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Effect of 460 and 532 nm Laser Light on the Erythrocyte Deformability of Anaemic Blood Samples

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ABSTRACT: *This study investigated the effect of 460 nm and 532 nm laser irradiation on anaemic blood compared with normal samples at different exposure times. Blood smears were prepared to study the effect of laser irradiation on erythrocyte deformability. Irradiation of normal and anaemic blood samples with 460 and 532 nm laser light significantly changed erythrocyte deformability. The deformability of both the normal and anaemic blood samples increased as the exposure time increased. The analysis also revealed that erythrocyte deformability is greater in anaemic blood than normal blood.*

Keywords: Laser, anaemic, erythrocyte, deformability, blood samples

1. INTRODUCTION

Lasers emit a beam of intense electromagnetic radiation that is essentially monochromatic or contains at most a few wavelengths that are only nearly monochromatic; the beam is typically only weakly divergent and is easily focused onto external optical systems.[1] From a medical point of view, lasers are a convenient but sophisticated source of light in the visible, ultraviolet, and infrared parts of the spectrum.[2] Easy to control, the light beam (of a single colour) can be focused into a small spot and, in many cases, the beam can be transmitted via flexible fibres, making internal delivery of light feasible.[2]

Low-intensity laser irradiation has primarily been shown to be useful in medical applications and is widely used in clinical practice.[3–5] Such widespread interest in lasers largely resulted from the fact that their creation was an interdisciplinary enterprise. Because of its positive effect, laser therapy treatment has become a popular phenomenon, especially in the cosmetics industry.[6,7] The use of visible light, which is considered safe, has attracted many to this treatment, including those who have anaemia, with the thought that it will not have any negative effect on them. However, in laser irradiation, a photon of light which is absorbed by a system is excited to another state and then releases its extra energy to achieve stability.[8,9] Thus, an effect or interaction will still occur unless it is not absorbed in the system.

Laser irradiation of the blood, especially intravenously, has been utilised clinically in sports medicine and to treat acute cerebral infarction, diabetes, psoriasis and rheumatoid arthritis.[10–12] The irradiation of red blood cells leads to deformability if it is given beyond a therapeutic level.[9] Deformability is a basic rheological property of the erythrocyte that allows its membranes to undergo morphological changes.[13] Erythrocyte deformability is an essential rheological feature that gives flexibility under shear stress; in particular, for blood in microcirculation, it allows erythrocytes to pass through narrow vessels.[14] However, permanent deformability affects erythrocyte function and causes serious vascular complications.[13,15,16] Thus, maintaining the appropriate percentage of deformability is crucial for the physiological function of red blood cells (RBCs).[17] Studies have shown that deformation of red blood cell also causes the release of Adenosine Triphosphate (ATP) to participate with the circulation.[16,18] A reduction in the ATP content of an erythrocyte can be associated with changes in shape, loss of membrane lipids, and an increase in cellular rigidity, which causes deformability.[15,16]

Studying the effects of low-level laser irradiation on the blood is very important, as blood is an important component of humans. Despite the positive effects of

low-level lasers on the blood, the safety of the procedure needs to be considered, especially with regard to red blood cells and anaemic patients. Thus, this study reports the effect of 460 nm and 532 nm laser irradiation on the percentage of erythrocyte deformability of anaemic blood samples.

2. EXPERIMENTAL

2.1 Blood Samples

Blood samples (~3 ml) from 32 males and 32 females ranging in age of 18–60 years old were selected based on the results of a full blood count (FBC). The samples were then categorised as normal or anaemic according to the haematological reference range, which was provided by the Haematology Laboratory, Hospital Universiti Sains Malaysia (HUSM). Three aliquots were prepared from each EDTA-treated blood sample. One served as a control (untreated), and the other two were irradiated with the 460 nm or 532 nm lasers.

