Measurements of RBC deformability and its effect on blood viscosity

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적혈구 변형성의 측정과 혈액 점도와의 상관관계 연구

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Abstract

A slit-flow apparatus with laser diffraction method has been developed with significant advances in ektacytometry design, operation and data analysis. In the slit-flow ektacytometry (or laser-diffractometry), the deformation of red blood cells subjected to continuously decreasing shear stress in slit flow is measured. A laser beam traverses a diluted blood suspension flowing through a slit and is diffracted by RBCs in the volume. The diffraction patterns are captured by a CCD-video camera, linked to a frame grabber integrated with a computer, while the differential pressure variation is measured by a pressure transducer. Both measurements of laser-diffraction image and pressure with respect to time enable to determine deformation index and the shear stress. The range of shear stress of 0 ~ 35 Pa and measuring time is less than 2 min. When deforming under decreasing shear stress, RBCs change gradually from the prolate ellipsoid towards a circular biconcave morphology. The Deformation Index (DI) as a measure of RBC deformability is determined from an isointensity curve in the diffraction pattern using an ellipse-fitting program. The advantages of this design are simplicity, i.e., ease of operation and no moving parts, low cost, short operating time, and the disposable kit which is contacted with blood sample.

1. Introduction

The red blood cell, a biconcave disc at rest, readily changes in shape when subjected to shear stress. The ability to undergo large deformations when subjected to stresses allows the red blood cells to pass through capillaries narrower than the resting RBC diameter. This property is also responsible for the surprisingly low viscosity at high shear rates in the large arteries, although the whole blood is consisted of almost 50 volume percent of the blood cells.(1) A slight decrease in red cell deformability may cause important disturbances in the blood circulation of micro-vessels, but also in blood vessels whose lumen is markedly diminished by atherosclerosis or thrombosis. A large variety of diseases have been described in association with less deformable RBCs.(2)

Therefore, various methods for measuring RBC deformability have been developed and can be found elsewhere.(3) A filtration method to pass RBC suspensions through micro pores (3~5 μm) has become popular due to its simplicity. In addition, there have been plural variations in filtration techniques, e.g., positive or negative pressure driving, filters having a different number of pores (several thousands to single pore), and fabricated from various material. Meanwhile, an instrument, the Extacytometer, using laser diffraction analysis of RBCs under varying stress has been developed and is commercially available (LORCA®, R&R Mechatronics, Hoorn, The Nether-lands).(4) The instrument consists of a laser diode, a thermostated bob-cup measuring system, step motor and a video camera attached to a microcomputer. The microcomputer also

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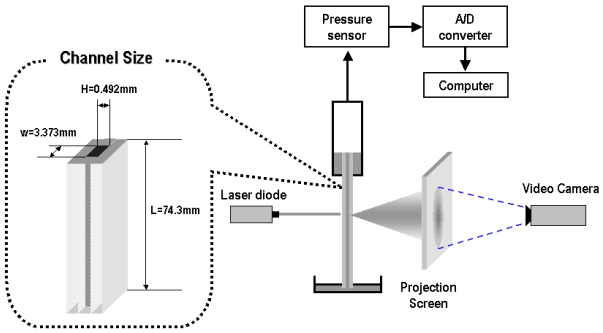

Fig.1 Schematic diagram of the laser-diffraction slit-rheometer(LDSR)

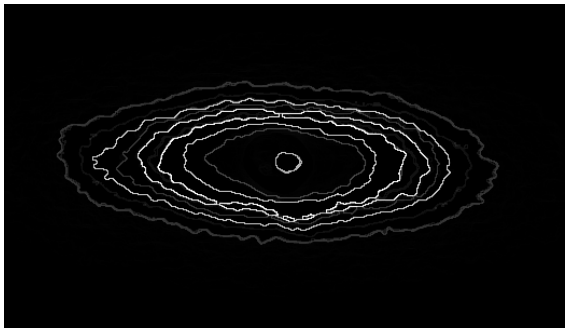

Fig. 2 Isointensity curves in a laser-diffraction pattern

controls the step motor, to generate various shear stress in the sample. The sample is sheared in a Couette system made of glass, with a gap of 0.3 mm between the cylinders. The diffraction pattern is analyzed by the microcomputer and elongation indexes (EI) are calculated for shear stress between 0.5 – 50 Pa.

