The Clinical Study of Red Blood Cell Deformability for Cardiovascular Disease Patients

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1. Introduction

Red blood cell (RBC) has an ability to undergo large deformations when subjected to stresses, which allows the RBCs to pass through capillaries narrower than resting RBC diameter. 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 diminished markedly by atherosclerosis or thrombosis. Deformability of red blood cell continues to be of hot issue in biophysics and clinical hemorheology. In fact, recent clinical observations reported that reduced RBC deformability is a common risk factor for circulation diseases including diabetes (1), hypertension (2), sickle cell anemia (3) and other circulation disorders.

Various techniques in measuring the deformability of RBCs have been proposed and comparisons regarding their effectiveness can be found elsewhere. Typical techniques can be summarized briefly as follows: (i) RBC filtration: This method has been widely used in measuring RBC deformability due to its similarity and simplicity (4). Other methods such as micropipette aspiration were also used to measure RBC deformability; (ii) Rheoscope (5): It is a direct observation of the shape of RBCs under a given shear stress. This method allows to confirm the tank treading of RBCs in shear flow. (iii) Ektacytometry (6): The principle of this technique uses laser diffraction analysis of the RBCs under varying stress levels. This method has several advantages compared to other techniques, in that it is relatively easy to perform, has acceptable precision, and is able to operate at various shear stresses.

Although there are many methods and instruments for measuring deformability as described above, most of the current techniques including ektacytometry require cleaning after each measurement. In order to measure cell deformability in a clinical setting, one needs to repeat the cleaning after each measurement, which leads to a labor-intensive and time-consuming process. Hence, the current techniques are not optimal for day-to-day clinical use. Therefore, there has been a need to develop a simple and labor-free disposable element which eliminates frequent cleaning.

This study describes a newly developed disposable-slit ektacytometer (Rheoscan-D), which is capable of measuring RBC deformability continuously over a broad range of shear stresses. The principle of the present slit ektacytometer is based on the combination of the laser-diffraction technique and a slit rheometry. Throughout the development of this technique an emphasis has been placed on the simplicity of the measuring process, i.e, designing of a disposable element that holds blood sample, so that the present technique can be applicable to the clinical environment. The technique is finally employed to measure the RBC deformability of a healthy individuals and of cardiovascular disease patients.

2. Materials and Method

Blood was obtained from normal, healthy volunteers (n = 54) who were not on any medications and who provided informed consent (age range 25-60 years and male/female participants). The blood samples used in the experiments were not pooled from more than one individual subject and all analyses were completed within 6 hours after blood collection. The samples of venous blood were drawn from the antecubital vein and collected into an EDTA containing Vacutainers (BD, Franklin Lakes, NJ). For measurement of RBC deformability, 8ml of blood was diluted in 1ml of a solution of 5.5% polyvinylpyrrolidone (PVP, MW=360,000, sigma, St. Louis, MO, USA) in phosphate buffered saline(PBS, pH=7.4, Osmolarity 290mOsm/kg).

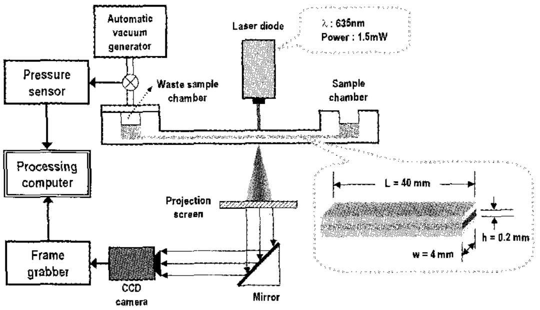

Fig. 1 Schematic diagram of Rheoscan-D

In addition, for measuring of RBC deformability for cardiovascular disease patients, 166 patients with diabetic mellitus (n=43), hypertension (n=46), chronic renal failure (n=36), stroke (n=20) and end stage renal disease (n=21) have been selected from the department of laboratory medicine of Kyungpook National University Hospital(105 males and 61 females).

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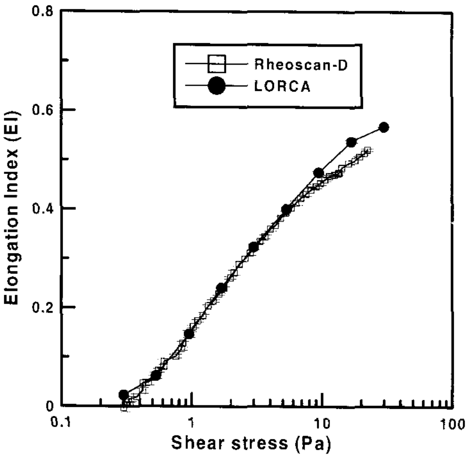

Fig. 2 Comparison of mean value of EI for healthy RBC measured with Rheoscan-D(□) and LORCA(●).

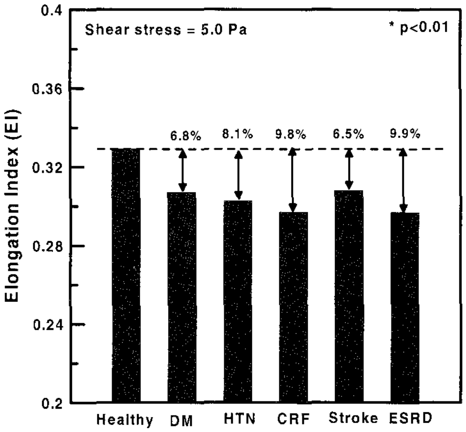

Fig. 3 shows the deformability alteration for the cells of healthy and of cardiovascular disease patients.

As shown in Fig. 1, the basic apparatus of Rheoscan-D contains the laser diode, a CCD video camera, screen, and the disposable slit. The laboratory setup also consisted of a computer. The laser diode (635 nm, 1.5 mW) 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 determined at the corresponding shear stresses (0 ~ 20 Pa).(7)

3. Results and Discussion

The slit ektacytometer, Rheoscan-D, was tested regarding validity, reproducibility, and errors of measurements for healthy red blood cells (n=54). The results were compared to the elongation data measured with the commercial rotating type ektacytometry, LORCA (R&R Mechatronics, Netherlands) as shown in Fig. 2. Open rectangle symbols indicate the EI measured with the LORCA; and solid circle symbols indicate those measured with the present slit ektacytometry. The test results from the slit ektacytometer, Rheoscan-D, were in close agreement (less than 5%) with those from the rotating ektacytometer in a shear stress range of 0 ~ 20 Pa.

Fig. 3 shows the deformability alteration for the cells of healthy and of patients-diabetic mellitus, hypertension, chronic renal failure, stroke and end stage renal disease at a shear stress of 5 Pa, especially. A significant difference ($p<0.01$) was found in RBC deformability in all levels of shear stress between healthy control and each diseases.

4. Conclusion

The newly developed slit ektacytometer can measure the RBC deformability with ease and accuracy. A straightforward combination of the slit rheometry and diffraction optics made it possible to measure the deformability of red blood cells in a clinical setting. The possible applications, in addition to the examples described here, include a test at home and at any point of care. The advantages of this design are in its simplicity, i.e., ease of operation and no moving parts, low cost, a short operating time, and a disposable kit which is in contact with the blood sample. In addition, dimension of actual device can be reduced to a hand-held size.

Acknowledgements

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