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Jinwon Mok, Seonghyun Han, and Hyunsook Lee

AFFILIATIONS
Department of Oriental Biomedical Engineering, Sangji University, Wonju 26339, South Korea

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Corresponding Author: Hyunsook Lee, hslee@sangji.ac.kr

ABSTRACT

It is widely known that abnormal peripheral blood circulation due to intravascular stenosis causes ischemic diseases and blood circulatory disorders. The purpose of this study was to investigate the influence of pulsed magnetic field (PMF) on hemodynamic characteristics in blood vessel with stenosis of 33% with diameter of 15 μm, with the change of PMF intensity and hematocrit (Ht) concentration. Stenosed microvascular channels were fabricated using polydimethylsiloxane (PDMS). Our PMF stimulator has the maximum intensity of 2700G at a transition time of 102 μs with pulse intervals of 1Hz. For the Ht altered RBCs suspension, the changes in blood flow and deformation of red blood cells (RBCs) were examined before and after PMF stimulus with various intensity of 200 ∼ 2700G. In stenosed channel, threshold intensity for improvement of blood flow seems to be 400G. The higher the Ht, the higher the viscosity and the slower the velocity of RBCs, but after PMF stimulus, RBCs movement overall increased by more than 9 ∼ 67% in both Ht 5% and 20% RBCs suspension. Our study shows that PMF plays an important role in treating cardiovascular diseases with blood circulation disorders caused by narrowing microvascular due to stenosis. In order to extend our results to clinical applications, we need develop more indicators for hemorheologic characteristics such as viscosity and resistance, and need further experiment with diverse PMF condition such as pulse shape, pulse duration, or repetition rate.

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I. INTRODUCTION

The capillary, a small vessel from 5 to 10μm in diameter, performs an important function by the blood circulation allowing to exchange oxygen and carbon dioxide with surrounding tissues. Peripheral artery disease, blood clots, diabetes, Raynaud’s disease and so on may lead to poor circulation. Particularly, atherosclerosis and coronary artery disease cause severe disorders of circulatory system, since the plaque buildup in arteries has caused the arteries to narrow and harden. Also blood clots can further block the arteries. It is reported that various cardiovascular diseases such as heart attack, arrhythmias, hypertension, angina, and thrombosis are highly correlated to the red blood cells (RBCs) aggregation.

Many studies for protection and clinical treatment against blood circulation disorders have been conducted in order to prevent cardiovascular-related diseases, the most common mortality from diseases. Narrowing and stiffening of the blood vessels cause stenosis, so it is necessary to study the hemodynamic changes such as blood flow and velocity characteristics in the stenosis. Simon assessed the rate of blood flow and a drop in pressure through a stenotic orifice: For high conductance of the peripheral vascular bed, the stenosis becomes the limiting factor and the stream is no longer laminar. Ku also reported that high shear stress in turbulent flow due to the stenosis might activate platelets and thereby induce thrombosis.

Among various non-invasive methods to improve blood circulation, many active researches were recently done using pulsed magnetic field (PMF) therapy. According to Graak et al., low power, low frequency pulsed electromagnetic field treatment has the potential to modulate neuropathic pain and nerve impulse,
thereby effective to the patients with diabetic polyneuropathy. And Shupak et al. reported that PMF therapy has been frequently used for the patients with chronic pain in order to relieve pain. When human umbilical artery smooth muscle cells were exposed to static magnetic field (SMT) of 5 mT, the proliferation, migration, and adhesion potential of cells were significantly decreased. This means SMT also plays a role in preventing cardiovascular disease.

Preliminary researches in our group have observed that stacked RBCs were disaggregated, their movements were fast, and blood flow in nailfold capillary vessel increased under strong PMF. It is also observed the blood sedimentation rate improved in the PMF stimulus. Since exposure to strong electromagnetic fields has long been controversial in the safety and animal testing, it is necessary to know the optimal stimulus intensity for blood circulation. In our previous study, RBCs mobility in straight microvascular channels increased in the intensity of 1000 and 2700G. It needs to expand our finding to the stenosed microvascular channels as well as to various PMF intensities.

