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Scientific Paper

Direct monitoring of erythrocytes aggregation under the effect of the low-intensity magnetic field by measuring light transmission at wavelength 800 nm

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Abstract

Interacting electromagnetic field with the living organisms and cells became of the great interest in the last decade. Erythrocytes are the most common types of the blood cells and have unique rheological, electrical, and magnetic properties. Aggregation is one of the important characteristics of the erythrocytes which has a great impact in some clinical cases. The present study introduces a simple method to monitor the effect of static magnetic field on erythrocytes aggregation using light transmission. Features were extracted from the time course curve of the light transmission through the whole blood under different intensities of the magnetic field. The findings of this research showed that static magnetic field could influence the size and the rate of erythrocytes aggregation. The strong correlations confirmed these results between the static magnetic field intensity and both the time of aggregation and sedimentation of erythrocytes. From this study, it can be concluded that static magnetic field can be used to modify the mechanisms of erythrocytes aggregation.

Key words: erythrocytes; aggregation; magnetic field; light; transmission.

Introduction

Aggregation is one of the characteristic behaviours of the erythrocytes. Erythrocyte aggregation is reversible and is dependent on the presence of some blood proteins such as fibrinogen. As well as chemical conditions can influence the aggregation behaviour of erythrocytes, also the physical conditions such as shear forces have a significant effect on erythrocytes aggregation [1,2]. The rate and size of erythrocytes aggregation could be used as a biomarker for many of clinical conditions and one of the vital signs of some diseases such as diabetes [3]. In type 2 diabetes, erythrocytes aggregate more readily than normal erythrocytes. Erythrocytes aggregation is one of the important complications that accrues to diabetes patients with poor glycemic control [4,5]. Deep vein thrombosis takes places due to the pathophysiological role of locally altered hemorheology results from increasing erythrocytes aggregation [6].

Many techniques are available to monitor and measure the rate and size of erythrocytes aggregation by both direct and indirect methods. One of these techniques is sedimentation rate which can be categorized as an indirect indicator of erythrocyte aggregation. Erythrocytes aggregometers are instruments that were developed for direct measurement of erythrocyte aggregation. Most of the erythrocytes aggregometers monitor light transmittance (LT) or light reflectance (LR) during the time course of aggregation. This time course during RBC aggregation is widely used to get aggregation parameters by mathematical analysis [7].

The living organisms rationally infer to be adapted to the weak geomagnetic field. Along with this assumption, there must be interactions between magnetic field (MF) and living tissues [8]. Particular attention was given to the potential of magnetism on the microcirculatory system. Many experiments had been done on both human and animals to understand the mechanism of the interaction between MF and microcirculation [9,10]. Also, some studies on the cellular level had been done to explore the cellular activities under the effect of MF [11]. Recently, it has been investigated the good benefits of MFs on human systems. For instance, it is documented that MF exposure can provide analgesia, decrease healing time for fractures, increase the speed of nerve regeneration, act as a treatment for depression, and provide other medical benefits [12-16]. Increased knowledge of the influence of MFs on microvascular function may have significant therapeutic potential [17, 18].

The effect of magnetic field on the rheological properties of blood is still under investigation. Blood viscosity was found to be decreased by exposure to MF. It has also been observed the improvement of blood flow in upper limp after exposure to MF [19,20].

One of the major factors that can influence the microcirculation is erythrocytes aggregation. Therefore, this study represents a simple, direct method to monitor the effect of static magnetic field (SMF) on erythrocytes aggregation. Also, this study evaluates the effect of the SMF on the rate and size of erythrocytes aggregation to explore a new physical method that can be used to control the erythrocytes aggregation.

Materials and Methods

Sample collection and preparation

Thirty blood samples were collected from healthy donors with the same age and gender. Five ml of blood was collected on EDTA as anticoagulants. All samples were checked for any abnormalities. All samples were centrifuged at 4000 rpm for 5 min to separate erythrocytes and plasma. Hematocrit was adjusted at 20%.

Transmission measuring setup

C shaped electromagnetic core generator (ECG) was designed with an iron core. The coil was wrapped up in the opposite side of the air gap of ECG. DC power supply was connected to the coil to control the magnetic field intensity. The wanted field was produced in the air gap between the two ends of the ECG with a value up to 500 mT. The ECG was built in the compartment of the spectrophotometer in the position that the two ends faced the source and the detector of the spectrophotometer. A hole at each end of the ECG allowed the light to be passed through the sample. The ECG was aligned in a position the glass sample holder to be at the center and introduced to the maximum intensity of the magnetic field. **Figure 1** represents the schematic diagram of the ECG. All the measurements were done in the range of magnetic field between 100 and 500 mT. Erythrocytes suspended in plasma (20% Hct) were introduced in the glass sample holder after stirring with a magnetic stirrer for 2 min for the complete destruction of aggregates. The light transmission was measured at the wavelength of 800 nm. The light transmission percentage (LT%) was calculated as:

$$LT\% = \frac{\text{light transmission of whole blood}}{\text{light transmission of plasma}} \quad \text{Eq. 1}$$

For each sample measurement of LT% was repeated under different intensities of the magnetic field (B) from 100 µT to 500 µT.

