# Effect of Aging on Deformability of Erythrocytes in Shear Flow

**Shigehiro HASHIMOTO, Shuichi MOCHIZUKI, Yusuke MORITA**
**Department of Biomedical Engineering, Osaka Institute of Technology**
**Osaka, 535-8585, Japan**
**hashimoto@bme.oit.ac.jp**

**and**

**Hajime OTANI, Hiroji IMAMURA, Toshiji IWASAKA**
**Cardiovascular Center, Kansai Medical University**
**Moriguchi, 570-8506, Japan**

## ABSTRACT

To study the effect of aging on deformability of an erythrocyte, rheological measurement has been performed after exposure to a shear field in vitro. Deformability was evaluated with shear stress responsiveness and with critical elongation calculated from an exponential curve between an elongation index and shear stress. Human erythrocytes were classified according to their density by a centrifugal method. Deformability decreases in erythrocytes of high density after shearing. Even after exposure to the shear field of 640 1/s for one hour, erythrocytes deform from biconcave to ellipsoidal and their deformability is maintained.

**Keywords:** Bio-measurement, Erythrocyte, Shear Flow, Aging, Deformability, Elongation Index, Shear Stress Responsiveness

## 1. INTRODUCTION

An erythrocyte deforms to pass through micro-circulation. It deforms from biconcave to ellipsoid in a Couette type of flow: e.g., a shear field between an impeller and a casing of a centrifugal pump of an artificial heart. Deformability varies with its contents and the condition of the membrane, and decreases after damage by shear stress. To study the effect of aging on deformability, rheological measurement has been performed after exposure to the shear field *in vitro*.

## 2. METHODS

### Exposure to shear field

A shearing machine was designed with a concavo-convex Couette flow system (Fig. 1) [1]. In the space between a stationary convex cone and a rotating concave cone, the sample blood was sheared in a uniform shear field, where the shear rate is constant regardless of the distance from the axes of rotation. Two cones were made of transparent polymethyl-methacrylate (Fig. 2). Variation was made on shear rate with the rotational speed of the convex cone. Human blood was drawn from volunteers with anticoagulant of ethylene-diaminetetraaceticacid and sheared for one hour at the shear rate of 640 per second at twenty degrees Celsius.

**Fig. 1:** Concavo-convex Couette flow system.

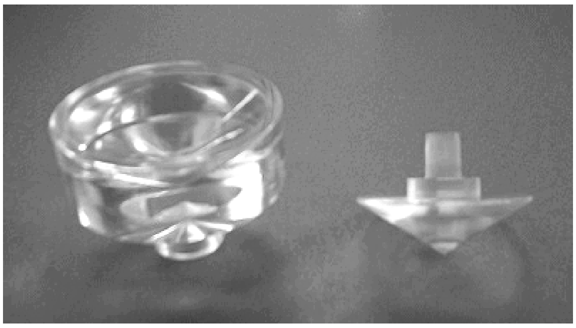

**Fig. 2:** Concavo and convex cones.

### Measurement of deformation

Before measurement of deformation, the cells were classified according to the density by a centrifugal method [2]. The density of cell's content increases with aging *in vivo*. The fluid of phthalate-ester with controlled density was used as a separator. The younger cells were collected from 10 percent of the supernatant section after centrifugation, where the older cells were collected from 10 percent of the bottom section after centrifugation.

The erythrocytes were suspended in a dextran aqueous solution to separate each other and to load high shear stress at low shear rate. The cells were sheared in the Couette flow between two counter-rotating parallel disks at twenty degrees Celsius (Fig. 3).

The shear stress is controlled between 0.6 Pa and 6 Pa with the rotating speed of the disks. The deformation was observed with a phase-contrast microscope and recorded with a video camera system. An erythrocyte deforms from biconcave into ellipsoid in the Couette type of shear flow.

Deformation was quantified with an elongation index (E), which was calculated from dimensions of the major (L) and minor (W) axes of the ellipsoid.

$$E=(L-W)/(L+W) \tag{1}$$

E becomes zero in a sphere (L=W), and approaches to unity as deformation advances (L>>W). The elongation index (E) was plotted as a function of shear stress (S), and the fitting exponential curve was calculated by

