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Effect of visible laser light on ATP level of anaemic red blood cell

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ABSTRACT

In this work we present influence of visible laser light on ATP level and viability of anaemic red blood cell (RBC). The visible laser lights used in this work are 460 nm and 532 nm. The responses of ATP level in anaemic and normal RBC before and after laser irradiation at different exposure time (30, 40, 50 and 60 s) were observed. Three aliquots were prepared from the ethylenediaminetetraacetic acid (EDTA) blood sample. One served as a control (untreated) and another two were irradiated with 460 nm and 560 nm lasers. Packed RBC was prepared to study ATP level in the RBC using CellTiter-GloLuminescent cell Viability Assay kit. The assay generates a glow type signal produced by luciferase reaction, which is proportional to the amount of ATP present in RBCs. Paired *t*-test were done to analyse ATP level before and after laser irradiation. The results revealed laser irradiation improve level of ATP in anaemic RBC. Effect of laser light on anaemic RBCs were significant over different exposure time for both 460 nm ($p = 0.000$) and 532 nm ($p = 0.003$). The result of ATP level is further used as marker for RBC viability. The influence of ATP level and viability were studied. Optical densities obtained from the data were used to determine cell viability of the samples. Results showed that laser irradiation increased viability of anaemic RBC compared to normal RBC.

1. Introduction

Low level laser intensity has been used widely in many field such as diagnostic and therapy while searching for non-invasive treatment. Laser interaction with tissue can either gives positive or negative effect depends on laser parameters. The parameters include laser line wavelength, power and exposure time [1]. Biostimulation effects of laser light have shown that laser irradiation has positive therapeutic effect such as wound healing, tissue repair, improve microcirculation of blood flow and local tissue perfusion, modified cellular processes, improved proliferation of cells and rheological properties [2–5].

Deformability is the basic rheological property of the RBC where its cell membranes undergo morphological changes. RBC flexibility undergoes deformation when subjected to shear stress especially for blood flow in microcirculation allowing erythrocytes to pass through narrow vessels. However, permanent deformability affects erythrocytes function and caused serious vascular complication [6,7]. Studies have shown that laser irradiation of deformed RBC improves the deformability [4,5]. It well known that anaemic RBC is deformed with decreased Adenosine Triphosphate (ATP). Adenosine triphosphate (ATP) is the energy currency in all metabolic cell reactions, and the key molecule for most cell processes [8]. The ATP measurement can be used as a marker for evaluation of RBC viability [9]. A reduce in ATP content in RBC can be associated with shape changes from discs to spheres, a loss of membrane lipid, and an increase in cellular rigidity. Thus, maintaining RBC integrity and flexibility is an energy-consuming process [10]. Several works have shown that laser irradiation has positive effect on RBC deformability and ATPase activity [4,5,11–13]. However, in this work effect of visible laser light on ATP level of anaemic RBC is presented. To analyse the response of RBC to the action of laser irradiation, the ATP level before and after laser irradiation on normal and anaemic RBC at different exposure time were studied. In addition, the influence of laser irradiation on anaemic RBC viability is also reported. The visible laser lights used in this work are 460 nm and 532 nm.

2. Experimental

2.1. Blood Samples

Blood samples (~3 ml) from 32 Males and 32 females range from 18 to 60 years old were selected based on Full Blood Count (FBC) result which is categorized into normal or anaemic blood samples according to haematological reference range. Three aliquots were prepared from the EDTA blood sample. One served as a control (untreated) and another two will be irradiated with 460 nm and 560 nm lasers. Blood samples were provided by Haematology Laboratory, Hospital Universiti Sains Malaysia (HUSM).

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Table 1
Level of ATP for normal red blood cells.

	Exposure time (s)				*p*-Value
	30	40	50	60	
Un-irradiated	0.0763 ± 0.0022	0.0788 ± 0.0005	0.0795 ± 0.0017	0.0708 ± 0.0010	
460 nm	0.0733 ± 0.0015	0.0708 ± 0.0015	0.0740 ± 0.0026	0.0728 ± 0.0024	0.004***
532 nm	0.0730 ± 0.0009	0.0695 ± 0.0013	0.0710 ± 0.0018	0.0723 ± 0.0020	0.001***

Statistical significance: ***$p < 0.01$.

2.2. Blood Irradiation

Lasers used in this research are 460 nm blue laser and 532 nm green diode pumped Solid State with output power, 100 mW. Laser was arranged vertically with the sample 6 cm to the later. Blood samples (~3 ml) were then irradiated with lasers for 40, 50, 60 and 70 s exposure time.

