

# Detect of Sublethal Damage with Cyclic Deformation of Erythrocyte in Shear Flow

Shigehiro HASHIMOTO

Biomedical Engineering, Department of Mechanical Engineering, Kogakuin University,  
Tokyo, 163-8677, Japan

shashimoto@cc.kogakuin.ac.jp <http://www.mech.kogakuin.ac.jp/labs/bio/>

## ABSTRACT

The deformation of an erythrocyte has been observed microscopically in the shear flow to detect the sublethal damage of an erythrocyte *in vitro*. A rheoscope system has been manufactured to observe the deformation of the suspended erythrocytes in the shear flow. The rheoscope consists of a pair of parallel disks and an inverted phase-contrast microscope. The human erythrocytes were suspended in the dextran aqueous solution, which has high viscosity. The erythrocytes are sheared in the Couette flow between a pair of counter rotating disks. The experiments with the rheoscope show following results. The erythrocytes deform from a biconcave to an ellipsoidal shape. The erythrocytes deform periodically at the double frequency of tank tread motion of the membrane of the deformed erythrocyte, when erythrocytes have the sublethal damage on the membrane.

**Keywords:** Biomedical Engineering, Erythrocyte, Shear Flow and Sublethal Damage.

## 1. INTRODUCTION

An erythrocyte has flexibility [1, 2] and deforms in the shear flow [3]. It also passes through micro-circulation, of which the dimension is smaller than the diameter of the erythrocyte. After circulation through the blood vessels for days, the erythrocyte is damaged and trapped in the micro-circulation systems.

The deformation of erythrocytes has been observed *in vivo* and *in vitro* with various methods: a micro-channel [1, 2, 4], a filter [5, 6], a micro slit [7, 8], and a rheoscope [3, 9]. While erythrocytes are exposed to the shear flow, they show tank tread motion at the membrane [10], and eject contents (hemolysis [11]) through the crevasse of the membrane, before fragmentation.

In the present study, the deformation of erythrocyte has been observed microscopically in the shear flow to detect the sublethal damage of erythrocyte *in vitro*.

## 2. METHODS

### Rheoscope System

A rheoscope system has been manufactured to observe the deformation of the suspended erythrocytes in the shear flow (Fig. 1). The rheoscope consists of a pair of parallel disks and an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) [3, 9]. The erythrocytes are sheared in the Couette flow between a pair of counter rotating disks made of

transparent polymethacrylate. The velocity of the middle plane between the counter rotating disks is zero in the shear field of Couette type flow, so that the erythrocyte in the plane is easily observed with the microscope.

The radius and thickness of the disk is 30 mm and 2 mm, respectively. The distance between the rotational axis and the observation point is 20 mm. The lower disk has a wall at the rim. With a DC motor, the rotational speed of each disk is controlled between  $0.05 \text{ rad s}^{-1}$  and  $0.3 \text{ rad s}^{-1}$ . The distance between two disks is adjusted between 0.02 mm and 0.1 mm. The distance is confirmed by the volume of the suspension filled between the disks (between  $0.06 \text{ cm}^3$  and  $0.3 \text{ cm}^3$ ), and by the calibrated focus positions with the microscope.

The shear rate  $G [\text{s}^{-1}]$  is calculated by the following equation.

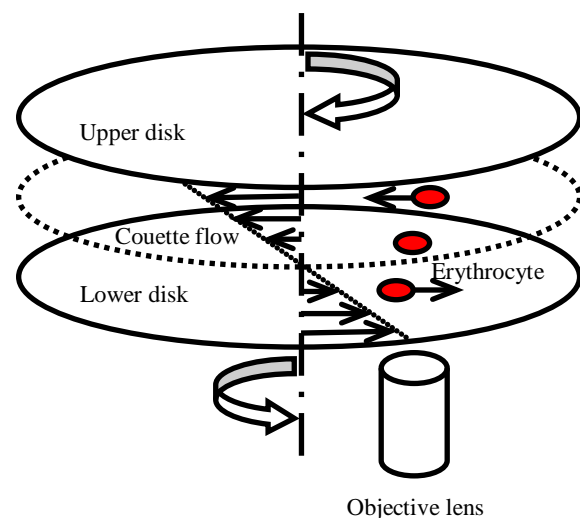
$$G = v / D \quad (1)$$

In Eq. 1,  $D$  is distance [m] between two parallel disks, and  $v$  is the circumferential velocity difference between of the disks [ $\text{m s}^{-1}$ ].

