IN-VITRO CHARACTERIZATION OF THE THROMBOTIC POTENTIAL OF WHOLE BLOOD USING AN IMPEDANCE METHOD

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Abstract

This study presents an impedance method of in-vitro characterization of the thrombotic potential of whole blood. Whole blood samples of 0.2 cc were put into a micro-cell with embedded three electrodes immediately after venepuncture at 37°C. Anti-coagulated blood samples were also collected for hematocrit and blood viscosity analyses. The rate of change of electron flow was measured, which indicates the inverse of the thrombotic potential. A sudden decrease in the rate of change of electron flow was observed at a time equal to approximately 110 seconds. This sudden decrease was significantly delayed in anti-coagulated samples. After the sudden decrease, the rate continued to decrease, reaching a minimum value in unadulterated samples while the change in the rate in the anti-coagulated ones was found rather moderate. Based on these preliminary findings, the present method may be of used as a new tool for the diagnosis of the thrombotic potential of whole blood.

INTRODUCTION

Blood coagulation is an essential biochemical process of hemostasis which refers to the complex process of forming clots to prevent excessive bleeding and to repair damaged blood vessels. However, the involvement of this process in the pathophysiological development of thrombosis is also beyond dispute [9]. Under pathophysiological conditions, blood coagulation can lead to increased hemorrhage or excessive thrombosis. Clinically, the most frequent cause of death is the development of thromboembolism, such as coronary thrombosis, peripheral deep venous thrombolism, and pulmonary embolus [5]. Adequate knowledge and understanding of coagulation mechanism is essential for an effective detection and management of these disorders. In relation with this, several blood analyzers have been developed to facilitate the detection of various diseases related to blood coagulation deficiencies and to monitor the rational use and effects of drugs such as anticoagulants. These devices were

theoretically based on the principles of mechanical impedance, photometry, and electromagnetics [10]. Yet, these devices simply give the endpoint of every coagulation test and the final output is the clotting time. Although hemostatic problems can be detected by measurement of the clotting time, yet an assay that will give the thombotic potential and extent of coagulation of the blood samples are equally important.

This study presents an impedance method of characterizing the thrombotic potential of whole blood and determining the extent of this potential under static condition. In the absence of specialized transducers, the response time is mainly governed by the clotting event.

MEASUREMENT PRINCIPLE

Human blood is a suspension of red cells, white cells, and platelets in plasma, an electrolyte containing a myriad of other dissolved and suspended substances, and its resistance to current flow is dependent on the percentage of the suspended cells and interaction of these cells with the suspending media [2]. Information about an electrochemical system is gained by applying an electrical perturbation to it and observing the resulting changes of its characteristics [1]. The present system utilized the potentiostat method of perturbation in which a potential step of known magnitude is applied and the behavior of the current response is systematically kept tracked. Inherent in this method is its exponentially decaying current with a time constant. This principle is accomplished in the present system by applying a short duration pulses every ~4 or 5 seconds upon contact of the electrodes with the blood samples. The maximum peak voltages from each pulse are monitored and recorded over 30 – 40 minutes period as illustrated in Fig.1. As the blood samples coagulate, the flow of electrons correspondingly decreases. Thus, the behaviors and changes of electrons flow signify the thrombotic tendency of the blood.

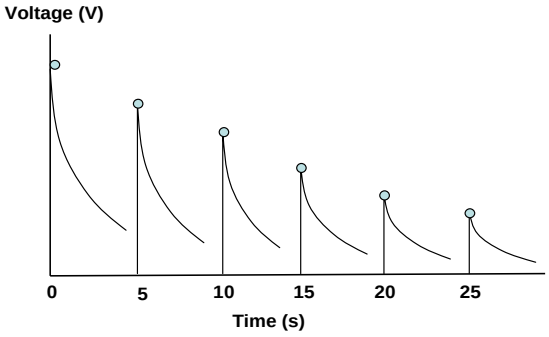

Figure 1- Sample of thrombotic potential measurement using the decay of electron flow after each pulse

EXPERIMENTAL SET-UP

Figure 2 shows the block diagram of the entire experimental set-up. It was composed mainly of impedance cell (EC), high speed and stability potentiostat (BioMechatron,

Jeonju, Jeonbuk, Korea), LabView (National Instrument) Data Acquisition System (DAS), and PC. Within the potentiostat were the impedance system, signal generator, and data processing system. The impedance cell was connected directly to the impedance system by copper connectors. During experimentation, the impedance cell was inserted in thermostat block to maintain the temperature of the samples at 37°C.

