Measurements of RBC Deformability using Laser-Diffraction Method

Yunhee Ku* · Sehyun Shin**

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 bobcup measuring system, step motor and a video camera attached to a microcomputer. The microcomputer also 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.

2. Materials and Methods

Fig. 1 is a schematic diagram of the laser-diffraction slit-rheometer(LDSR), which consisted of a vacuum chamber, glass slit, receptacle, pressure transducer, laser diode, screen, CCD video camera, and a computer data acquisition system. The blood sample is sheared in the slit channel made of glass with a gap of 0.49 mm and width of 3.7 mm. The glass slit integrated with a vacuum chamber is 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 0.5-35 Pascal (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.

Prior to the viscosity measurement, the atmospheric pressure (P_A) and the total volume of the vacuum chamber (V_0) are determined. Typical tests are conducted as follows: At time t = 0, the data acquisition system is enabled and

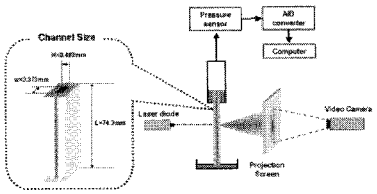

Fig.1 Schematic diagram of the laser-diffraction slit-rheometer (LDSR)

* Kyungpook National Univ., School of Mech. Eng.
** Kyungpook National Univ., School of Mech. Eng.

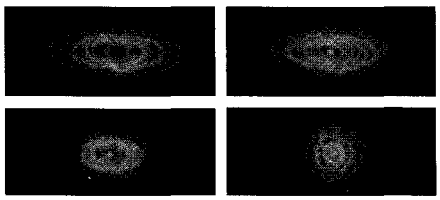

Fig. 2 Laser diffraction patterns at various shear stresses (a) τ_w=31 Pa, (b) τ_w=12 Pa, (c) τ_w=3 Pa, and (d) τ_w=0 Pa.

the valve between the preset vacuum 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 (P_h = 1.05 *kPa*), the test fluid stops flowing.

While the fluid 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 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.

The essential feature of the LDSR was the use of a precision pressure transducer (Validyne DP15TL) to measure the pressure in the vacuum chamber, *P(t)*, every 0.1s with a resolution of 1Pa. The instantaneous pressure was recorded in a computer data file through an analog-to-digital data acquisition system (NI DAS-16) with respect to time. In addition, another essential feature of the LDSR is the use of an optical diffraction to obtain the deformed RBC images under various stresses.

3. Result and Discussion

Fig. 2 shows the change in diffraction patterns during fluid flowing at room temperature. As the shear stress decreases, the RBCs change gradually from a prolate ellipsoid towards the circular morphology. It is worthy to note that the diffraction pattern images are oriented perpendicular to the orientation of the elongated cells.

Fig. 3 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 Fig. 2 using an ellipse-fitting program. The *EI* is defined as (X-Y)/(X+Y), where X and Y are the major and minor axes of the ellipse, respectively. The *EI* is continuously obtained over a range of shear stresses (0~31 Pa). The decrease of shear stress from 31 to 0 *Pa* results initially in a slow decrease of EI until 10 Pa, followed by a rapid decrease. The LDSR is calibrated

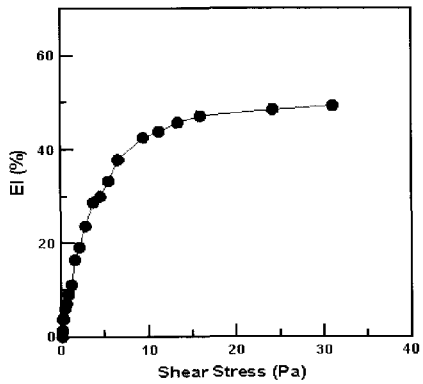

Fig. 3 Elongation index versus wall shear stress

with 5 m diameter polymer microspheres having an EI = 0.

4. Conclusion

Present study introduced a new method of measuring RBC deformability using laser diffraction slit rheometer over a range of shear stresses continuously. Using a precision pressure transducer, one can measure the variation of pressure in the vacuum chamber, P(t), from which the shear stress and shear rate are mathematically calculated. The diffraction patterns are captured by a CCD-video camera, linked to a frame grabber integrated with a computer. The Elongation Index(EI) as a measure of RBC deformability is determined from an isointensity curve in the diffraction pattern sing an ellipse-fitting program. The advantages of the 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.

Acknowledgments

This work was supported by a grant from the National Research Laboratory of the Ministry of Science and Technology, Korea.

References

(1) S. Oka, *Cardiovascular Hemorheology* (Cambridge University Press, 1981).
(2) F. C. Mokken, M. Kedaria, CH. P. Henny, M.R. Hardeman, A.W. Gelb, Ann. Hematol. 64, 113 (1992).
(3) D. Bareford, P.C.W. Stnoe, N.M. Caldwell, H.H. Meiselman, J. Stuart, Clin. Hemorheol. 5, 311 (1985).
(4) M.R. Hardeman, P.T. Goedhart, N. H. Schyt, Clin. Heomrheol. 14, 619 (1994).
(5) S. Shin, D.Y. Keum and Y. H. Ku, KSME Int. J., 16, 1719 (2002).
(6) C. W. Macosko, *Rheology: Principles, Measurements, and Applications* (Wiley, New York, 1994).