Anemia hypoxia, ischemic heart disease, erythrocytosis, etc. are classified according to hematocrit (Ht), the volume ratio of RBCs in the blood. It is also important to know the effect of PMF stimulus on these diseases. As above mentioned, the formation of a stenosis may cause blood circulation disorders, but the studies on microstenosis are still insufficient. It is necessary to understand completely the relationship between blood flow and symptoms for cardiovascular stenosis. Hence, the purpose of this study was to determine if PMF stimulus would have a significant effect on the rate of blood flow in stenosed microvascular model with the change of PMF intensity and Ht %. Stenosed microvascular channels that mimic the in vivo hemodynamic environment were fabricated using polydimethylsiloxane (PDMS).

The changes in blood flow and deformation of RBCs were examined before and after PMF stimulus. These hemodynamic information on RBC passing through the stenotic region might provide the treatment of blood circulatory disorders. Since Zeta potential decreases due to the protein concentration in plasma, which causes RBCs aggregation and rouleaux, Ht concentration might be very important factor in blood viscosity. Therefore, it is meaningful to observe the effect of PMF intensity on the different concentrations of Ht. Another crucial factor of blood viscosity is RBC deformability. Accordingly, our study shows that PMF plays an important role in hemorheologic and hemodynamic properties, through understanding the relationship between RBCs movement and deformability according to PMF intensity and Ht concentration.

II. EXPERIMENTAL METHODS

Microchannel with stenosis of 33% with diameter of 15μm was designed using autoCAD (Fig. 1(b)). Straight microchannel and stenosed microchannel was fabricated by means of MEMS (Micro-Electro Mechanical Systems) process and PDMS. Syringe Pump (NE-300) was used to inflow blood constantly into the channel. Flow rate was set up to 1 μl/min. The thickness and height of the channel were measured using a scanner (RH-2000 NPS Co., Ltd. Hirox Korea).

Healthy human blood of 6 ml was periodically obtained from Kangwon Blood bank of Korean Red Cross after reviewing IRB exemption in Sangji University Bioethics Committee. In order to extract RBCs only, centrifugation (3000 rpm, 10 min) was performed twice to remove plasma and leukocytes from whole blood. RBCs suspension of Ht of 5% and 20% using phosphate buffer saline (PBS) and plasma was prepared to see the effect of PMF according to Ht %. Since PBS is protein-free buffer and prevents RBC agglomerating, and plasma is in an aqueous solution of organic molecules, proteins and salts, non-Newtonian hemorheologic factors such as viscosity and deformability could be compared with each other in the RBCs suspended in the PBS and plasma.

![Artificial micro-channels using PDMS](a) straight microchannel with diameter of 15 μm (b) microchannel pattern with stenosis of 33%, narrowing from 15 μm to 10 μm. (c) Schematic diagram of our PMF device and time-varying pulsed wave, which has maximum peak magnetic field intensity of 2700 G, 5 mm away from coil. The magnetic field pulse duration was 306 μs, including 3 micro-pulses, and pulse repetition rate was 1 Hz.
In order to obtain RBC flow rate, the time interval and moving distance between images of one selected RBC were measured, respectively, from continuously captured microscopic images in one selected micro-channel. It was investigated before and after PMF stimulus with the intensity of 200G ~ 2700G. In order to observe RBC deformability (RBCD), RBCs suspension of 1% Ht using PBS was filtered using pluriStrainer (pluriSelect: Germany) having a pore size of 5μm, and counted the numbers of RBCs which passed through the filter. The passing time was measured exactly for 1min. and then the passed RBCs were counted using Hemocytometer.

Our PMF stimulator system consisted of the single-layered coil of 10 turns with an elliptical shape of 12 cm × 4.5 cm. The maximum intensity is 2700G, 5 mm away from coil and the pulse duration was 102μs, including 3 micro-pulses and pulse repetition rate was 1 Hz. Since the waveforms of generated PMF show rapidly decayed sinusoidal signal, averaging magnetic fields over a period were calculated as 0.058 Gauss (Fig. 1(c)). Therefore, our PMF stimulator would deliver approximately 800 mW/cm² at the skin surface. PMF stimulus was applied to the sample for 3 min. in all experiments. Since it is valuable to obtain the threshold magnetic field intensity, which is harmless and safe for the human body, in this study, the improvement of RBCs flow in the stenosed microchannel was monitored by changing the intensity of the magnetic field from 2700 G to 200G.

