미세유동 채널에서의 적혈구 응집성의 동적 특성 측정

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Dynamic measurement of Erythrocyte Aggregation using Microfluidics

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1. Introduction

The aggregation characteristics of red blood cells (RBCs) play an important role in microvascular flow system and increased RBC aggregation has been observed in various pathological diseases, such as thrombosis and myocardial infarction. This paper presents a novel methodfor evaluating the aggregability of RBCs by directly measuring aggregating force (critical shear stress) to overcome shearing force due to flow in a microchannel. This methodfunctionality is based on a microfluidic slit rheometry with decreasing pressure-driven flow mechanism with laser light backscattering technique. The present RBC aggregation index shows a higher sensitivity to small changes of aggregation modulated by amount of fibrinogen in blood plasma. The direct dynamic measurement of RBC aggregating force represents a new concept for the field of blood rheology and should prove beneficial for basic science and clinical applications.

2.Materials and Method

Blood was obtained from healthy volunteers who provided informed consent. Venous blood samples were drawn from the antecubital vein and collected into Vacutainers (6ml, BD, Franklin Lakes, NJ, USA) that contained anticoagulant, (K2)EDTA. After that, a series of process were conducted to vary the amount of fibrinogen in blood plasma. We prepared

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four different RBC suspensions (hematocrit: 45%) with varying Fbc (fibrinogen concentration: 0, 50, 200, and 400 mg/dL) in serum.

Plastic microfluidic chips were fabricated by Sewon Meditech., Inc (Korea). The microfluidic chip consists of the microchannel, a sample reservoir and a waste sample reservoir. The channel was 200μm deep, 4mm wide and 40mm long. This single channel chip loads the sample volume of 200μl.

The basic apparatus consists of the microchip, vacuum generation unit, laser-light scattering detection system, pressure transducer, and data acquisition system as shown in Fig.1. With the vacuum generation unit, the blood sample in the sample reservoir flows through the micro-channel. A precision differential pressure transducer is employed to measure the pressure differential variation between two chambers with respect to time, $\Delta(t)$, from which average shear stress were calculated mathematically.

The laser-light scattering detection system consists of a diode laser (637 nm, 1.5mW, Lanics, Seoul, Korea) and two photodiodes (FDS1010, ThorLabs, Newton, NJ,USA). The laser beam was perpendicularly directed into the center of the microchannel. The beam diameter was narrower than the channel width of 4mm to avoid scattering from the channel walls. Two photodiodes were mounted along the microchannel centered at the laser beam. The setup was interfaced with a PC and a data acquisition card (PCI-MIO-16E-4, NI, USA). Data was collected over a period of 250sand

processed with software based on Labview.

Typical tests were conducted as follows: the sample chamber is filled with the sample and sealed with a rubber cap. At time t=0, the valve between the vacuum generating system and the waste chamber is opened, allowing the sample to flow through the microchannel and to be collected in the waste chamber as driven by the differential pressure. The fluid stops flowing while the differential pressure reaches equilibrium. Whenthe fluid is flowing through the microchannel, the back-scattered light intensity and the differential pressure are measured every 0.1s simultaneously. The pressure measurement over time can determine shear stress, whereas the light intensity-time curve, called syllectogram1, determines the aggregation indexes. Thus, the present method can provide the dynamic characteristics of erythrocyte aggregation over a range of shear stresses.
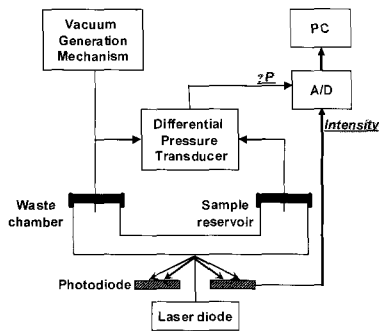

Fig. 1   Schematic diagram of the apparatus

A detailed description of the stress-pressure relation can be found in our previous studies2. Wall shear stress can be written as

$$\tau_w(t) = \frac{\Delta P(t) H}{2\left(1 + H/W\right)L} \qquad (1)$$

Thus, it is worth noting that the optical measurement of anisotropy in the laser-backscattering reflects the RBCs aggregated at all depths in the pressure-driven slit flow. Since RBC aggregates experience shear levels from zero up to the wall shear stress, average shear stress was adopt throughout the present study to represent shear flow characteristics in the microchannel.

