RESEARCH ARTICLE

The effect of a low-level yellow laser on improving the stability of stored whole blood

Safa Hassan Mohamed, Mustafa Salih. Al Musawi, Bassam Al-Gailani

Abstract

Objective: To explore if low-level laser 589nm may stabilise stored whole blood and red blood cell suspension in vitro.

Method: The interventional study was conducted from November 2021 to April 2022 at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from healthy adults. The samples were obtained put in tubes containing anticoagulant citrate-phosphate dextrose-adenine. Each sample was divided into eight equal aliquots and stored for 21 days at 4ºC. Of the 8 aliquots, 4 were takebn as controls, while the other 4 were irradiated by 589nm laser beam with a radiation dose of 50J/cm² for 15min. Stability test measured by the percentage of haemolysis of an overnight stored red blood cell in saline solution was done on days 0, 7, 14 and 21 for non-irradiated and radiated aliquots. Data was analysed using SPSS 24.

Results: Eight subjects (4 males and 4 females) were included, with mean age 21±3 years (range: 19-24 years). Whole blood samples showed a significant reduction in haemolysis by 40%, 20% and 42% compared to the control samples at days 0, 7 and 14, respectively ($p<0.05$). Red blood cell suspension showed a significant reduction in haemolysis by 9% compared to the control samples only at day 21 ($p<0.05$).

Conclusion: Low-level laser 589nm beam at 50J/cm² radiation dose was more effective on the stability of stored whole blood than the red blood cell suspension.

Key Words: Saline, Hemolysis, Erythrocytes, Adenine, Anticoagulants, Citrates, Glucose, Phosphates, Radiation, Lasers.

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Introduction

Low-level laser irradiation (LLLI) or low-level laser therapy (LLLT) is a type of laser therapy in which a low-powered laser is used to achieve therapeutic results[1]. The therapy does not damage hydrogen bonds in tissues, and does not cause any changes other than photochemistry effects by increase in cell metabolism[2]. The ability of LLL intensity to enhance microcirculation and blood rheology has been used with different powers, wavelengths and exposure times[3-4]. Several studies have been conducted on the effects of LLLs on RBCs[5]. LLLT has no effect on living cells since it has no thermal effect[6]. To detect LLLI impact on living biological systems, photons should be absorbed by electrical absorption bands belonging to a molecular chromophore or a photoacceptor[7]. The interaction of LLL radiation with blood remains unknown[8]. More research is needed to better understand how LLLI affects human blood cells, because examining the effects of LLLI on blood is crucial to determining how lasers interact with biological tissues[9]. Regardless of the fact that human blood's response to LLL radiation provides essential information about the cell's interaction mechanism, not much research has been devoted to such investigations in living tissues even though there is a lack of understanding on the response parameters, such as the response of red blood cells (RBCs) to LLL radiation[10]. When RBCs absorb more photons, they become more receptive, and, hence, haemoglobin (Hb) may be the focal point of laser irradiation[11,12]. In the field of haematology, the use of laser irradiation to rejuvenate preserved blood has been proposed because laser blood irradiation is practical, accurate, cost-effective and non-invasive, with no blood contamination[13]. During storage, the storage lesion is a collection of well-defined alterations that may influence blood quality[14]. As a result of storage conditions, such as low adenosine triphosphate (ATP) content, RBC membranes change visually and physiologically. The osmotic fragility of preserved RBCs is higher, and they are less deformable in general[15].

The current study was planned to investigate the effect of a yellow diode pumping solid-state (DPSS) LLL at 589nm wavelength on the stability of stored whole blood and RBC suspension.

Materials and Methods

The interventional study was conducted from November

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2021 to April 2022 at the Postgraduate Medical Physics Laboratory, Mustansiriyah University, Baghdad, Iraq, and comprised blood samples from eight healthy subjects (4 males and 4 females) with mean age 21±3 years (range: 19-24 years). After approval from the institutional ethics review committee, and consent from the volunteers, blood samples were collected from these healthy adults having no history of major illnesses or of taking medications for major diseases. Whole blood samples 10ml were obtained by venipuncture into tubes containing 1.4ml of anticoagulant citrate-phosphate dextrose-adenine (CPDA-1) (MacoPharma Ltd, India).

