RESEARCH ARTICLE

A comparison study of 589 and 650 nm on improving stored whole blood stability by irradiation with a low-power diode pumping solid-state laser

Safa Hassan Mohammed, Mustafa Salih Al Musawi, Bassam Talib Al-Gailani

Abstract

Objective: To assess the effects of diode-pumped solid-state laser irradiation with 589nm and 650nm wavelengths on the stability of stored red blood cells in vitro.

Method: This is an intervention study that was conducted from April to July 2021 at the Physiology and Medical Physics Laboratory, College of Medicine, Mustansiriyah University, Baghdad, Iraq, and comprised samples of healthy, adult human blood that were put in tubes with citrate-phosphate dextrose-adenine as an anticoagulant. The blood sample was divided into eight equal aliquots and stored for 21 days at 4ºC. The stability test was done on days 0, 7, 14 and 21 of storage time for non-irradiated and radiated aliquots. For 15 minutes, the irradiated aliquots were subjected to a diode-pumped solid-state laser with a wavelength of 589nm or a laser with a wavelength of 650nm at frequencies of 30, 50 and 70 J/cm^2. Data was analysed using SPSS 24.

Results: Exposure of the whole blood to 589nm and a radiation dose of 70J/cm^2, 50J/cm^2 and 30J/cm^2 was associated with a significant reduction in the percentage of haemolysis that ranged 23-42% throughout the whole storage time. Exposure of the whole blood to 650nm wavelength low-level laser therapy and a radiation dose of 70J/cm^2, 50J/cm^2 and 30J/cm^2 was associated with much less reduction in the percentage of haemolysis that ranged 5-9% throughout the whole storage time.

Conclusions: Both 589nm and to some extent 650nm low-level laser irradiation generally reduced haemolysis. The wavelength of 589nm lasers had a more effective influence than the 650nm counterpart in improving the stored red blood cells.

Key Words: Lasers, Solid-State, Hemolysis, Light Therapy, Erythrocytes, Anticoagulants, Radiation, Phosphates, Citrates, Glucose, Adenine

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Introduction

Laser therapy is now widely employed in a variety of medical fields, including cosmetics, diagnosis, surgery and other medical procedures[1,2]. Low-level laser therapy (LLLT) is a term used to describe a therapeutic approach. In LLLT, low-power beams are used for extended periods of time. The healing mechanism has been reported to be linked to photochemical interactions[3,4]. LLLT has been used in blood therapy for a range of therapeutic purposes due to its potential to improve microcirculation and modify blood rheology[5,6]. It is necessary to investigate the effects of LLLT on human blood in order to have a better understanding of the factors that influence how laser radiation interacts with biological tissues[7]. The living cells are not damaged by LLLT because this therapy does not have thermal effects[8]. Reaction characteristics, like the reactivity of red blood cells (RBCs) to LLL radiation, are still mostly unknown[9]. When RBCs absorb more photons, they become more receptive, hence, haemoglobin (Hb) may be the focal point of laser irradiation[10,11]. Recently, LLLT exhibited high efficacy in the field of blood irradiation as well as its direct effect on parameters of all blood cells, such as viscosity, stability and deformability[12]. Defining a membrane's stability is based on the maximum amount of deformation a membrane can tolerate before failing to return to its original shape, whcih means that under typical circulation conditions, erythrocytes can circulate without fragmentation as long as their membranes are stable. As a result, it is a term used to describe the low fragility of RBCs, or the tendency of RBCs to rupture when exposed to particular stresses, like osmotic and mechanical stress[13]. In haematology, the possible use of laser irradiation to rejuvenate the preserved blood has been proposed because laser blood irradiation is viable, efficient, inexpensive and non-invasive, ensuring that no blood is contaminated[14].

More research is required on the impact of LLL irradiation on these parameters. To reduce the risk of blood transfusion, scientists are always working to improve the transfusion procedure and blood storage conditions. During the storage period, well-defined alterations occur that are commonly referred to as storage lesions which would affect the quality of the blood[15].

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The current study was planned to assess the effects of diode-pumped solid-state (DPSS) laser irradiation with 589nm and 650nm wavelengths on the stability of stored RBCs in vitro.

Materials and Methods

This is an intervention study, conducted from April to July 2021 at the Physiology and Medical Physics Laboratory, College of Medicine, Mustansiriyah University, Baghdad, Iraq. After approval from the institutional ethics review committee, and informed consent from the particpants, blood samples were drawn from healthy adults with no history of past illnesses or medication for a chronic disease. A venipuncture was used to draw thet samples that were then placed in tubes containing anticoagulant citrate-phosphate dextrose-adenine (CPDA-1) (MacoPharma, USA), and processing began as soon as the samples were collected.

