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Rheology and Morphological Study of Anaemic Red Blood Cell Parameters Irradiated with Blue and Green Laser Beam

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Abstract: ***Numerous studies have explored the effects of low-level laser therapy (LLLT) on blood parameters over the past decade. This study investigates the in vitro effects of blue and green laser light on the rheological properties and morphology of anemic human red blood cells (RBCs). A total of 36 blood samples were collected and analyzed before and after laser irradiation at exposure times of 30, 60, and 90 seconds. Key blood parameters, including hematocrit (HCT), mean cell volume (MCV), and mean corpuscular hemoglobin (MCH), were measured immediately after irradiation using a hematology analyzer, followed by morphological evaluation. Significant changes were observed in HCT and MCV for both blue and green laser irradiation. Notably, RBC deformability increased, particularly after 90 seconds of laser exposure. The findings demonstrate that anemic samples are more affected by laser irradiation than normal samples, with green laser showing a greater impact on RBC morphology compared to blue laser.***

Keywords: Low Level Laser, Red Blood Cells Parameters, Rheology, Bio-Stimulation

1. Introduction

The use of lasers as a medical modality has increased significantly over the past decades. Numerous experimental studies have demonstrated positive responses to laser-induced biostimulation in living organisms. Lasers used in biostimulation are typically classified based on power level and beam characteristics, both of which influence their effects. Most lasers employed in biostimulation fall under low-level laser therapy (LLLT), with power ranges between 1 and 500 mW [1][2].

The mechanism of interaction in LLLT involves the absorption of laser energy by intracellular chromophores, leading to photochemical reactions that enhance cellular metabolic activity. Specifically, the absorbed energy increases the production of adenosine triphosphate (ATP) within cells. This process occurs as energy stored through electron excitation in chromophores is utilized to perform various cellular functions [2]–[4].

Blood therapy using low-intensity laser irradiation has gained popularity across various clinical applications due to its ability to modulate blood rheology, improve erythrocyte function and microcirculation, and enhance the activity of multiple enzymes [5], [6]. Despite these promising effects, understanding the specific responses of blood components to laser biostimulation remains crucial, particularly in different pathological conditions. Limited information exists regarding the response of key blood parameters—such as white blood cells, red blood cells, platelets, and their components—to low-level laser irradiation[7].

The potential to treat conditions using laser light across different wavelengths (e.g., red, green, infrared, blue, and yellow) and frequencies introduces novel therapeutic strategies and unexplored research areas. However, critical parameters, such as the safe irradiation levels and exposure durations for various blood components, have yet to be clearly defined [8]. This study focuses on evaluating the effects of low-level laser therapy on the rheological and morphological properties of red blood cell components, comparing samples from normal and anemic conditions.

2. Materials and Method

Blood Sampling

A total of 36 blood samples from 18 males and 18 females range from 18-60 years old provided by Haematology Laboratory, Hospital Universiti Sains Malaysia (HUSM) were used in this research. Samples were selected and categorized into normal or anaemic blood samples according to haematological reference range based on Full Blood Count (FBC) result. The bloods were received in the morning from the patient (~3ml) then transferred into a tube containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Three aliquots were prepared from the EDTA blood sample. One was served as a control (untreated) and another two were irradiated with blue and green laser.

Blood Irradiation

Laser used in this research are Laser diode and Diode pumped Solid State (DPSS) with the wavelength of 460 nm (blue) and 532 nm (green) respectively and the output power for both lasers are 100 mW. Beam diameter for both laser is 5 mm. Laser was arranged vertically with the sample 6 cm to the later. Both lasers were calibrated and blood samples then were irradiated with 30 s, 60 s and 90 s exposure time.

Blood test

All blood samples selected were tested for complete blood count (CBC) using Sysmex XE-5000 Automated Hematology Analyzer before and after laser irradiation.

Morphological Analysis

Blood smear was prepared for control and irradiated samples to study the morphological properties of blood before and after irradiation. A small drop of blood was placed on a glass microscope slide. A spreader slide was positioned at an angle and slowly drawn toward the drop

of blood. The slide then was dried and stained using Wright's stain and phosphate buffer pH 6.8. Slides were examined under microscope and the analysed.

Statistical Analysis

In this study, paired t test was used to analyse the significance of difference for each sample pair (control and irradiated). All statistical analyses were performed using IBM SPSS Statistics 22.

3. Results and Discussion

The primary effects of laser irradiation on red blood cell parameters at different exposure times are summarized in the following tables. Data were statistically analyzed using a paired t-test to compare differences between control and irradiated samples. Table 1 presents cell count readings for the normal blood sample group irradiated with a blue laser, while Table 2 contains results for irradiation with a green laser.

In Table 1, significant changes were observed in hematocrit ($p = 0.003$) and mean cell volume (MCV) ($p = 0.000$) following blue laser irradiation. However, no significant difference was noted in mean corpuscular hemoglobin (MCH) ($p = 0.602$). Both hematocrit percentage and MCV showed a decrease in values after blue laser irradiation. Similarly, Table 2 indicates significant changes in hematocrit ($p = 0.013$) and MCV ($p = 0.000$) after green laser irradiation, while MCH remained unaffected ($p = 0.710$).