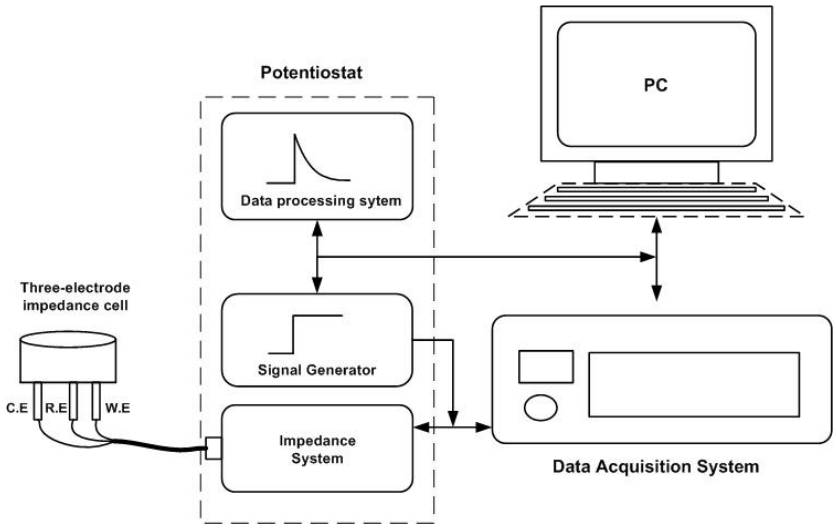

Figure 2 - Block diagram of the impedance measurement system

The body of the impedance cell was made in polychlorofluoroethylene (KEL-F) which had an active surface of 10 mm in diameter. This material had excellent electrical properties suitable for this application. Embedded in it were the three electrodes: counter electrode (C.E), reference electrode (R.E) and working electrode (W.E). The three electrodes were all made of gold.

An important part of the impedance system was the stable and fast rising potentiostat which had a slew rate of 1 GV/s. It performed two major tasks. Firstly, it measured the potential difference between a working electrode and a reference electrode without polarizing the reference electrode, and compared the potential difference with a preset voltage. Secondly, it injected current flowing from a counter electrode to a working electrode, in order to counteract the difference between the preset potential and the existing working electrode. After completing the two tasks, the output of the potentiostat was the current flowing from a counter electrode to a working electrode.

EXPERIMENTAL METHODS

Informed consents were obtained from 4 healthy volunteers, 2 female and 2 male. Personal data were gathered from each volunteer. Prior to the start of the analysis, needed parameters such as step voltage, sampling time, and sampling rate were inputted in the control panel, and the impedance system was stabilized. Upon stabilization, using minimal tourniquet, venous blood of 2 cc was collected from each volunteer. Immediately after venepuncture, 0.2 cc was put into the impedance cell through a 3 cc plastic syringe. When the three-electrode cell made contact with a pool of blood, the EC system was run for 30 minutes. Separately, whole blood samples were also collected

into 3 cc Vacutainers (BD, Franklin Lakes, NJ, U.S.A) containing 5.4 mg K2 ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. The anti-coagulated samples were analyzed using the same procedure as with non-anticoagulated samples. A portion of these samples were used to measure hematocrit and viscosity. Hematocrit measurements were performed within 2 hrs after blood collection using the microhematocrit method while viscosity measurements of each blood sample were measured using a rotating viscometer (Brookfield Co. DV-III Rheometer) at normal body temperature, 37°C. Shear rate was set at 0.1 s^{-1} to resemble the low flow or near static condition.

RESULTS AND DISCUSSIONS

The variation of voltage with time for unadulterated and anticoagulated samples for both male and female volunteers were shown in Fig. 3. Apparently, the degree of decay, as well as the initial voltage, was considerable in anticoagulated samples than the unadulterated samples. It was also evident that the female voltage profiles had higher initial values than those of the male. This difference was due to the lower hematocrit values of female blood compared to male blood as shown in Table 1. Okada and Schwan [7] reported that the resistivity of blood samples is dependent on the percentage of cells suspended in plasma. Yet, it is intriguing to observe that female blood voltage profiles decay faster than male blood that implies its greater thrombotic tendency as shown in the dV/dt curves. Several studies had investigated the sex-related contribution of the rheological properties of blood and its implication to its thrombotic tendency. Sagesaka [8] suggested that male bloods were prone to thrombosis formation due to the significant influence of hematocrit and viscosity on hemostasis. However, other studies mentioned that among the risk factors of thrombosis formation is fibrinogen level [4]. Lowe [6] pointed out that increased concentration of fibrinogen and factor VII in women consequently contributes to greater tendency of thrombosis.