Although these methods and instruments are able to measure RBC deformability, the most current technology, while useful in a research setting, is not optimal for day-to-day clinical use. Furthermore, most current techniques produce deformability measurements for one shear stress at a time. In order to measure blood viscosity that is shear-dependent, one needs to repeat the measurement over a range of shear stress by varying either the rotating speed or driving pressure, which is a time-consuming process. Therefore, there has been a need to develop a simple and labor-free instrument that can measure the deformability of RBCs over plural shear stresses at a time.

This study describes an innovative approach to slit rheometry that is capable of measuring RBS deformability continuously over a broad range of shear stresses. The flow-rate and pressure-drop measurements that are usually required for the operation of a slit rheometer are replaced with a single measurement of pressure variation with time. Throughout the development of this technique an emphasis has been placed on the simplicity of the design, i.e, ease of operation, no moving parts, and low cost. Furthermore, this study investigates the effects of red blood cell deformability on whole blood viscosity over a wide range shear rates. The deformability of red blood cell measured by laser-diffraction slit rheometer for normal red blood cell suspension and hardened red blood cell suspension. Then it was measured blood viscosity using pressure-sensing capillary viscometer for each suspension with wide range shear rates.

2. Materials and Methods

The basic apparatus, containing the laser, CCD video camera, screen, and pressure driven slit rheometry, is shown in Figure 1. The laboratory setup consisted further of a computer. Details of the pressure-driven slit rheometry consists of a vacuum chamber, connecting needle, collecting chamber, receptacle, pressure transducer and a computer data acquisition system. With vacuum suction, the blood sample flows through the slit made of glass with a gap of 0.49 mm and width of 3.7 mm. The glass slit integrated with a collecting chamber is designed to be disposable. The diode laser (650 nm, 5mW) and a CCD camera (SONY-ES30) combined with a frame grabber were used to obtain a laser-diffraction pattern. The diffraction pattern is analyzed by an ellipse-fitting-program and the elongation indices (EI) are calculated for shear stress between 0 ~ 35 Pa. The length and gap of the slit were chosen to ensure that the friction loss in the slit was the dominant loss in the system.

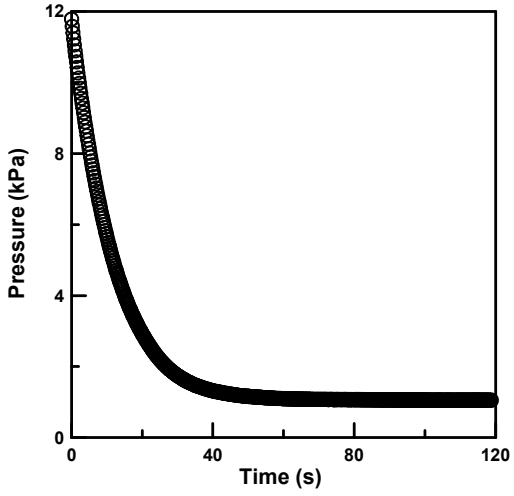

Fig.3 Differential pressure variation versus time for a diluted blood sample

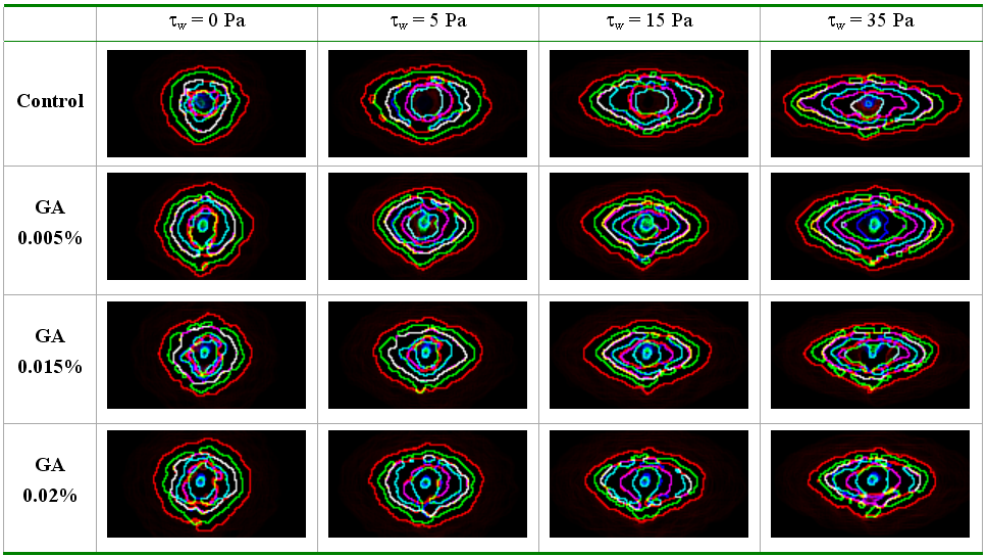

Fig. 4 Laser diffraction patterns at various wall shear stresses for four different blood samples