2.3. Red Blood Cell Purification

To separate RBCs from plasma, whole blood was centrifuged at 2500 rpm for 5 min. The supernatant and "buffy coat" were removed using pipette. RBCs packed cells were diluted with blood buffer (10 mM glucose and 0.015 mM Bovine Serum Albumin (BSA) in PBS) to preserve the normal shape of RBCs. The RBCs were washed two times by blood buffer and centrifuged at 2500 rpm for 3 min. Final dilution of the RBCs packed cells by blood buffer were 3 μl:1 ml [9]. RBCs were then seeding into 96-well plate at a density of $1x10^6$ in 75 μl of diluted RBCs to be tested.

2.4. Adenosine Triphosphate (ATP) Measurement

The level of ATP in RBCs was determined using CellTiter-GloLuminescent cell Viability Assay kit (Promega). The assay generates a glow type signal produced by luciferase reaction, which is proportional to the amount of ATP present in cells. The volume of reagent inserted is equal to the volume of diluted RBCs present in each well. The contents were mixed for 2 min in Enzyme Linked Immunosorbent Assay (ELISA) reader and were shake to induce cell lysis. The plate was allowed to incubate at room temperature for 10 min to stabilize luminescent signal and recorded using ELISA reader at the peak values of 560 nm.

2.5. Ethics Approval

This research has been approved by Human Research Ethics Committee, Universiti Sains Malaysia (FWA Reg. No: 00007718; IRB Reg. No: 00004494).

2.6. Statistical Analysis

The data were statistically analysed using paired *t*-test and Oneway ANOVA to compare ATP level before and after laser irradiation. Then the values of standard deviations in the same group were statistically calculated. And then *p* value was determined accordingly to analyse the significance of difference for each sample pair. All statistical calculations and analyses were performed with the statistical package for social science (SPSS) 22.0 software.

3. Results and Discussion

Both normal and anaemic blood samples were irradiated with 460 nm and 532 nm lasers. ATP Level of RBC was measured by optical density. The ELISA reader measures intensity of emittance produce by CellTiter-GloLuminescentin terms of optical density. The result of laser irradiated normal RBC are shown in Table 1. Unlike the un-irradiated samples of the normal RBC, after irradiation ATP level of the normal RBC decreases except the sample irradiated for 60 s. This reveals that laser irradiation reduced the ATP level of a normal RBC. The decreased in ATP level can be associated with a shape change [9]. This shows laser irradiation of normal RBC can have negative effect and non-therapeutic effect on the RBC [14]. So, we assumed laser irradiation of healthy RBC can damage the RBC morphology and decreased the level of ATP.

From the data shown in Table 1, we assumed 0.0763 is minimum value of ATP for un-irradiated normal RBC. As shown in Table 2, the level of ATP in un-irradiated abnormal RBC is lower than that of normal RBC (control sample of normal blood) for anaemic blood sample. Unlike the un-irradiated (control) samples in Table 2, the level of ATP increased in all the irradiated anaemic RBC. The irradiation of RBC with 460 nm (Table 1) shows an increased in ATP level after 30 s (3% increment), 40 s (7% increment), 50 s (10% increment) and 60 s (6% increment) of exposure times. Unlike for 60 s, the results demonstrate that the amount of ATP level increased with increased in exposure time (30 s, 40 s, 50 s). As for RBC irradiation with 532 nm (Table 2), it can be seen that increased in ATP levels is significant after irradiated with 30 s (1% increment), 40 s (9%), 50 s (3%) and 60 s (2%) exposure times. The percentage of increment at 40 s exposure times for 532 nm is nearly the same with 460 nm. However, this shows less time is needed by 532 nm to give nearly similar effect of 460 nm laser light. Comparing Tables 1 and 2, ATP level below minimum value of un-irradiated normal RBC increased in the level of ATP after laser irradiation. For normal blood sample, at 60 s (Table 1), un-irradiated control sample has 0.0708 which is below minimum amount of normal ATP in RBC. Although, this sample is from normal blood sample, the ATP level in the RBC is below assumed minimum level. Consequently, laser irradiation improves its ATP level. At 30 s exposure time, ATP level increment of anaemic RBC is small (1% increment). This is due to the level of ATP is very low (0.0665). However, at 60 s exposure time, ATP level for un-irradiated anaemic RBC is also very low but percentage of increment is higher, 6%. Herein, we observed biostimulating effect is proportional to the exposure time [11]. Since the ATP level is low, more exposure time is needed to increase the ATP level in RBC as there are thresholds for laser light to be

Table 2
Level of ATP for anaemic red blood cells.

	Exposure time (s)				*p*-Value
	30	40	50	60	
Un-irradiated	0.0665 ± 0.0010	0.0735 ± 0.0013	0.0705 ± 0.0006	0.0678 ± 0.0010	
460 nm	0.0678 ± 0.0005	0.0790 ± 0.0022	0.0775 ± 0.0017	0.0720 ± 0.0014	0.000***
532 nm	0.0673 ± 0.0015	0.0798 ± 0.0018	0.0728 ± 0.0019	0.0690 ± 0.0033	0.003***

Statistical significance: ***$p < 0.01$.