The circumferential velocity difference is calculated by Eq. 2.

$$v = r w \quad (2)$$

In Eq. 2,  $r$  is the radius [m], and  $w$  is the angular velocity difference [ $\text{rad s}^{-1}$ ] between the disks.



**Fig. 1:** Rheoscope system.

At the observation point ( $r = 0.02$  m), the shear rate ( $G$ ) is in the range between  $20 \text{ s}^{-1}$  and  $600 \text{ s}^{-1}$ , calculated with  $w$  (between  $0.1 \text{ rad s}^{-1}$  and  $0.6 \text{ rad s}^{-1}$ ) and with  $D$  (between  $0.02$  mm and  $0.1$  mm) in the present experiment.

### Erythrocyte Suspension

Erythrocytes were sparsely suspended in the dextran (molecular weight: 200000-300000) aqueous solution, which has high viscosity.

The shear stress ( $S$  [Pa]) in the suspension is calculated by Eq. 3.

$$S = N G \quad (3)$$

In Eq. 3,  $N$  is the viscosity [Pa s] of the fluid, and  $G$  is the shear rate [ $\text{s}^{-1}$ ] between the disks.

Variation was made on the shear stress (between  $5$  Pa and  $30$  Pa) with  $N$  (between  $0.1$  Pa s and  $2.6$  Pa s) and with  $G$  (between  $20 \text{ s}^{-1}$  and  $600 \text{ s}^{-1}$ ) in the present experiment. The viscosity of the dextran aqueous solution was measured with a cone and plate type of viscometer.

The human erythrocytes from volunteer are used in the present experiment. Before measurement of deformation, the cells were classified according to the density by a centrifugal method [2]. The density of content in cells 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.

The erythrocytes were suspended in a dextran aqueous solution to separate each other and to load the high shear stress at the low shear rate. The cells were sheared in the Couette flow between two counter-rotating parallel disks at  $298$  K.

The deformation of cells was observed with the microscope and recorded with a video camera system. The long-focus objective lens is used to observe erythrocytes suspended near the middle plane.

## 3. RESULTS

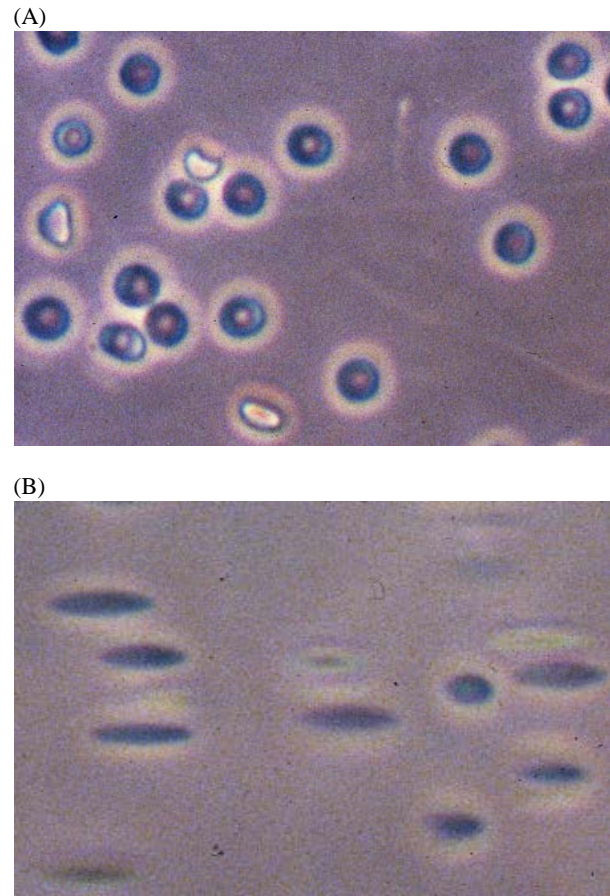
Fig. 2 exemplifies deformation of erythrocytes from a biconcave to an ellipsoid, which are observed with the rheoscope system manufactured in the present study. The media moves from left to right in the front layer, while the media moves from right to left in the rear layer. The erythrocytes in the middle layer are able to be observed for a long time within the focused area with the microscope, while the erythrocytes are sheared in the Couette flow (Figs. 2-5). Most of erythrocytes keep the biconcave shape moving along the flow of the slow velocity in the low shear stress field. Some of the erythrocytes roll over and deform to the parachute shape (Fig. 2(A)). Every erythrocyte, on the other hand, deforms to ellipsoid in the high shear stress field (Fig. 2(B)). The ellipsoid is elongated, as the shear stress increases. The direction of the major axis of the ellipsoid is oriented in parallel to the flow.