Prior to the test, the atmospheric pressure (P_A) and the total volume of the vacuum chamber (V_0) are determined. Typical tests are conducted as follows: the system is turned on and connected to a computer. The data acquisition system on the computer is enabled. At time t = 0, the preset vacuum chamber is pushed into the connecting needle so that the preset chamber and the slit is opened, allowing the fluid to flow through the slit and be collected in the vacuum chamber as driven by the differential pressure. When the differential pressure reaches equilibrium with a pressure head, the test fluid stops flowing.

While the blood is flowing through the slit, a laser beam emitting from the laser diode traverses the diluted RBC suspension and is diffracted by RBCs in the volume. The diffraction pattern projected on the screen is captured by a CCD-video camera, linked to a frame grabber integrated with a computer. While the differential pressure is decreasing, RBCs change gradually from the prolate ellipsoid towards a biconcave morphology. The Elongation Index (EI) as a measure of RBC deformability is determined from an isointensity curve in the diffraction pattern using an ellipse-fitting program as shown in Figure 2. It is worthy to note that the diffraction pattern images are oriented perpendicular to the orientation of the elongated cells.

Typically, it took approximately two minutes for a pressure difference to reach an asymptote for blood in the present study. In fact, the time to complete a run should vary depending on types of liquid and dimension of slit. It is worth noting that the initial pressure in the vacuum chamber was chosen to produce the maximum shear stress of approximately 35 Pa. If it is required to expand to higher shear stress, an initial vacuum pressure can easily be lowered.

The essential feature of the present ektacytometer is the use of a disposable slit, which is designed to plug-in and -out to the collecting chamber. The disposable slit can be made of the various materials including glass, silicon, PMMA, etc. Another essential feature of the present ektacytometer is the use of precision pressure transducer to measure the decreasing differential pressure in the collecting chamber, $P(t)$, every 0.1 s with a resolution of 1 Pa. The instantaneous pressure was recorded

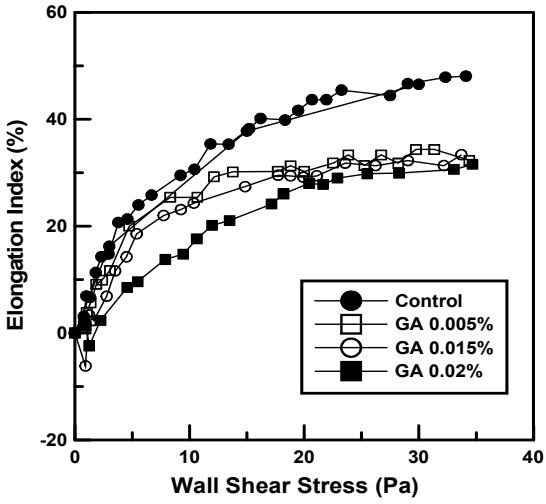

Fig. 5 Elongation Index versus wall shear stress for four different blood samples

in a computer data file through an analog-to-digital data acquisition system (NI PCI-6014) with respect to time. In addition, another essential feature of the present ektacytometer is the use of an optical diffraction to obtain the deformed RBC images over a range of stress at a time.

Figure 3 shows the differential pressure variations over time for the diluted RBC suspension. As time passes, the differential pressure between the collecting chamber and atmosphere decreases since the collecting chamber is filled with the flowing fluid from the slit. Typically, it takes approximately two minutes to reach an asymptote for the diluted RBC suspension.

3. Results and Discussion

The present study measured the RBC deformability with the present slit-flow ektacytometry. In order to vary the deformability of RBCs, the RBCs were exposed in three different concentrations (0.005, 0.015, 0.02%) of glutaraldehyde (GA). It has been known that the higher concentration of GA is used, the less deformable RBC becomes. Figure 4 shows the change in diffraction patterns of RBCs during fluid flowing changing shear stress for different RBCs. As shown in Figure 4, the diffraction patterns of RBCs change gradually from the circular morphology towards a prolate ellipsoid as the shear stress increases. However, as the shear stress increases, there are differences in the shape of ellipsoid. For example, at a high shear stress (τ_w= 35), the diffraction pattern for controlled RBCs shows the largest deformation among them, whereas that for RBC exposed to 0.02% GA shows the smallest deformation. As the concentration of GA increase, the diffraction patterns show less flat ellipsoid. In the above analysis, it is necessary to quantify the deformability in terms of shape of RBC diffraction pattern. Thus, an elongation index *EI* is defined as (X-Y)/(X+Y), where X and Y are the major and minor axes of the ellipse, respectively. Figure 5 shows the Elongation Index (*EI*) as a measure of the RBC deformability along shear stress. The *EI* is calculated from an isointensity curve in the diffraction pattern as shown in Figure 2 using an ellipse-fitting program. The *EI* is continuously obtained over a range of shear stresses (0 ~ 35 Pa). As the wall shear stress increases, the *EI* curves increase and asymptotically approach a plateau value (EI_{max}). As shown in Figure 5, the controlled RBCs show the largest plateau value of *EI* at a high shear stress. The higher concentration of GA is used, the smaller *EI* value shows.