The blood samples were stored for 21 days at 4°C. Each sample was divided into 8 equal aliquots of 1ml each; 4 served as controls and the other 4 aliquots were irradiated by laser beam for 15min. Separation of blood components was done by centrifugation of blood at 3000 x g for 10 minutes. The plasma, buffy coat, and top layers of packed RBCs were discarded. The RBCs were washed with a 0.9% sodium chloride NaCl solution one time through re-suspension and re-centrifugation of the RBC suspension.

The blood sample was irradiated with a yellow DPSS LLL (Changchun Dragon Lasers Co., China) at a wavelength of 589nm and an output power of 50mW. Each irradiation sample received a total dose of $50J/cm^2$ for 15 minutes. The laser was directed at the blood sample tubes in a regular manner. Irradiation was done at mean room temperature 23±2°C.

Hb released after 24 hours of storage of RBC suspension at 4°C with or without irradiation with a laser wavelength of 589nm was used to measure the stability indirectly. Two blood samples were allocated for each day of storage period of 0, 7, 14 and 21. At each storage day, a non-irradiated sample was used as a control. The remaining aliquots received $50J/ cm^2$ radiation doses.

At the end of the wash process, 1ml normal saline was added to the packed RBC. At day 0, 50ul of blood from the control sample was then taken and added to 5ml of distilled water to achieve 100% haemolysis. Another 50ul of blood from the control sample was taken and added to 5ml of the normal saline. Both RBC suspensions were kept at 4°C for 24hr for the stability test in line with literature[13]. Both blood suspensions were then centrifuged at 3000g for 10 minutes by bench centrifuge. The supernatants OD (Optical density) were measured by a spectrophotometer (SP-300 Optima, Japan) at a wavelength of 540nm. The percentage of 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 (i.e. 100% haemolysis). This process was repeated for the irradiated sample. All OD measurements were done in duplicate and the average was taken for comparison. The whole procedure was repeated for all blood samples stored on subsequent days. For each tested day, the day-specific sample and the rest of the intervention samples were exposed to the specified dose and wavelength of the laser light.

Data was analysed using SPSS 24. Data was presented as mean ± Standard deviation. Student's t test was used to assess differences across the variables. $P<0.05$ was taken as significant.

Results

Whole blood samples showed a significant reduction in haemolysis by 40%, 20% and 42% compared to the control samples at days 0, 7 and 14, respectively (Figure 1).

RBC suspension showed a significant reduction in haemolysis by 9% compared to the control samples only at day 21 (Figure 2).

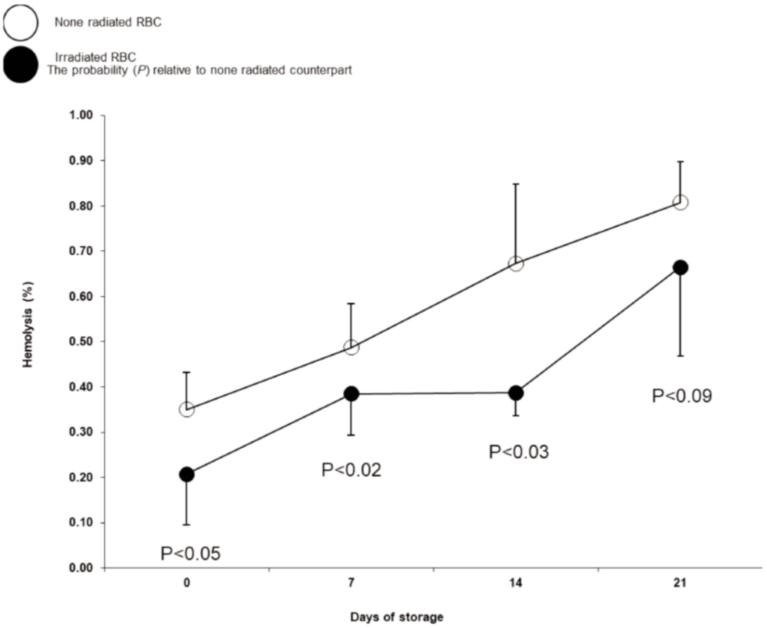

Figure-1: The percentage of haemolysis of stored whole blood with and without irradiation by 589nm wavelength laser light at a dose of $50J/cm^2$ (N = 4).

