 5 samples were taken out of the refrigerator and centrifuged at 1200xg for 10 minutes. The supernatants of all the 5 samples were collected and the absorbance (optical density) was measured using a spectrophotometer (PD-303, Aple Co. Ltd., Japan) by reading the optical density of the supernatant at a wavelength of 540nm. The percent haemolysis of each sample was calculated by comparing the optical density of the sample with the optical density of RBCs haemolysed with distilled water.

Data was analysed using SPSS (Version 24).

Results

There were 6 subjects aged 18-60 years. There was a decrease in the viscosity of RBC suspensions after exposure to LLL radiation compared to the control samples (Figure 1). The radiation dose of 22.5J/cm² was the most effective for both wavelengths, where the decrease was significant by 3% for the yellow wavelength and 2% for the red wavelength. Exposure to residual radiation doses of 15J/cm² and 33.5J/cm² for the 2 wavelengths led decrease in viscosity by 1% and 2% for both yellow and red wavelengths. There was no statistically significant variation in the viscosity of suspended erythrocyte (p>0.05).

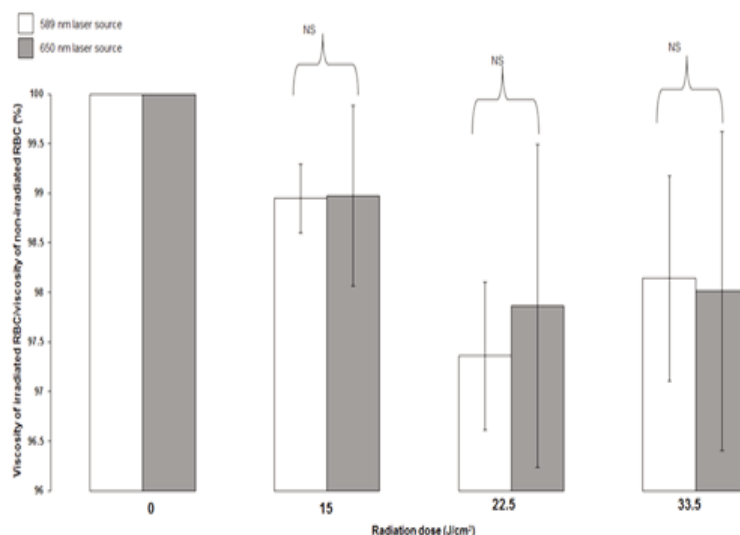


Figure-1: Comparison of 20% haematocrit (Hct) red blood cell (RBC) suspension viscosities.

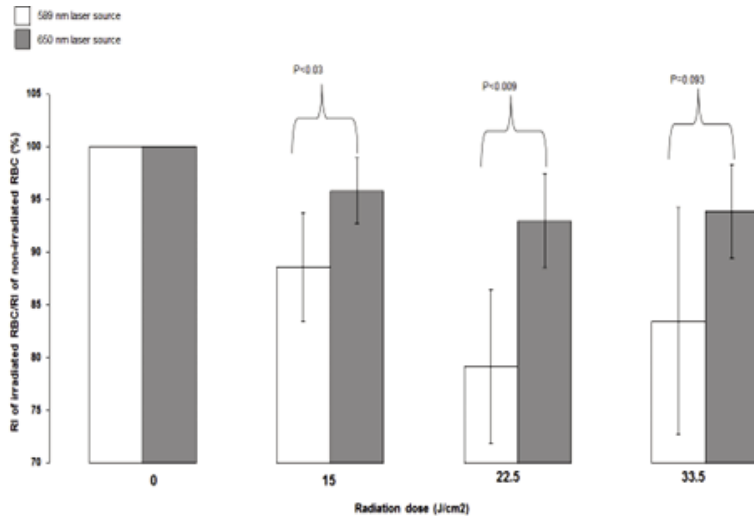


Figure-2: Comparison of 10% haematocrit (Hct) red blood cell (RBC) suspension rigidity index (RI).

