Measurement of Red Blood Cell Aggregation by Vibration aided Optical Analysis

Juhee Jang*, Myungsu Park*, Yunhee Ku*, Sehyun Shin**

1. Introduction

Red blood cells (RBCs) in normal human blood tend to form linear and branched aggregates. Such aggregation is frequently referred to as rouleaux formation, which is similar to a stack of coins. Since the RBC aggregation has been known to be a major determinant of the in vitro rheological properties of blood, it continues to be of interest in the field of hemorheology[1-2]. In sepsis[3], diabetic mellitus[4] and myocardial ischaemia[5], increased RBC aggregation is observed but the causes of the diseases are different. The major cause of aggregation is the presence of large plasma-proteins, especially fibrinogen[6].

Various techniques for measuring RBC aggregation have been developed and are described elsewhere[7]. A photometric method to record light intensity has been widely employed to quantify aggregation due to its simplicity. Additionally, aggregometers using phtometric analysis after sudden cessation of shear stress have been developed, which are commercially available such as Erythroaggregometer (Regulest, France), Myrenne aggregometer (Myrenne, Germany), and LORCA (R&R Mechatornics, Netherlands). These commercial instruments employ different geometries for the rotational shearing system such as cone-plate, parallel plates and concentric bob-cup systems. These instruments analyze the syllectogram using a curve-fitting program and determine the aggregation indices such as *AI (aggregation index), half-time ($t_{1/2}$), M-index,* etc.

These rotational shearing systems cause the instruments to be expensive and difficult to design. In addition, they require labor-intensive cleaning after each measurement. Hence, these current techniques, while useful in a research setting, are not optimal for day-to-day clinical use.

Therefore, it is necessary to develop a simple and labor-free instrument that can measure the aggregation index of RBCs with minimal blood sample. The current study describes an innovative approach to a vibration-aided optical biosensor to detect RBC aggregation. The rotational shearing system is replaced with a simple vibration-aided disposable element containing a blood sample. The advantages of this design are its simplicity, low cost, and easy to use.

* Kyungpook Nat'l Univ., School of Mech. Eng.
** Kyungpook Nat'l Univ., School of Mech. Eng.

2. Materials and Methods

The present study developed a backscattered-light sensor and system for detecting RBC aggregation as shown in Fig. 1. The system consists of a disposable test slit with an inlet reservoir, vibration mechanism, laser diode, photodiode, and a computer data acquisition system. The blood sample (8 μl) is placed on the micro-slit with a gap of 0.2 mm, a width of 1.2 mm and a length of 40.0 mm. The slit, which is made of glass, is designed to be disposable. A laser diode (650 nm, 1.5 mW) and a photo-diode are used to obtain laser-reflection intensity. The vibration mechanism consists of a function generator, amplifier, and a speaker. In the present study, the vibrating frequency and amplitude are fixed at 150 Hz and 0.5 mm, respectively. A jig, attached to the speaker diaphragm, is connected to the slit. The detail description can be found elsewhere[8].

The older the cells are, the higher the density red cells become. Thus, through high speed centrifugation (15,000 rpm, 20 min), a density-based separation can be achieved.

Typical tests were conducted as follows: The test fluid (8 μl) is placed on a test slit and sealed with a sealant. Then, the test slit is mechanically mounted onto the jig, which is attached to the speaker diaphragm. For disaggregating the RBC aggregates, the defined vibration is applied for 40 s and is then stopped. Then, the laser beam emitting from the laser diode traverses the blood sample and is backscattered from the blood sample. The backscattered light is detected by the photodiode which is linked to the data acquisition system by a computer. When the vibration stops suddenly, the disaggregated RBCs start to aggregate. The light intensity is recorded over time, which is called

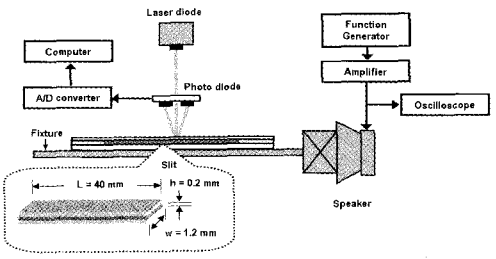

Fig. 1 Schematic diagram of the light-reflection aggregometer with vibrating mechanism

the syllectogram. Indexes of the aggregation as a measure of the RBC aggregation are determined from the syllectogram using a curve-fitting program.