2.2 Blood Irradiation

The lasers used in this research were a 460 nm blue laser diode and a 532 nm green diode-pumped solid-state laser with an output power of 100 mW. The beam diameter apertures of the 460 and 532 nm lasers were approximately 2.5 and 2.1 mm, respectively, with a divergence of less than 2.8 and 2.0 mrad, respectively. Laser was arranged vertically with the sample 6 cm to the later. The blood samples, approximately 3 ml each, were then irradiated with the lasers for exposure times of 30, 50, 70 and 90 s.

2.3 Morphological Analysis

A blood smear was prepared from each control and irradiated sample. The slides were then examined under a microscope and were analysed. Using these slides, we observed changes in erythrocyte shape before and after irradiation by the lasers. The percentage of erythrocyte deformability before and after irradiation was measured and recorded.

2.5 Statistical Analysis

The data were statistically analysed using paired t-tests to compare the effect of the 460 and 532 nm irradiation on erythrocyte deformability before and after irradiation. All statistical calculations and analyses were performed with the Statistical Package for Social Science (SPSS) software version 22.0.

3. RESULTS AND DISCUSSION

Samples of both the normal and anaemic blood were irradiated at 460 nm and 532 nm to compare these two types of blood. The control was the unirradiated blood. Table 1 presents the effect on erythrocyte deformability before and after laser irradiation for the normal and anaemic samples at 460 nm and 532 nm. The table shows that laser irradiation of the normal and anaemic blood samples significantly changed the erythrocyte deformability (p-value = 0.000).

Table 1: Erythrocyte deformability before and after laser irradiation.

Exposure time (s)	Normal samples			Anaemic samples		
	Control	460 nm	532 nm	Control	460 nm	532 nm
30	97.93 ± 0.10	96.98 ± 0.10	98.03 ± 0.17	39.03 ± 0.25	41.00 ± 0.09	38.98±0.13
50	98.20 ± 0.34	95.00 ±0.14	88.00 ± 0.10	91.03 ± 0.13	79.00 ± 0.08	80.97 ± 0.21
70	86.03 ± 0.10	82.00 ± 0.08	81.00 ± 0.08	81.00 ± 0.08	71.00 ± 0.08	81.00 ± 0.08
90	94.98 ± 0.17	92.95 ± 0.06	73.98 ± 0.13	85.00 ± 0.08	78.98 ± 0.17	62.97 ± 0.13
p-value		0.000***	0.000***		0.000***	0.000**

*Statistical significance: ***p<0.01*

The percentage of erythrocyte deformability for the normal and anaemic blood samples irradiated at 460 nm is shown in Figure 1. The normal samples irradiated at 460 nm showed increased deformability as the exposure time increased, except at the 90 s time point. For the anaemic samples, the erythrocyte deformability increased at 30 s and 50 s but started to drop slightly at 70 s and 90 s. Comparing both blood categories, the anaemic sample was affected more by the 460 nm laser irradiation.

Figure 2 shows the percentage of erythrocyte deformability for the normal and anaemic samples irradiated at 532 nm. After 30 s of exposure, only 1% erythrocyte deformability was observed for the normal samples, but it was 2% for the anaemic samples irradiated at 460 nm. By contrast, the deformability of both of the samples was unaffected after irradiation with the 532 nm laser, as shown in Figure 2. Erythrocyte deformability increased as the exposure time increased for the normal blood samples, with the exception of the 70-s exposure time point. Like the normal samples, the erythrocyte deformability of the anaemic blood samples increased as the exposure time increased. The highest percentage of deformability of the normal samples irradiated at 460 and 532 nm was observed

at 70 s (4%) and 90 s (21%), respectively. However, for the anaemic samples, the highest deformability was observed at 50 s (12%) and 90 s (22%) when the samples were irradiated at 460 and 532 nm, respectively. At 90 s, irradiation of the blood samples at 532 nm resulted in greater deformability of both the normal and anaemic samples.