In order to neglect the offset effects and to rationally compare the results, the instantaneous slope of the smoothed voltage/time curve was calculated as shown in Fig.4. The dV/dt curves signified the rate of change of electron flow. The coagulating process offered resistance to the flow of electrons across the electrodes. As the samples coagulate, electron flow was retarded. The generated curves had three distinct identical parts: initial, rapid downtrend, and highly variable. The initial part begun at time 0 and had an almost constant trend that culminated at the start of the rapid downtrend part. This second part was characterized by very steep downward slope. This slope was considerably higher in unadulterated samples than the anti-coagulated ones. An important feature at the onset of this part was the occurrence of a sudden drop in the dV/dt curves that was observed at a time equal to approximately 110 seconds. This sudden decrease was significantly delayed in anti-coagulated samples. These features have invaluable information on the thrombotic potential of blood. The authors hypothesized this trend as good characterization of the anti-coagulant effect. In intrinsic pathway of hemostatic initiation, calcium ions are required for promotion or acceleration of the hemostatic reactions [3]. However, this action was reduced due to the strong and irreversible bonding of EDTA to calcium ions. The last part was made up of highly variable trends and the variations were unique for each sample. Moreover, this

part was not clearly defined in coagulated samples. Yet, the authors believed that the highly variable part was equally important. It was hypothesized that this variation in electron flow implied meaningful information about the end condition of the formed thrombus. This could be clearly observed from the variation between anti-coagulated and adulterated samples. In anti-coagulated samples, blood cells only formed aggregates affected by certain plasma components with high molecular weights and changes of the blood cell structure but were not capable to coagulate due to the retarding action of EDTA as discussed above. These aggregates also increased the impedance of blood but not comparable to the impedance offered by the coagulated blood resulting to intense erratic behavior of electron flow in adulterated samples.

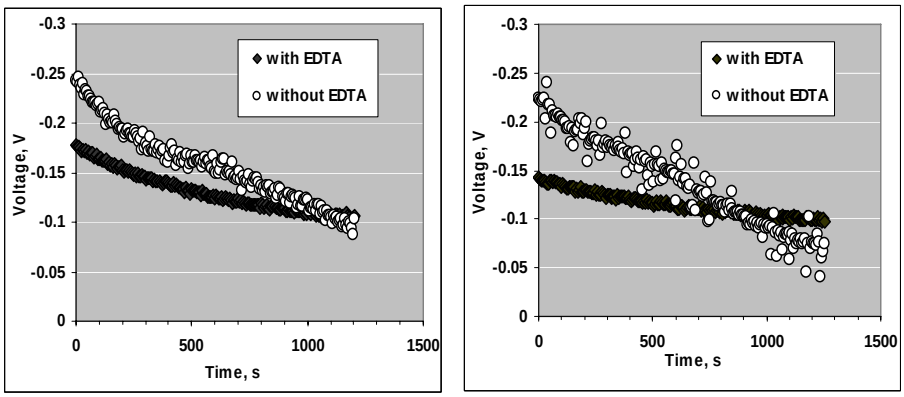

Figure 3 - Response voltage profiles of whole blood samples with and without EDTA for (a) female and (b) male

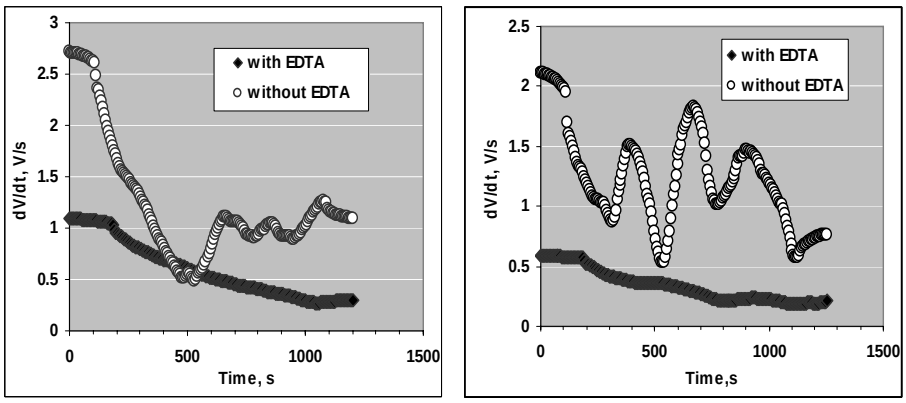

Figure 4 - Dependence of rate of change of electron flow with time of blood samples with and without EDTA for (a) female blood sample and (b) male blood sample.

Table 1 – Mean volunteer characteristics.

Sex	Age (yr)	Viscosit (cP)	Hematocrit (%)
Female	24±2	18±6	39.5±1.5
Male	25±1	23.5±11	49±1

The present study had inherent limitations as well. Other related parameters should have been determined and more samples should have been gathered to further investigate the current results to other factors.Yet, the consistency of the trend followed in accordance with previous studies give impetus for further future study.

CONCLUSION

Based on these preliminary findings, the present impedance method may be used as a new tool for the diagnosis of the thrombotic potential of whole blood. The distinct part of the dV/dt curve would give invaluable information about the process of coagulation and would give practical approach in characterizing the thrombotic potential of blood. Moreover, the method was sensitive to anti-coagulant effect which would be useful in the management and monitoring of anti-coagulant therapy for thrombotic related diseases.

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