Meanwhile, the effect of GA concentration on *EI* values do not show large difference at high shear stress but at an intermediate shear stress, as shown in Figure 5. Thus, it is required a new parameter to quantify the deformability, which can represent the deformability over a range of shear stress. The present study proposes a new deformability index (*DI*) as an area under the *EI* curve between 0 and τ_{10Pa} (τ_w = 0~10 Pa), which is expected to be important in physiological conditions. The *DI* indicates an integral deformability over a low range of shear stress. Thus, the *DI* may be comprehensive and representative index in a sense to quantify the deformability. Figure 6 shows the deformation indices for four different blood samples. As indicated in Fig. 6, there are apparent differences of *DI* between controlled blood and blood samples with hardened RBCs. In addition, as the concentration of GA increases, the *DI* decreases linearly.

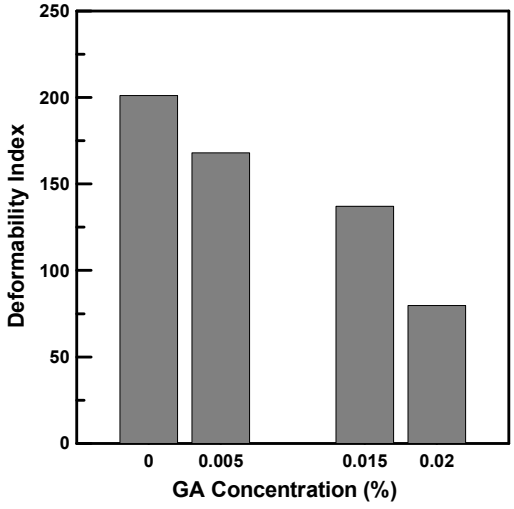

Fig. 6 Deformation index versus concentration of GA

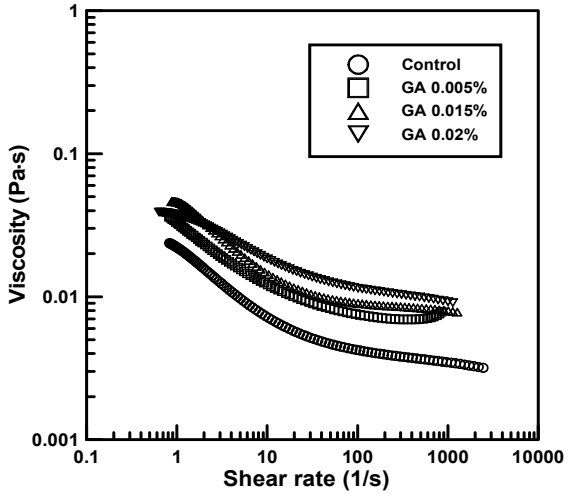

Fig. 7 Viscosity versus shear rates for several RBC suspension

In Figure 7, it shows that with an increase in shear rate, the viscosity decreases for suspensions of normal RBC in control suspension, but not for the hardened RBC relatively. This shear dependent deformation concept also seems relevant to the viscometric behavior Glutaraldehyde treated RBC: increasing Glutaraldehyde level results in RBC whose resting shape is discocytic but whose ability to deform and align is progressively impaired. Thus for such cells with a diminished ability to deform, there would be a smaller decrease of viscosity at high shear.

4. Conclusion

Novel features of the slit-flow laser-diffracting method described here include 1) use of a disposable slit to measure shear stress; 2) decreasing pressure differential in the collecting chamber, resulting in plural shear stress at a test; 3) the use of laser diffraction through the slit for detection. The test time required is approximately less than 1 min. Straightforward combination of the slit rheometry and diffraction optics made it possible to measure the deformability of red blood cells in a clinical setting. Possible applications, in addition to the examples described here, include the test at home and any point of care.

Acknowledgement

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