The freshly drawn whole blood (10mL) was mixed with CPDA-1 (1.4ml). The blood sample was stored for 21 days at 4°C. Each sample was divided into 8 equal aliquots, and divided into non-irradiated control samples, and intervention samples that were irradiated by yellow or red LLL light at days 0, 7, 14 and 21 of the storage time.

A low-power DPSS laser light was used with a continuous wave wavelength of 589nm and 650nm (Model F series, Changchun Dragon Lasers Co., China).The irradiation sources had an output power of 50mW and doses of 30, 50 and 70J/cm^2 for laser radiation for 15 minutes. The Fluence equation18 was calculated thus:

Dose (J/ cm^2) = power (w) × Time (Sec)/ Area of spot (cm^2).

The laser beam was directed typically to the centre of the test tube containing the blood sample. A power meter was used to measure the output power of a laser (type Gentec -E, Maestro, Canada). The irradiation was conducted at mean room temperature 23±2°C.

The stability was measured indirectly by the Hb released after 24 hours of storage of blood suspension at 4°C with or without irradiation.

At day 0, 50ul of blood from the control samples was taken and added to 5ml of distilled water to achieve 100% haemolysis. Another 50ul of blood from the control samples was taken and added to 5ml of normal saline. Both blood suspensions were kept at 4°C for 24hr for the stability test. Both blood suspensions were then centrifuged at 3000g for 10 minutes using bench centrifuge. The supernatants optical density (O.D) were measured by a spectrophotometer (Optima SP-300 Optima, Japan) at a wavelength of 540nm. The percent haemolysis in the sample was calculated by comparing the optical density of the blood suspension in normal saline to the optical density of the blood suspension in distilled water (100% haemolysis). This process was repeated for the irradiated sample. All the O.D measurements were done in duplicate and the average was taken for comparison. The whole procedure was repeated for all the blood samples stored on subsequent days. For each test day, the same-day sample and all the rest of the blood samples that were designated to be as irradiated samples were exposed to the specified dose and wavelength of the laser light and then placed bacl to complete their storage time.

Data was analysed using SPSS version 24. Data was presented frequencies and percentages and as means with standard deviations, as appropriate. To compare variables, student paired t-test was used. $P<0.05$ was considered significant.

Results

Of the 8 aliquots, 4(50%) each were control and interventions samples. Exposure of the whole blood to 589nm wavelength LLL and a radiation dose of 70J/ cm^2 was associated with significant reduction in the percentage of haemolysis by 31%, 33%, 23%, and 28% relative to their none-irradiated counterparts at 0, 7, 14 and 21 days of storage time, respectively (Figure 1A). This type of behavior was also observed with the use of LLL radiation doses of 50 J/cm^2 with significant reduction in the percentage of hemolysis by 40%, 20%, and 42% relative to none-irradiated counterparts at 0, 7, 14 and 21 days of storage time, respectively (Figure 1B). The same findings were recorded when the whole blood was exposed to LLL radiation doses of 30J/cm^2 in which there were significant reductions in the percentage of haemolysis by 33%, 29%, 31%, and 34% relative to non-irradiated samples at 0, 7, 14 and 21 days of storage time, respectively (Figure 1C).

In addition, exposure the whole blood to 650nm wavelength LLL and a radiation dose of 70J/cm^2 was associated with a significant reduction in the percentage of haemolysis by 8%, 9%, 9%, and 12% relative to non-irradiated samples at 0, 7, 14 and 21 days of storage time, respectively (Figure 2A). The same was observed when the whole blood was exposed to LLL radiation doses of 50J/cm^2 in which there were significant reductions in the percentage of haemolysis by 6%, 5%, 7% and 6% relative to the non-irradiated samples at 0, 7, 14 and 21 days of storage time, respectively (Figure 2B). In contrast, when the whole blood was exposed to LLL radiation doses of 30J/cm^2 there were no significant reduction in the