Tables 3 and 4 present cell count readings for anemic blood samples irradiated with blue and green lasers, respectively. Blue laser irradiation of anemic samples resulted in significant changes in hematocrit ($p = 0.021$) and MCV ($p = 0.000$), as shown in Table 3. For green laser irradiation, significant changes were detected in hematocrit ($p = 0.000$) and MCV ($p = 0.000$), as indicated in Table 4. Both hematocrit and MCV showed decreased values after irradiation with both blue and green lasers.

Notably, a significant change in MCH ($p = 0.045$) was observed in anemic samples irradiated with green laser light, while no significant change ($p = 0.138$) was found following blue laser irradiation. When comparing exposure times, irradiation for 60 seconds produced more prominent changes in red blood cell parameters in normal blood samples. Conversely, anemic blood samples exhibited greater changes after 90 seconds of irradiation.

Table 1 Measurement of cell count at different exposure time for blue laser (Normal group)

Exposure time	HCT		MCV		MCH	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
30	38.70±4.66	37.90±5.05	85.33±4.57	83.70±4.10	30.13±1.37	29.93±1.60
60	39.90±2.08	39.00±1.51	83.17±3.58	81.47±2.09	27.70±0.60	27.63±0.15
90	40.80±3.86	40.13±3.90	85.13±10.45	83.53±10.10	28.40±2.88	28.43±3.02
p-value	0.003[a]		0.000[a]		0.602	
Statistical significance: [a]p<0.01						

Table 2 Measurement of cell count at different exposure time for green laser (Normal group)

Exposure time	HCT		MCV		MCH	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
30	38.77±4.85	38.03±5.45	85.33±4.76	83.77±4.53	30.33±2.06	30.37±2.06
60	38.27±2.48	37.77±2.23	83.13±3.73	81.20±3.99	27.70±0.95	27.77±0.95
90	40.67±3.55	40.20±4.16	85.00±10.33	83.70±10.11	28.53±2.90	28.53±3.02
p-value	0.013[b]		0.000[a]		0.71	
Statistical significance: [a]p<0.01; [b]p<0.05						

Table 3 Measurement of cell count at different exposure time for blue laser (Anaemic group)

Exposure time	HCT		MCV		MCH	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
30	22.77±2.35	22.37±2.66	90.57±4.89	88.6±4.50	29.47±0.75	29.10±0.89
60	28.10±5.05	27.63±5.05	83.07±3.27	81.63±3.55	25.83±2.10	26.03±2.08
90	23.57±0.85	23.10±0.85	82.10±6.35	79.93±5.61	26.87±1.27	26.70±1.15
p-value	0.021[b]		0.000[a]		0.138	
Statistical significance: [a]p<0.01; [b]p<0.05						

Table 4 Measurement of cell count at different exposure time for green laser (Anaemic group)

Exposure time	HCT		MCV		MCH	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
30	22.57±2.55	22.27±2.70	90.23±4.77	88.17±4.60	29.23±0.75	28.97±0.96
60	27.97±5.08	27.63±4.93	82.70±3.45	81.30±3.56	26.10±1.87	25.77±2.11
90	23.50±0.90	23.10±0.89	81.80±5.90	80.00±5.80	27.00±1.44	26.73±1.62
p-value	0.000[a]		0.000[a]		0.045[b]	

Statistical significance: [a]p<0.01; [b]p<0.05

Rheological properties of red blood cells (RBCs) are essential for ensuring their function as oxygen and nutrient transporters. Blood viscosity, which directly correlates with hematocrit levels, plays a key role in maintaining efficient blood flow. Hematocrit, defined as the proportion of RBCs in the total blood volume, determines blood viscosity. An increase in hematocrit leads to a corresponding rise in blood viscosity. In this study, the results demonstrate a reduction in hematocrit percentage following laser irradiation. This indicates a decrease in the proportion of RBCs per unit volume of blood, consequently lowering blood viscosity. Blood viscosity is a critical physiological parameter, often used to assess health conditions related to blood flow. Elevated blood viscosity has been reported in patients with peripheral arterial disease, coronary artery disease, stroke, and hypertension [9]–[11]. Therefore, blood irradiation may serve as an alternative therapeutic approach to enhance blood flow and address these conditions.

Mean cell volume (MCV), a measure of the average size of RBCs, was also evaluated. The findings reveal that blue laser irradiation induces geometric deformability in RBCs. Specifically, MCV values decreased after laser exposure, suggesting that laser irradiation may alter RBC morphology. This phenomenon could be attributed to laser-induced disruption of the cell membrane, potentially opening Ca^{2+}-dependent K^{+} channels and reducing RBC volume [12][13].

Mean corpuscular hemoglobin (MCH) represents the average hemoglobin content per RBC, measured in weight. This parameter is clinically relevant for assessing the oxygen-carrying capacity of blood. The results of this study indicate a reduction in MCH following laser irradiation. This decrease is likely associated with the concurrent reductions in MCV and hematocrit.