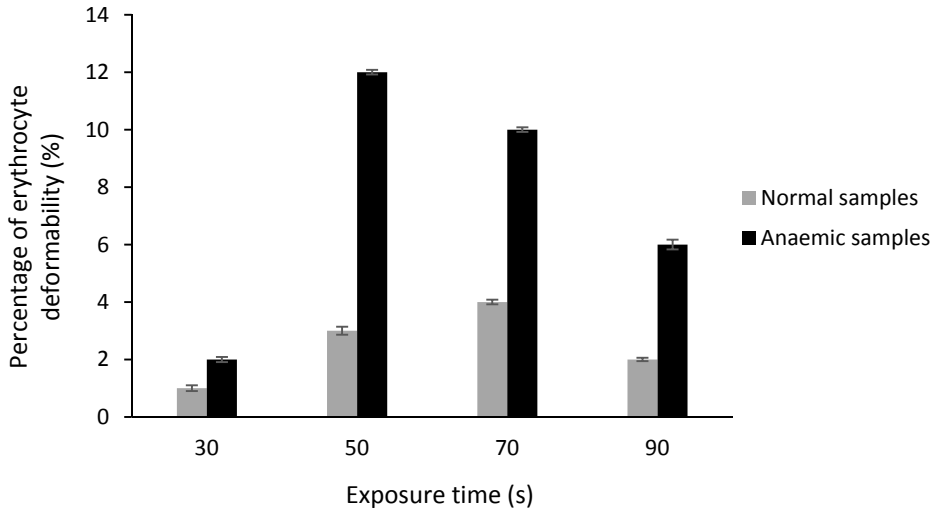

Figure 1: Percentage of erythrocyte deformability in normal and anaemic samples irradiated at 460 nm.

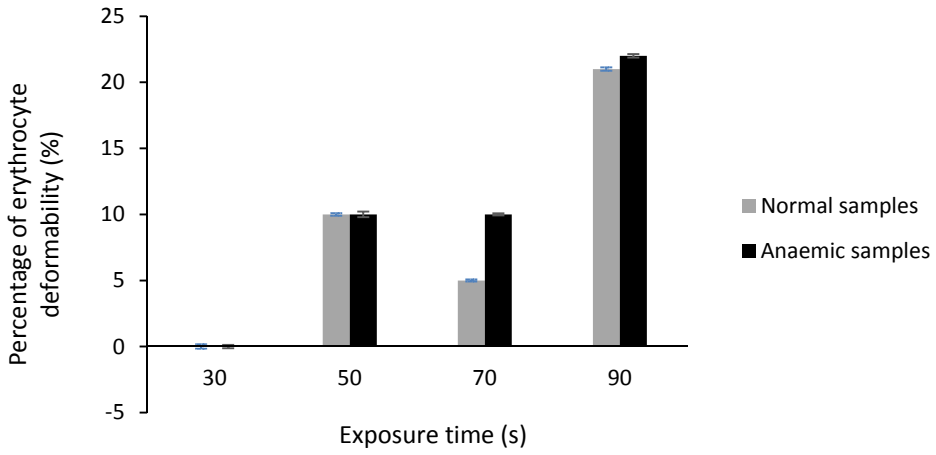

Figure 2: Percentage of erythrocyte deformability in normal and anaemic samples irradiated at 532 nm.

The anaemic blood was affected more than the normal blood samples. Morphologically, both the 460 and 532 nm lasers caused erythrocyte deformability, especially for the anaemic samples. We assumed that the 532 nm laser would affect erythrocyte deformability more than the 460 nm laser, as the greatest deformability percentage was observed after 90 s of irradiation with 532 nm. This effect is because the effective tissue penetration is maximised at 532 nm compared to 460 nm. Haemoglobin, which acts as a chromophore, absorbs more photons at higher absorption bands from the 532 nm laser than the 460 nm laser.[9] Thus, the effect is more prominent. Previously, it was shown that laser irradiation improved red blood cell deformability.[10,19,20] However, the radiation must be administered at a therapeutic dose and not beyond that.[9] The correct dose will stimulate a positive effect, but a higher dose will cause damage to and inhibit the erythrocytes.[21]

Figure 3 shows images of blood smears from the normal blood samples. Figure 3A is the control unirradiated sample. The erythrocytes in this sample are in good condition. After irradiation for 50 s with the 460 nm laser (Figure 3B) and the 532 nm laser (Figure 3C), deformability of the erythrocytes was observed. Among the abnormal erythrocytes observed in the smears in Figure 3B and 3C are keratocytes, dacrocytes, boat-shaped cells and echinocytes. Irradiation at 532 nm affected the erythrocytes of the normal samples more than the 460 nm laser.