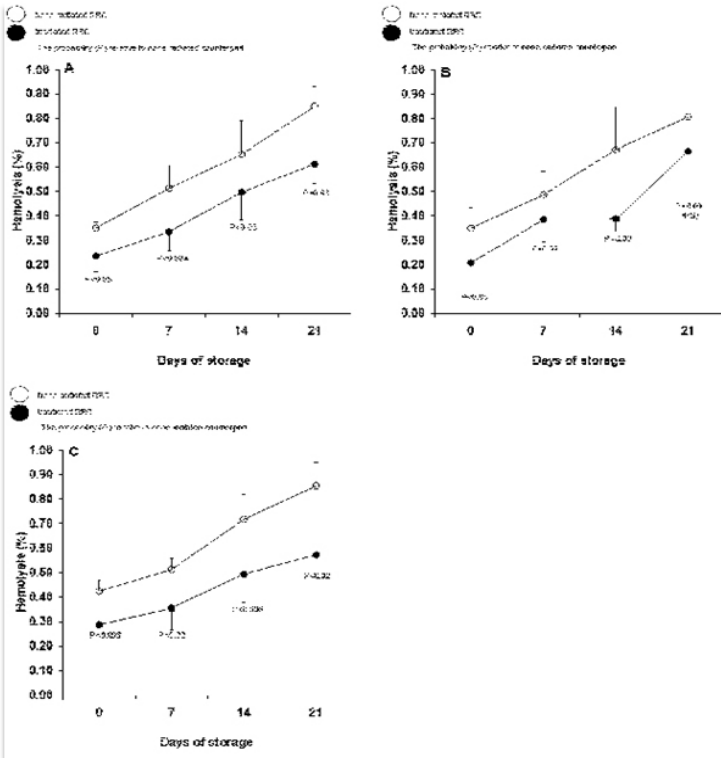

Figure-1: The haemolysis of stored whole blood with and without irradiation by 589nm wavelength laser light at a dose of 70J/cm^2 [A], 50J/cm^2 [B], and 30J/cm^2 [C].

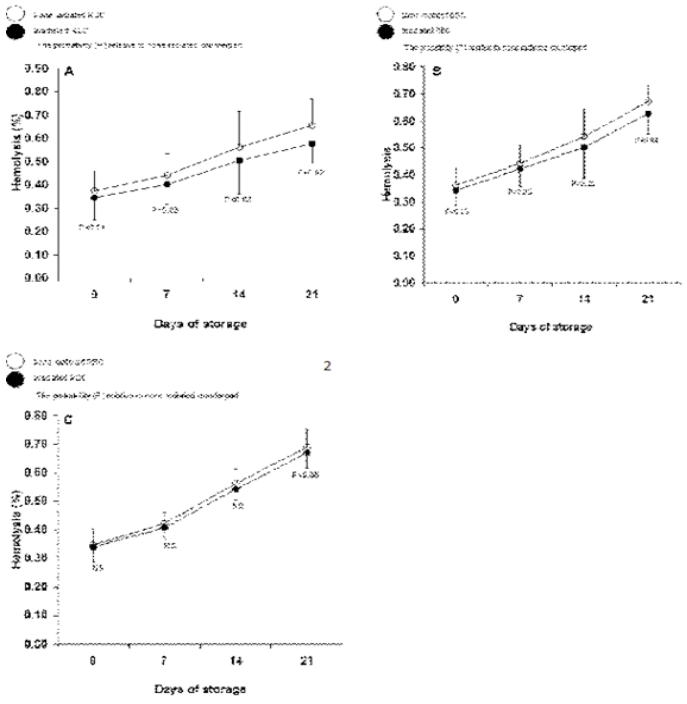

Figure-2: The haemolysis of stored whole blood with and without irradiation by 650nm wavelength laser light at a dose of 70J/cm^2 [A], 50J/cm^2 [B] and 30J/cm^2 [C].

percentage of haemolysis relative to the non-irradiated samples across the storage time (Figure 2C). The wavelength of the 589nm lasers showed more effective influence ($p<0.05$) than its 650nm counterpart in improving the stored blood's stability.

Discussion

The current study showed that both 589nm and 650nm wavelengths had sufficient laser fluency necessary for the induction of human blood stability, as reported earlier[16,17]. The maximum decrease in the percentage of haemolysis was observed with a dose of 50J/cm^2 with 589nm, and 70J/cm^2 with 650nm. The results observed with 589nm were consistent with the biphasic dose-response concept. The biphasic curve is useful for determining the threshold dose, or the amount of energy needed to achieve optimum bio-stimulation When the dose level is much higher than the threshold dose, bio-stimulation is replaced by bio-inhibition[18,19]. The relationship between blood stability and wavelength was demonstrated in the current study. A significant decrease in haemolysis with different wavelengths has been reported[20]. The current results showed that the 589nm wavelength was more effective than 650nm. This is due to the fact that Hb molecules absorb more photons in the yellow visible region than in the red visible region. In other words, the shorter the wavelength, the higher the energy of the absorbed photons, and the stronger the response[21].

Hb molecules control the RBCs and are thus responsible for photon absorption when low-power laser photons are absorbed by Hb, which is the major target in RBCs. As a result, there is a larger response when more photons are absorbed[8]. Because of the significant absorption, laser action has a strong influence on RBCs, but without damaging the RBCs. The membranes of RBCs are protected by LLL irradiation, reducing hypotonic haemolysis and stabilising cell membranes[22,23]. Several studies have shown that irradiating RBCs with LLL light improves blood rheological properties and improve microcirculation[24]. Several studies have revealed that LLL treatment can change the rheological properties of blood based on low-energy radiation parameters[8]. Although the use of LLL is still unclear, it has become more common in therapeutic applications in recent years[25].

Conclusion

Exposing blood samples to LLL in vitro for 15 minutes had a positive effect on blood stability. Wavelength 589nm was found to be more effective than 650nm.

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Conflict of Interest: None.

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