$$E=C(1-\exp(-S/R)) \tag{2}$$

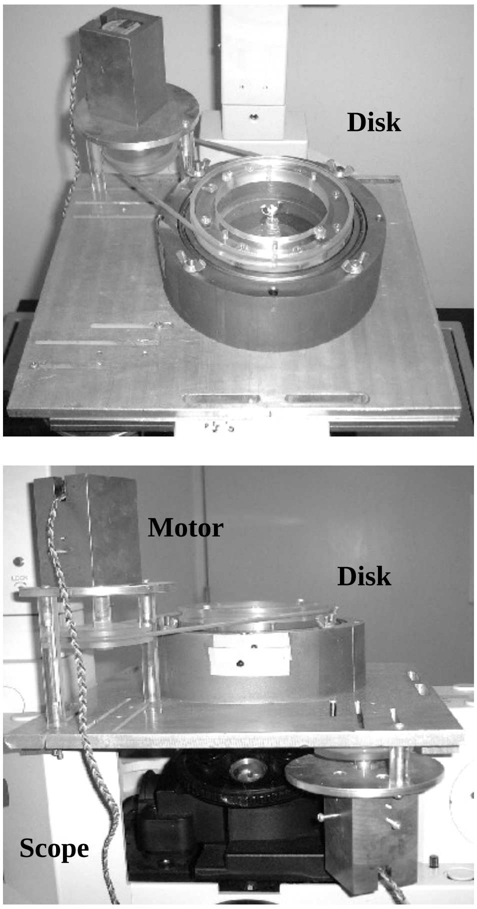


**Fig. 3:** Rheological Couette flow system of counter-rotating parallel disks.

where C is the critical elongation and R is the shear stress responsiveness. C indicates the maximum deformation with the high shear stress, and R indicates shear stress to deform to 63 percent of the maximum deformation. When C is similar in cells, the cell with low R has higher deformability.
Shear stress is the product of the viscosity and the shear rate. The shear rate is calculated from the rotating speed of the disk divided by the distance between two disks. The viscosity of the dextran aqueous solution was 0.24 Pa s, which was measured with a cone and plate type of viscometer.

## 3. RESULTS

Experimental results before exposure to shear fields are shown in Figs. 4 & 5. Figure four shows deformed erythrocytes suspended in the shear field. The cell deforms from biconcave to ellipsoid. Each plotted value shows the mean index and the standard deviation calculated from ten cells in Fig. 5. Figure five shows that the elongation index increases with shear stress, and saturates at high shear stress. Shear stress responsiveness is smaller in young cells (1.8 Pa) than in old cells (2.0 Pa) in Fig. 5, which shows that young cells are more compliant than old ones. Critical elongation index is larger in young cells (0.43) than in old cells (0.36) (Fig. 5), which shows that young cells saturate at highly deformed shape.