Figure 4 shows images of blood smears from the anaemic blood samples. Figure 4A is an image of the control unirradiated sample. The erythrocytes from this sample are in poor condition. Abnormal erythrocytes, such as echinocytes and dacrocytes, can already be observed. Irradiation for 50 s with the 460 nm and 532 nm lasers caused more deformability to the erythrocytes than in the control sample, as shown in Figure 4B and 4C, respectively. Among the abnormal erythrocytes observed in the smear in Figure 4B and 4C were schistocytes, acanthocytes, keratocytes, target cells and echinocytes.

Based on all the blood smears prepared, most of the abnormal cells observed were echinocytes, which are crenated compared to the other abnormal red blood cell variants. The echinocytes can become spherocytes as they lose membrane vesicles, which leads to a greater loss of surface area and volume and finally leads to haemolysis.[22–24] Therefore, a suitable laser irradiation parameter must be carefully chosen after weighing the benefits and the risks.

The red blood cell lifespan depends on an adequate oxidative stress response. Red blood cells are made of protein, and denaturation can occur when the local heat is high, which causes shear stress to the red blood cell membrane and thus changes

the shape of the red blood cell. Echinocytes also form because of decreased ATP generation, resulting in the loss of water and potassium from the red blood cells. Red blood cells have deformable structures that allow them to recover their initial shape after passing through very small capillaries, which is an important and essential feature for their blood flow properties.[14,18,25,26] However, if the alteration and deformation of the red blood cell is severe, it may affect the ability of the cell to function properly. Thus, maintaining the percentage of deformability of red blood cells after laser irradiation is crucial.