**III. RESULTS AND DISCUSSION**

The rate of RBCs velocity in various intensity of PMF stimulus inside the stenosed channel and straight channel were observed in order to find the optimal peak intensity of PMF for the improvement of RBCs flow, with RBCs suspension of 5% Ht using PBS. (Fig. 2) The rate of RBCs velocity means the ratio of RBCs velocities under PMF stimulus and non-PMF condition. In straight channel, the flow of RBCs exposed to peak intensity of PMF in the range of 200G to 2700G was improved, but in stenosed channel, threshold intensity for improvement of blood flow seems to be 400G, because the rate of RBCs velocity was rather reduced to ~46% in the 200G. This means that low intensity of 200G is not sufficient to improve blood circulation in stenosed microchannel. In stenosed channel narrowing 15 to 10μm in diameter, RBCs in the form of biconcave disks about 5μm in diameter, have space limitation in the presence of stenosis as well as lower deformability of RBCs in smaller blood vessels.

RBCs with reduced deformability increase blood viscosity and in result the RBCs impair the proper systematic perfusion. Using 2ml RBCs of Ht 1% in PBS with cell strainer of 5μm in pore size, RBCs deformability was measured and compared the results under PMF stimulus and non-PMF condition. Fig. 3 showed the numbers of passed RBCs through filter after PMF exposure of the intensity of 200G to 2700G. RBCs not exposed to PMF stimulus are defined as control group, to determine whether RBCs deformability is effective after PMF stimulus. The passed numbers of RBCs exposed to low intensity of 200G were rather reduced, compared with control group. Even though it is not clear to figure out the lack of PMF effect on RBCs deformability at 200G, unlike the higher intensities of 400 to 2700 G, these results are in agreement with those of stenosis channel in Fig. 2. It need further experiment to identify the effect of PMF with lower peak intensity on the deformability of RBCs in the microchannel.

Fig. 4 shows RBCs velocity in RBCs suspension with Ht concentration of 5% and 20% using plasma, and 5% using PBS inside the stenosis channel, exposed to selected peak intensities of PMF. As expected, the velocity of RBCs with Ht 5% suspended in PBS increased as the increasing magnetic field intensity compared to the control group. Meanwhile, the velocity of RBCs with Ht 5% suspended in plasma was significantly lower than that of Ht 5% RBCs in PBS. This means that the proteins in the plasma, such as fibrinogen and albumin, have prevented blood flow and increased viscosity in stenosed microchannel. However, overall RBCs velocities increased
after PMF stimulus, compared to the control group. Also the velocity in RBCs suspension of Ht 20% using plasma was much lower than that of Ht 5% RBCs in plasma, due to the high viscosity caused by the shear stress in the microchannels.

IV. CONCLUSION

This present study has tried to investigate the influence of PMF on hemodynamic characteristics in blood vessel with stenosis of 33% with diameter of 15 μm. The threshold intensities of PMF stimulus for the improvement of blood circulation was found by observing RBCs flow rate, which were 200 G in straight channel and 400 G in the stenosed channel, respectively. In addition, RBCs deformability experiment using cell strainer further confirmed the optimal PMF intensity for improving the blood flow in the capillaries and aggregation of RBCs. High instantaneous flux change rate from $16 \times 10^6$ to $106 \times 10^6$ G/s in our PMF system affects the negative charges generated around RBCs, thus releasing the aggregation and rouleaux, and RBCs velocity increased.

Since alterations of Ht significantly contribute to hemorheological variations in diseases and in certain extreme physiological conditions, the effect of magnetic field strength on blood according to Ht concentration was studied. In Ht 5% RBCs suspension, increased plasma compared to in Ht 20% causes RBCs aggregation to deteriorate the blood flow, but in fact, due to high shear rate in the microchannel, the aggregation rate was released. Hence, the effect of PMF stimulus on the velocity of RBCs was observed in the stenosed microchannel. In addition, the change of Ht from 5 to 20% increases blood viscosity, thereby decreasing RBCs velocity, but overall increased by more than 9-67% after PMF stimulus. Our results have shown that PMF stimulus might have a positive effect in treating cardiovascular diseases with blood circulation disorders caused by narrowing microvascular due to stenosis. In order to examine the effect of PMF in the human body, hemorheological characteristics such as blood viscosity and RBCs deformability as well as blood flow should be considered.

For further clinical application for blood circulation, it is necessary to be done with various pulse shape of magnetic field, exposure time and frequency, as well as diverse-shaped stenosed microvascular channels that mimic the in vivo hemodynamic environment.

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