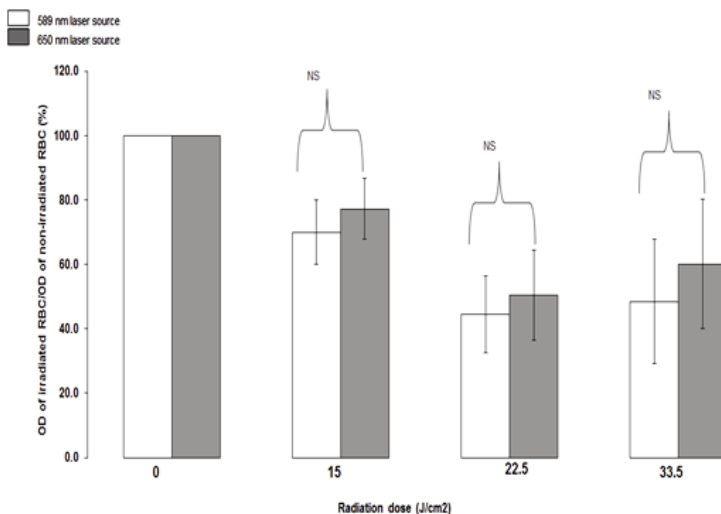


Figure-3: Comparison of haemolysis of red blood cell (RBC) suspension after storage for 24 hours at 4°C with nominal haematocrit (Hct) of 1.25%.

LLL irradiation lowered blood viscosity and it increased erythrocyte deformability (Figure 2). The effect of LLL irradiation at wavelengths of 589nm and 650nm on RBC deformability was confirmed by a reduction in rigidity index (RI) compared to the control samples. Both lasers improve RBC suspension denaturation. The 589nm laser performed better than the 650nm laser, and the relative difference was 12%, 22% and 18%, respectively, for the yellow laser, while for the red laser the values were 4%, 7% and 6%, respectively.

On the other hand, both wavelengths of the laser light had a comparable effect on RI, and there were significant differences ($p < 0.009$) in RI observed between them across all the laser doses. At radiation dose of 22.5J/cm²,

maximum reduction in RI of RBC was obtained in both wavelengths.

Irradiation resulted in a lower percentage of suspended haemolysis of erythrocytes compared to the non-irradiated control sample (Figure 3). Despite the positive effect of both laser wavelengths, there was no significant change ($p > 0.05$). The laser effect with 589nm was 25%, 55% and 38%, while the effect of 650nm was 26%, 50% and 42%, respectively.

Discussion

The current findings indicated a decrease in the RBC suspensions and RI, and the dose of 22.5 J/cm² was found to be the most effective for both wavelengths. The results are consistent with previous studies¹¹, and clearly compatible with the biphasic dose-response concept. The importance of the biphasic curve in distinguishing the dose of the threshold is to obtain the appropriate amount of energy for appropriate bio-stimulation. If the dose level is higher than the threshold, bio-stimulation is replaced by bio-inhibition^{12,13}.

The relationship between blood viscosity and wavelength indicated a significant decrease in viscosity¹¹. The current results proved that the 589nm wavelength was more effective than its 650nm counterpart. The reason is that haemoglobin (Hb) molecules absorb more photons falling in the yellow visible region than the red region, or, in other words, the shorter is the wavelength, the greater is the energy of the absorbed photons,

leading to better response¹⁴. This may suggest that higher Hb in RBCs lead to greater viscosity and less deformability¹⁵ because deformability susceptibility is a strong contributor of blood viscosity^{16,17}. When LLL radiation photons were absorbed by Hb which is the primary target in RBCs¹⁸. Haemoglobin (Hb) attached membrane. The adherence of Hb to the cell membrane determines blood viscosity and RBC deformability, therefore the stronger this link is, the higher will be the RI¹⁹. Laser irradiation regulates the rheological properties and thus enhances microcirculation^{20,21}. The current results are consistent with previous studies²² which indicated a significant decrease in erythrocyte

sedimentation rate (ESR) for 3 wavelengths, especially 405nm. ESR changes according to the viscosity of the blood, and a decrease in ESR post-exposure has a direct effect on decreasing blood viscosity, thus improving erythrocyte deformability.

Conclusion

The LLL beam had a clear positive effect on the blood rheological parameters (viscosity and deformability) of suspended erythrocytes. The 589nm and 650nm laser wavelengths enhanced the rheological characteristics of erythrocytes and reduced the percentage of haemolysis of RBC suspension profoundly.

Acknowledgments: We are grateful to the participants from the Medical Physics Department at the College of Medicine, Mustansiriyah University, Baghdad, Iraq.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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