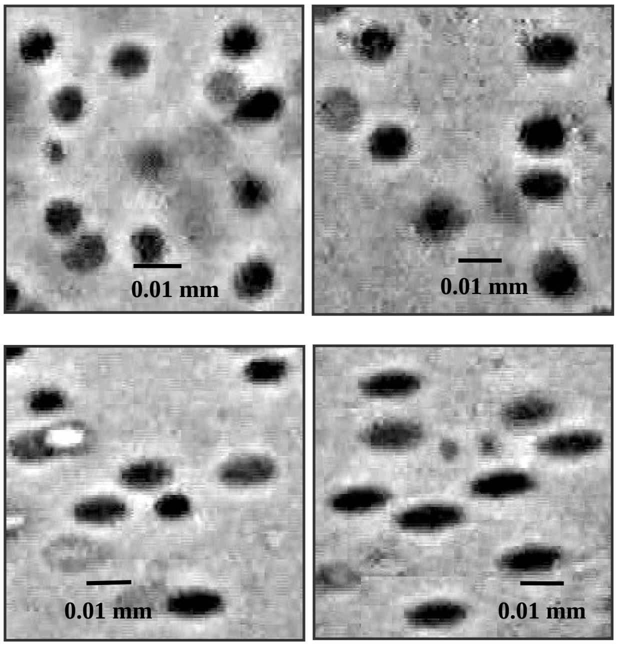


**Fig. 4:** Deformed erythrocytes in Couette flow. Old, left; young, right. Shear stress: 1.1 Pa, upper; 4.5 Pa, bottom.

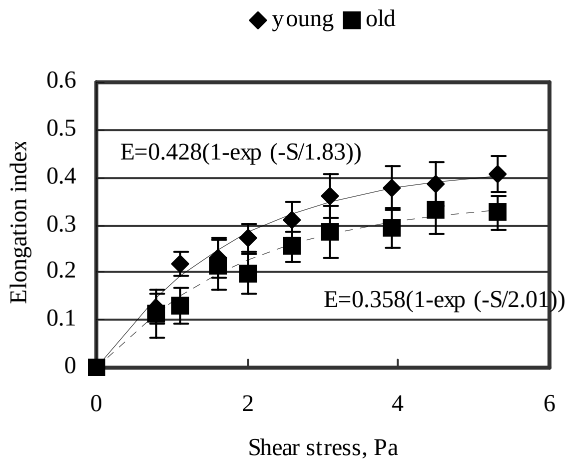


**Fig. 5:** Elongation index vs. shear stress.

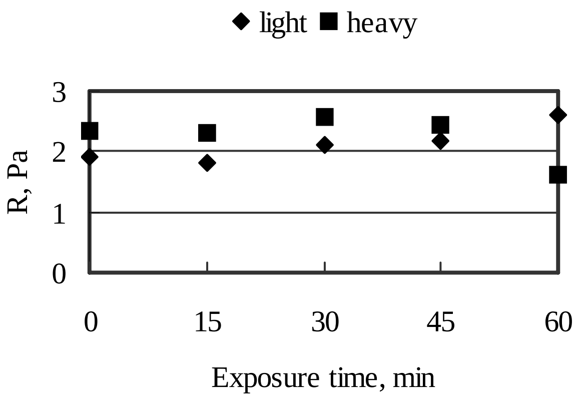


**Fig. 6**: Shear stress responsiveness (R) vs. exposure time to shear rate of 640 1/s.

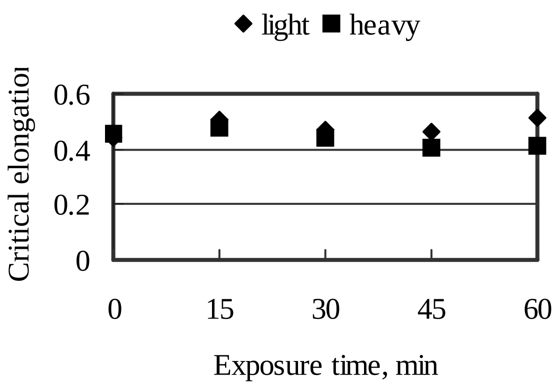


**Fig. 7**: Critical elongation vs. exposure time to shear rate of 640 1/s.

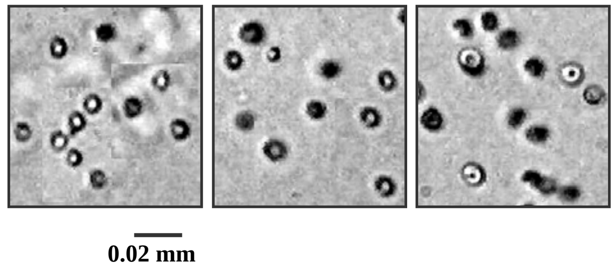


**Fig. 8:** Heavy erythrocytes after shear: control (left), exposure time 30 min (middle), 60 min (right).

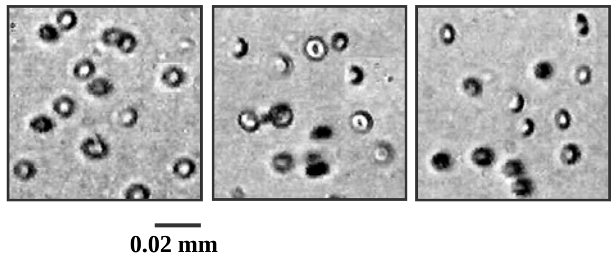


**Fig. 9:** Light erythrocytes after shear: control (left), exposure time 30 min (middle), 60 min (right).

The results of deformation test after exposure to a shear field of 640 1/s are shown in Figs. 6-9. After exposure to the shear field, cells were classified with density before deformation tests. Data points at zero of exposure time show data before exposure to the shear field (Figs. 6 & 7). Figure six shows that shear stress responsiveness does not vary during one hour of exposure to the shear field. Figure seven shows that critical elongation index is kept around 0.45 during one hour of exposure to the shear field. The tendency does not depend on the group of cell density: light or heavy in Figs. 6 & 7.

Many erythrocytes keep biconcave shape even after exposure to the shear field (Figs. 8 & 9). The shape of some cells vary into crenated shape after shear.

## 4. DISCUSSION AND CONCLUSION

The Couette-type shear flow is widely used to study mechanical properties of erythrocytes [1,3]. The parallel disks type of rheoscope has an advantage to keep geometrical accuracy compared to the cone and plate type.

The previous study shows that the density of erythrocytes increases as aging [4]. Deformation of the young cell is larger than that of old one, and young cell is more compliant than old one. The experimental results show that the deformability decreases with aging.

Although several investigations have been performed to decrease hemolysis in the flow path of artificial circulatory assist devices, sublethal damage might occur during perfusion with these devices [5,6].

Deformation of cell might depend on environment and state of the membrane [7]. The property of membrane might change after exposure to a shear field [8].

The present study shows that erythrocyte deforms from biconcave to ellipsoid and its deformability is maintained even after exposure to the shear field of 640 1/s for one hour, although the previous study shows that one percent of erythrocytes, however, are hemolyzed with the shear field of 640 1/s in one hour *in vitro* [1].

## 5. ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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