3. Results

Due to the characteristics of the decreasing shear flow, the test proceeds from high to low shear stress over the course of the experiment. During the high shear process, the backscattered light intensity increases since the progressive break-up of the cellular aggregates occurs, then, the intensity tends to decrease rapidly due to re-aggregation of RBCs below a shear stress level, which is defined as a critical shear stress (CST). CST can be easily determined as corresponding shear stress at peak point of intensity. The corresponding inflexion pointcan be described as the aggregation time (AT), which indicates the length of time that would take for a blood sample to re-aggregate during a decreasing shear flow.

In this study, CST and AT showed a strong dependence of fibrinogen concentration. CST value increases with increase of fibrinogen concentration as shown in Fig.2. CST range from 0.2 ~ 0.9Pa for 0 ~ 400mg/dL fibrinogen concentrations, while the present CST measurement precision is 0.01Pa. At low concentrations (i.e., 0 and 50mg/dL), CST measurements do not show apparentdifference. However, as fibrinogen increases (200 and 400 mg/dL), CST measurements show distinct differences compared to 50 mg/dL (203% and 353% increased, respectively). These significant differences at high fibrinogen concentrations provide good capability to detect RBC aggregability for hyper-aggregated blood sample, which may frequently obtained from diabetic or thrombotic patients.

In contrast to CST, AT value decreases with decrease of fibrinogen concentration as shown in Fig.3. The higher concentration of fibrinogen in serum, the less time it takesto reaggregate RBCs in flowing condition. In addition, the higher concentration of fibrinogen shows rapid decrease of light intensity than the lower one. This fact implies
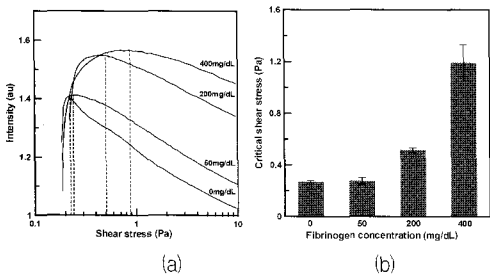
(a)   (b)

Fig. 2 Comparison of CST at varying Fbc
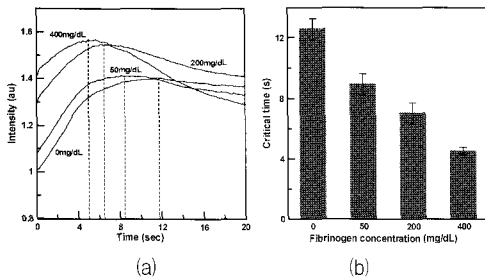

(a)   (b)

Fig. 3 Comparison of AT at varying Fbc

faster RBC aggregation process and higher concentration of fibrinogen.

4.Conclusion

The present study provides a conceptually new method to evaluate dynamic RBC aggregability by directly measuring the aggregating force in decreasing shear flow. Using present method, RBC aggregability can be evaluated within a short period of time. Furthermore, present study introduce two new aggregation index to determine RBC aggregability by direct measurement of AT and CST, in which RBC overcome disaggregating shear force and start to aggregate.

Reference

(1)   Hardeman, M.R., Goedhart, P.T., Dobbe, J.G.G. and Lettinga, K.P., 1994, "Laser-assisted optical rotational cell analyzer(L.O.R.C.A). A new instrument for measurementof various structural hemorheological parameters", Clin. Hemorheology, Vol. 14, pp. 605-618.

(2)   Shin, S., Park, M.S., Jang, J.H., Ku, Y.H. and Suh, J.S., 2004, "Measurement of red blood cell aggregation by analysis of light transmission in a pressure-driven slit flow system", Korea-Austrailia Rheology Journal, Vol. 16, pp. 129.