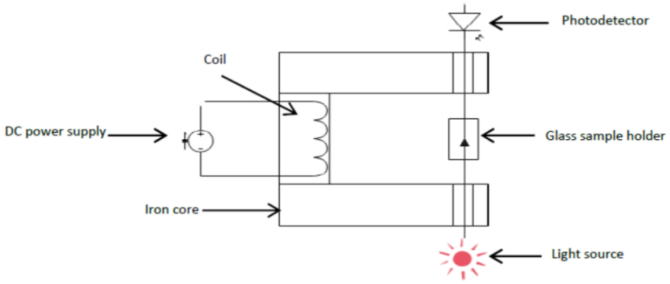

Figure 1. Schematic diagram of C shaped electromagnetic core generator

The following parameters were measured to evaluate the effect of SMF on the erythrocytes aggregation:

- The amplitude (Amp) was calculated as the difference between the minimum and maximum value of LT%.
- The area under the curve (AUC) was calculated for the curve drawing for the relationship between (LT%) and time (t). AUC was proportional to the aggregation formation.
- Time of aggregation (t_{agg}) represented the time of complete formation of erythrocytes aggregation.

Statistical analysis

All the values were represented as the mean ± SD. The relationships were examined with linear regression and correlation coefficient (R^2) was calculated. $R^2 \geq 0.6$ was considered as strong correlation. $0.5 \leq R^2 \leq 0.6$ was considered as moderate correlation. $R^2 \leq 0.5$ was considered as weak correlation.

Results and Discussion

Different techniques have been used to monitor erythrocytes aggregation using light transmission, and different parameters were used to describe the aggregation rate and intensity [7,21-23]. Technique established by M.R. Hardeman et al. was used to directly monitor the erythrocytes aggregation [22]. This method based on measuring the light reflectance and transmission through the blood under static and dynamic conditions. From light transmission and reflectance, many parameters could be extracted to describe the rheological properties of blood including the rate of aggregation and sedimentation [7]. Later on, Oguz K. Baskurt et al. used the same technique to monitor erythrocytes aggregation under different light wavelengths to optimize the conditions that give the best investigation of erythrocytes characterizations [21, 23]. The wavelength survey was done previously by Oguz K. Baskurt et al. and they found the best wavelength used to study light transmission through blood was 800 nm [23]. **Figure 2** shows the relationship between the LT% and the time. The curves obtained can be divided into two main parts. The first part due to aggregation and this part ended at the minimum value of LT%. The second part because of sedimentation and this part continued until LT% reached its maximum value. As shown in **Figure 2** LT% decreased until it reached its minimum value and after a period, it began to increase again. As shown in **Figure 2** the LT% went faster toward minimum value as the SMF intensity was increased. Amp of LT% decreased as the SMF increased. The results obtained in **Figure 2** were matched with the results in the previous studies. That confirmed the possibility to use light transmission technique using a spectrophotometer to check the behaviour of erythrocytes under different conditions. The amplitude of the relationship between LT% and time expressed the interaction between SMF and erythrocytes.

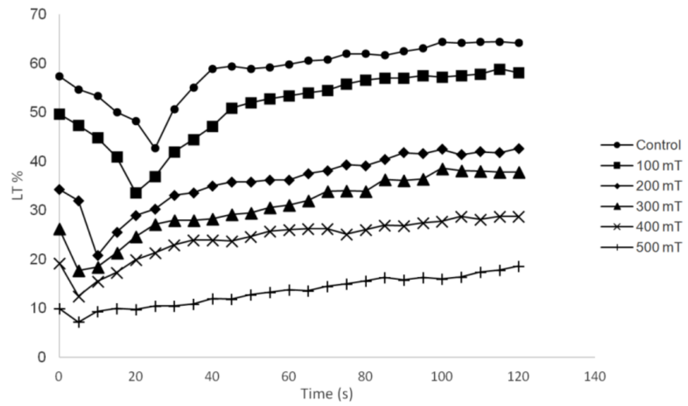

Figure 2. The time course of light transmission at different values of the magnetic field and compared with control (LT% without magnetic field).

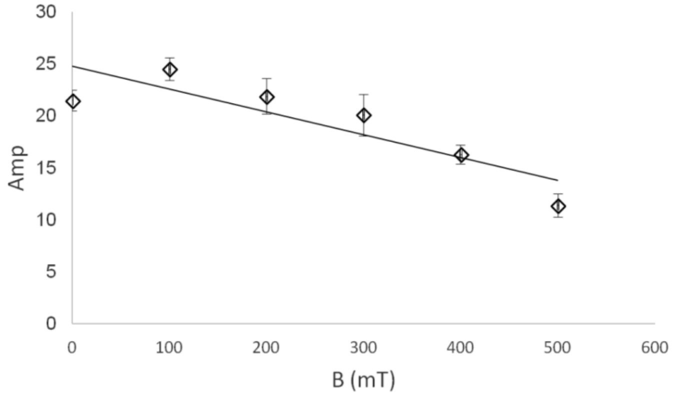

Figure 3. Light transmission amplitude and magnetic field intensity were inversely proportional. The solid line was the linear regression (R^2 = 0.76).