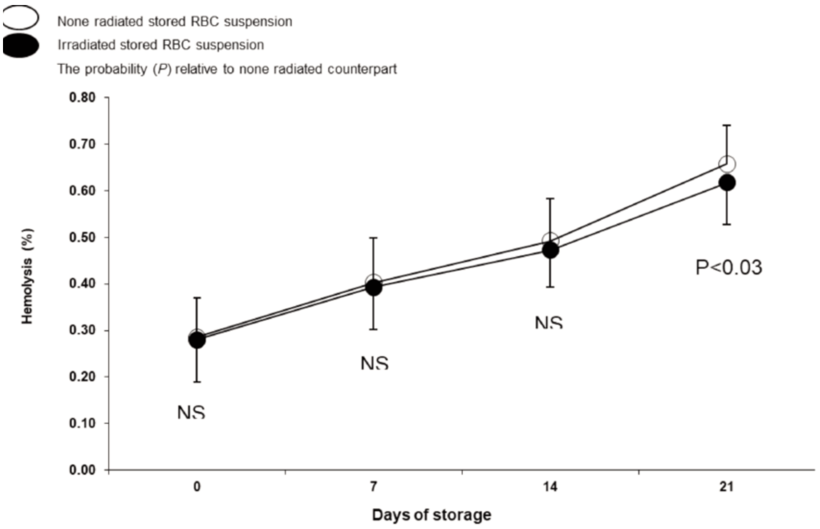

Figure-2: The percentage of haemolysis of stored red blood cell (RBC) suspension with and without irradiation with 589nm wavelength laser light at a dose of $50J/cm^2$ (N = 4).

Discussion

The present study revealed that the same dose of $50J/cm^2$ produced better results on whole blood samples than RBC suspension samples in terms of stored blood stability. LLLT can generate a variety of biological effects in living cells, most of which are caused by biochemical bio-modulation rather than a direct thermal effect[9]. The laser light can potentially influence the structure of RBC membrane proteins, resulting in denaturation of protein and cell lysis. The intensity of these impacts is mostly determined by the period of irradiation and the energy doses received[5]. Nevertheless, the basic mechanisms that promote LLLI's activities in tissues are still unclear[2]. A number of studies have demonstrated that irradiating RBCs with a low-energy laser have positive effects[11]. The laser light is absorbed through Hb, and is the main target in RBCs. As a result, as more photons are absorbed, the response becomes stronger[2]. Photons are absorbed by membrane-bound Hb (Hbm) and RBC membrane. The most essential component in the determinants of RBC deformability is Hbm[11]. Because the connection between Hb and the RBC membrane is weak, when LLL photons fall and are absorbed by Hbm, the Hbm link vibrates, and Hbm becomes Hb[16]. Because of the high absorption, laser action has a major effect on RBCs, although it does not destroy them. Low-power laser irradiation protects the membranes of RBCs[17]. Several studies have indicated that irradiating RBCs with low-energy laser light enhances blood rheological properties and thus microcirculation[18]. Blood maintained in blood banks improves biochemical and haematological characteristics in stored blood. Some studies suggest that processing transfused blood for an extended period of time raises the risk of transfusion problems[19]. As a result, using an LLL beam to extend the length of blood preservation to extended periods of time without haemolysis of blood cells is a serious concern. Already, DPSS lasers are increasingly widely employed in medical applications, especially for blood irradiation, due to the long-term stability of the exposure radiation beam [20]

Conclusion

Irradiating the whole blood with a yellow DPSS laser at 589nm wavelength caused a significant reduction in the percentage of haemolysis compared to its non-irradiated counterparts at various storage periods. The irradiation of whole blood at a dose of $50J/cm^2$ showed a more effective influence than its RBC suspension counterpart in keeping the stored blood stablised.

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