Morphological evaluations of RBCs were performed on blood samples to compare pre- and post-irradiation changes under blue and green laser light for both normal and anemic samples. Representative examples of irradiated RBC morphology for normal and anemic samples are shown in Figure 1. The percentage of normal versus abnormal RBC shapes was calculated and is presented in Table 5.

Table 5: Percentage of normal erythrocyte out of abnormal erythrocyte shapes

Exposure time (s)	Normal samples			Anaemic samples		
	Control	Blue	Green	Control	Blue	Green
30	97.97 ± 0.06	97.00 ± 0.10	97.97 ± 0.06	78.93 ± 0.21	61.00 ± 0.06	78.90 ± 0.06
60	99.93 ± 0.06	95.93 ± 0.06	93.97 ± 0.06	78.03 ± 0.06	49.03 ± 0.06	62.03 ± 0.06
90	95.00 ± 0.20	92.97 ± 0.06	74.03 ± 0.06	84.97 ± 0.06	78.90 ± 0.10	63.03 ± 0.06
p-value		0.001[a]	0.021[b]		0.001[a]	0.005[a]

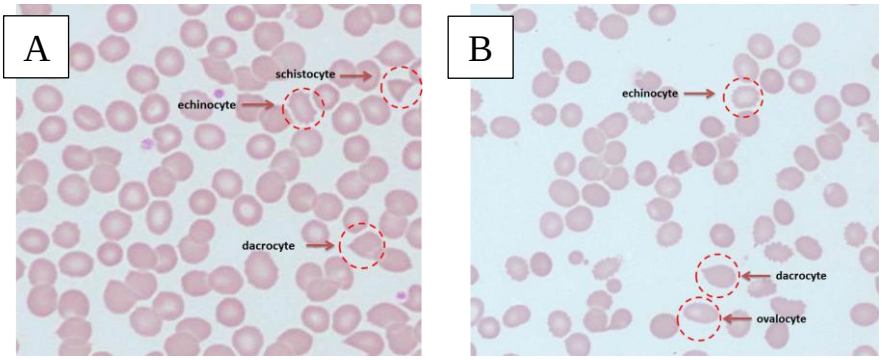

Figure 1: Blood smear after laser irradiation on (A) normal blood cells (B) anaemic blood cells

Morphological changes were observed in irradiated samples, highlighting the significant effects of laser light on RBCs, particularly in anemic samples. RBC shapes from normal blood samples remained largely intact at early exposure times. At 30 seconds of laser irradiation, deformability was minimal, with changes observed in less than 1% of cells. However, deformability increased with longer exposure durations. At 90 seconds, the highest percentage of deformability was recorded for both normal (20.97%) and anemic (21.94%) samples irradiated with green laser light. Comparatively, green laser irradiation resulted in greater RBC deformability than blue laser irradiation.

This study demonstrates that anemic blood samples are more affected by laser irradiation compared to normal samples. Moreover, green laser light was found to induce greater morphological changes in RBCs than blue laser light. The primary abnormality observed in

the prepared blood smears was the formation of echinocytes, or crenated cells, rather than other RBC variations.

Red blood cells exhibit deformability under mechanical stress, enabling them to traverse narrow capillaries efficiently. However, excessive deformability can result from alterations in cytoplasmic viscosity, cellular metabolism, or the physicochemical properties of the surrounding plasma [14]. The presence of echinocytes is often associated with reduced ATP generation, leading to water and potassium loss from RBCs and a subsequent decrease in erythrocyte survivability. Echinocytes may further transform into spherocytes as they lose membrane vesicle elasticity, resulting in cell shrinkage and eventual hemolysis [15][16].

The rheological changes observed in this study, including reductions in hematocrit and mean cell volume (MCV), align closely with the morphological alterations in red blood cells (RBCs) following laser irradiation. The decrease in hematocrit percentage may be attributed to morphological changes such as echinocyte formation, spherocyte transformation, and eventual cell shrinkage, which reduce the effective number of functional RBCs in circulation. Similarly, the reduction in MCV correlates with structural deformities in irradiated RBCs, likely caused by membrane disruptions and loss of intracellular water and potassium.

These morphological changes not only impact RBC deformability but also influence blood viscosity. As deformable cells are replaced by less flexible and abnormally shaped RBCs, the rheological properties of blood are altered. This is particularly evident in anemic samples, where the more fragile RBCs exhibited greater sensitivity to laser-induced stress, amplifying both rheological and morphological changes. Therefore, these factors showed important needs to carefully optimize laser parameters to harness therapeutic benefits without compromising RBC functionality.

4. Conclusion

This study indicated blue and green laser affected red blood cell parameters rheological and morphological. The exposure of red blood cells with laser has reduced its viscosity and volume based on CBC reading tested. Red blood cell deformability also has been observed after laser irradiation especially on anaemic samples after 90 s exposure times. Laser irradiation affected most in anaemic samples compared to normal blood samples and green laser affected red blood cell morphology more compared to blue laser.

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