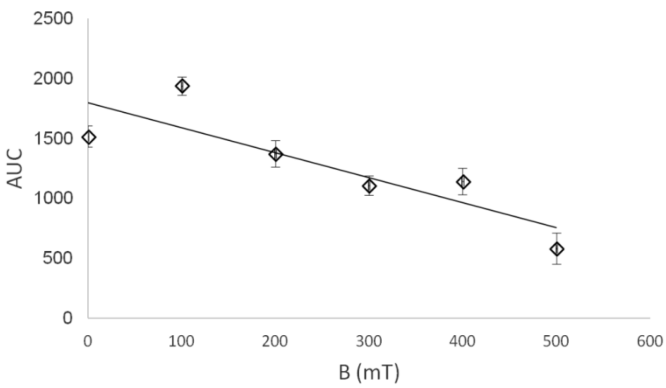

Figure 4. The size of erythrocytes aggregates decreased as SMF intensity increased. The solid line was the linear regression (R^2 = 0.74).

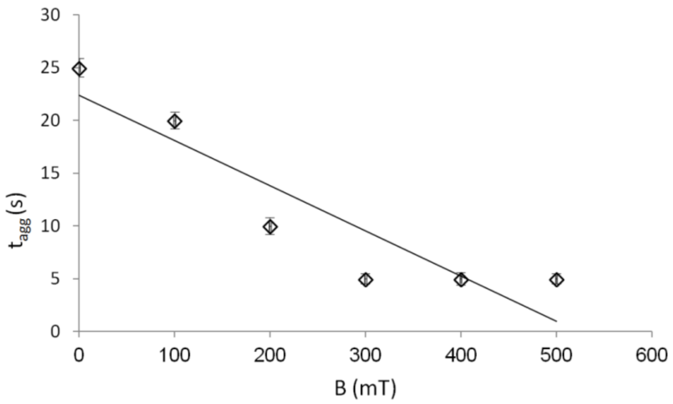

Figure 5. The rate of the erythrocytes aggregation formed faster when acted with SMF. The solid line was the linear regression (R^2 = 0.84).

As the intensity of SMF increased, Amp decreased as shown in **Figure 3**. This can be explained as the SMF speeded up the aggregation rate with limit in aggregation size. Strong negative correlation (R^2=0.76) between Amp and B was obtained in this study.

Haik, Y.V. et al. studied the effect of magnetic field on human blood viscosity. Experimental results of their study showed that magnetic field of 10 T could decrease the flow rate by 30 % in comparison with the blood flow rate under the gravity. Haik, Y.V. et al. concluded that this decline in blood flow rate is because of the increase of apparent viscosity of the blood [24]. Xu et al. assessed the effect of SMF on the blood velocity. In their experiment done on mice, they found that exposure to 10 mT (50 Hz) for 10 min could increase blood velocity by a 20-45%. They showed that different microcirculatory effects were possible because of the interaction between blood and magnetic field [25]. Gmitrov et al. found that SMF exposure (0.25 T, 40 min exposure) led to a 20-40% increase in microcirculation [26-28]. Tanimoto, Y. and Y. Kakuda found the sedimentation rate of blood was changed by 10-20% by application of the magnetic field. This result was interpreted regarding the magnetic force acting on the cells [29]. No contradictory between the results was got and plotted in **Figure 3** and **Figure 4**. This points out the possibility that SMF could increase the rate of formation of erythrocytes aggregation but with small size. The present study showed an inverse relationship between AUC and B. AUC measured the size of erythrocytes aggregation. The correlation between AUC and B was strong (R^2 = 0.74). This can be explained by the magnetic field influences the iron content of the erythrocytes and causes the erythrocytes to spin. Spinning the erythrocytes can lead to collisions between the erythrocytes, therefore, increase the chance to form an aggregation. However, this action contributes to the formation of small aggregates rather than big ones. Also **Figure 5** showed the dependency of t_{agg} on the intensity of SMF. t_{agg} and B was strongly correlated (R^2 = 0.84), and inverse proportionality between them was indicated.

Conclusion

Various physiological alterations can be produced in the living cells because of the exposure to the electromagnetic field. Changing in transmembrane potential and surface charge are examples of these alterations. Living cells in the electromagnetic field attract one another because of an increase in dipole-dipole interaction energy. This variation of the interaction energy between the living cells such as erythrocytes used in this study can arise from both internal and external factors. As pointed out in this study, SMF is one of the external factors can alter the aggregation of the erythrocytes.

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