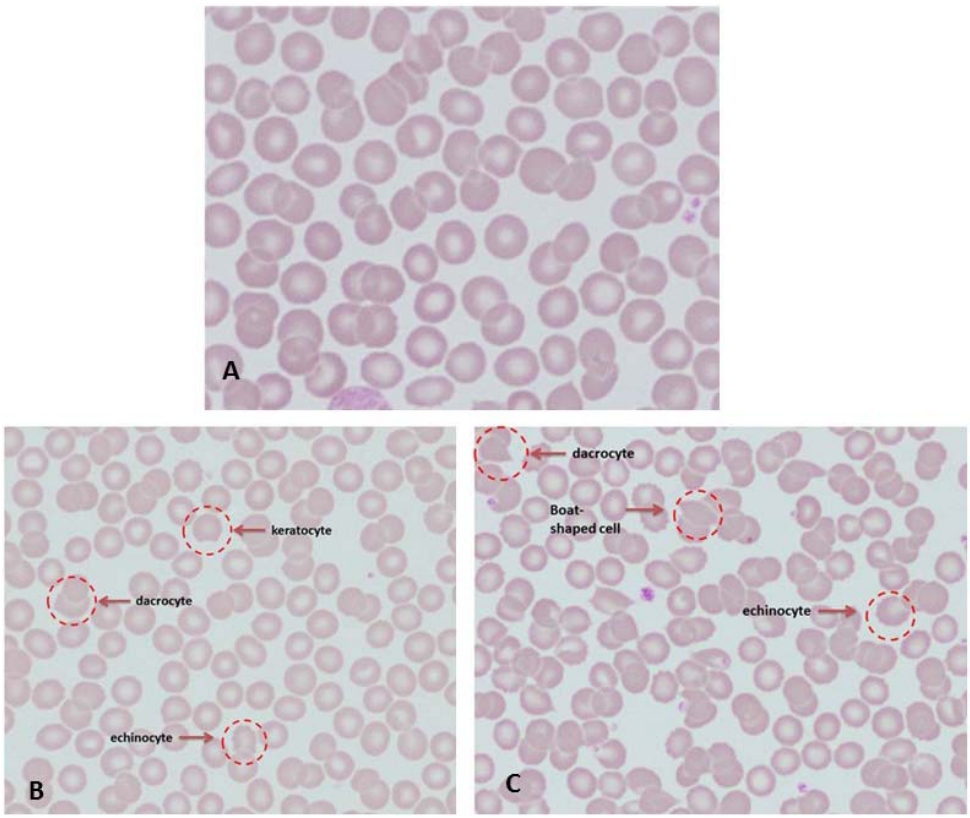

Figure 3: Images of blood smears from normal blood samples of A) control unirradiated sample; B) irradiated at 460 nm; and C) irradiated at 532 nm.

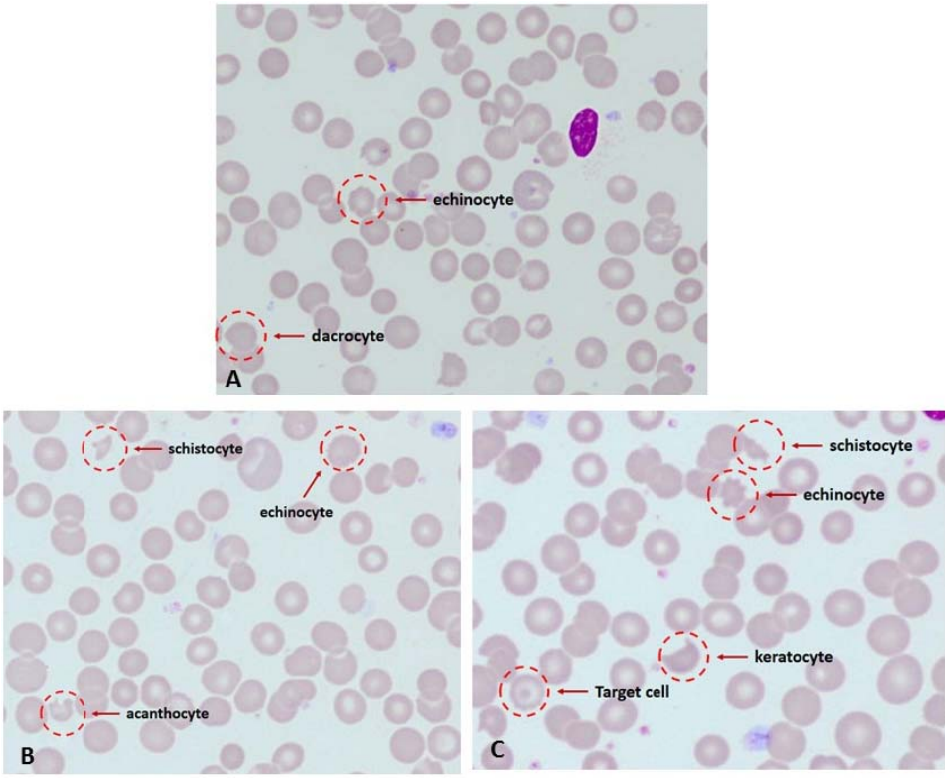

Figure 4: Images of blood smears from normal blood samples of A) control unirradiated sample; B) irradiated at 460 nm; and C) irradiated at 532 nm.

4. CONCLUSION

Red blood cells are important cells for humans that regulate the functions of the whole body. Irradiating normal and anaemic blood samples with 460 and 532 nm lasers significantly changes their deformability. The deformability of both the normal and anaemic blood samples increased as the exposure time increased. The analysis also revealed that the deformability of anaemic blood samples is greater than that of normal blood samples. Therefore, a suitable laser irradiation parameter must be carefully chosen after weighing the benefits and the risks.

5. ACKNOWLEDGEMENT

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