Aggregation indexes are determined from a syllectogram using a curve-fitting program. The measured parameters of the aggregation kinetics are well defined in a previous study[9]: *Amplitude (Amp)* is the difference between the maximum light intensity and the light intensity at 120s, indicating the extent of RBC aggregation. The *Half time* ($t_{1/2}$) is defined as the time required to reach a light intensity of "minimum intensity + 1/2 Amp." The *M-index* is the area under the syllectogram over a 40s time period and the *Aggregation Index (AI)* is the ratio of the area under the syllectogram to the total area over a 10 s time period.

3. Results

The reproducibility of the present apparatus from 10 repeated measurements on the aliquots of the same blood sample. The most reproducibility was found in the Amp index, with a CV of 0.4%. The *M index, half time* and *AI* showed high reproducibility with low CV values (less than 2.56%). Other repeated measurements on different samples yielded a similar pattern of precision for the different aggregation parameters. In addition, Table 1 compares the values for the RBC aggregation indexes measured by the present aggregometer and a commercial aggregometer (LORCA). It was found that the test results provided a good correlation between the two instruments with less than a 5.0 % error rate.

Table 1 shows the effect of cell density on RBC-aggregation by comparing the two aggregation indexes of young and old cells suspended in an autologous plasma. In fact, cell density is directly related with cell age. In other words, the older the cells are, the higher the density red cells become. Thus, through high speed centrifugation (15,000 rpm, 20 min), a density-based separation can be achieved. The *M-index* and the *AI* of the old cell suspension increased significantly, 15.32% and 14.3%, from that of young cell suspension, respectively even though the *Amp* of both suspensions showed a slight difference (1.8%). These results indicate that the old cells have a higher aggregability than the young cells.

4. Conclusion

The present aggregometer is a new tool that can probe RBC aggregability with high resolution and reproducibility. The aggregation indexes measured with the present aggregometer were in close agreement to those obtained with the LORCA aggregometer. The present instrument could also differentiate between high (old)- and low-density (young) cells in one blood sample. With proper technological improvements including automation, measuring the aggregability of several hundred samples per day now seems possible.

Table 1 Aggregation indexes and percentage difference for young and old cells suspended in autologous plasma

Aggregation Indexes	Young cell	Old cell	Percentage Difference
Amp (au)	55.6	56.2	1.80 (%) ↑
$t_{1/2}$ (s)	7.57	5.91	21.9 (%) ↓
M Index	194.2	224	15.3 (%) ↑
AI (%)	35	40	14.3 (%) ↑

Acknowledgments

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

References

(1) H. J.Meiselman, O. K. Baskurt, S. O. Sowemimo-Coker, and R. B. Wenby, 1999, " Cell electrophoresis studies relevant to red blood cell aggregation," Biorheology, pp. 427~432.

(2) J. F. Stoltz, M. Singh, and P. Riha, 1999, "Hemorheology in Practice," IOS Press, Amsterdam.

(3) O. K. Baskurt, A. Temiz and H. J. Meiselman, 1997 , " Red blood cell aggregation in experimental sepsis," J. Lab. Clin. Med., pp.183~190.

(4) R. M. Bauersachs, S. Shaw, A. Zeidler and H. J. Meiselman, 1987, "Hemorheological findings in uncontrolled type □ diabetes mellitus," Clin. Hemorheol., pp. 432.

(5) C. Rainer, D. T. Kawanishi, P. A. N. Chandraratna, R. M. Bauersachs, C. L. Reid, S. H. Rahimtoola and H. J. Meiselman, 1987, "Changes in blood rheology in patients with stable angina pectoris as a results of coronary artery disease," Circulation, pp. 15~20.

(6) Rampling, M.W., 1999, "in Cardiovascular flow modeling and measurement with application to clinical medicine, edited by S.G. Sajidai, G.B. Nash and M.W. Rampling," Clarendon Press, Oxford, pp. 183-193.

(7) Zhao, H., Wang, X. and Stolz, J.F., 1999, "Comparison of three optical methods to study erythrocyte aggregation," Clin. Hemorheol & Microcirc., Vol. 21, pp.297-302.

(8) S. Shin, J.H. Jang, M.S. Park Y.H. Ku, and J.S. Suh, "A light-transmission aggregometer using a vibration-induced disaggregation mechanism," Review of Scientific Instrum. (in press).

(9) M.R. Hardeman, J.G.G. Dobbe and C. Ince, 2001, "The Laser-assisted Optical Rotational Cell Analyzer (LORCA) as red blood cell aggregometer," Clin. Hemorheol., pp. 1~12.