After exposure to the high shear stress field for several minutes, some of erythrocytes are destroyed into fragments. Some

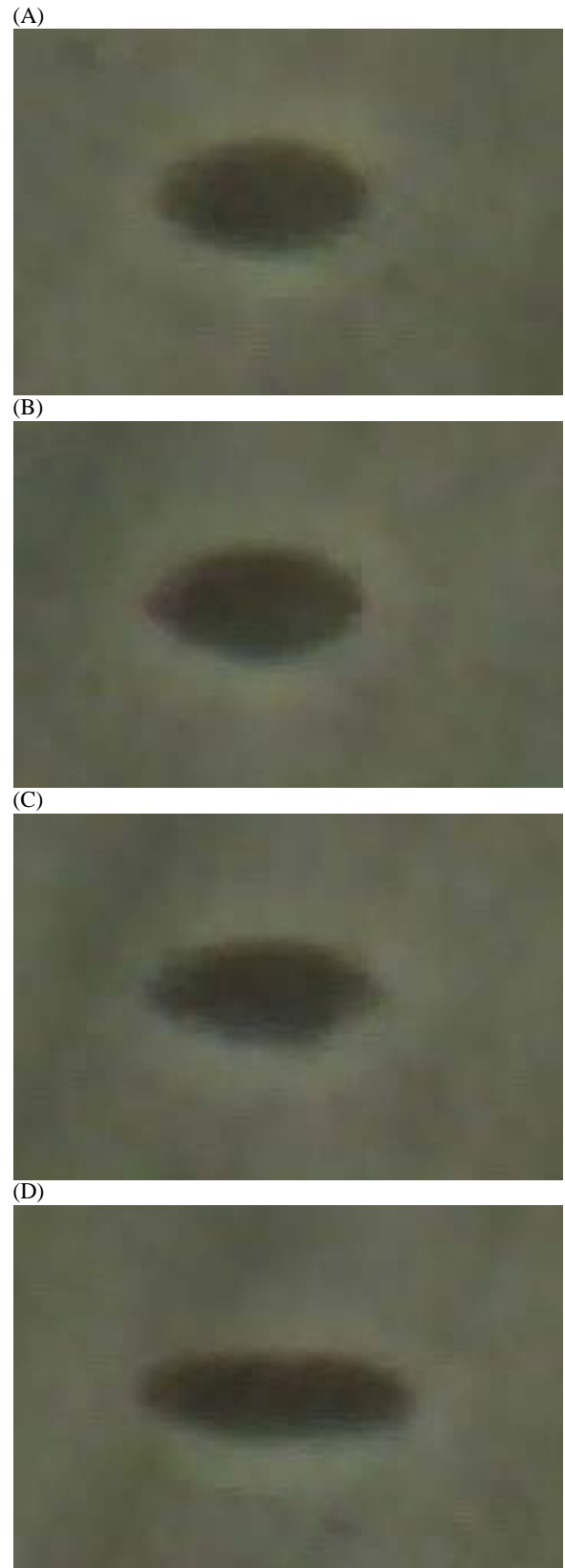
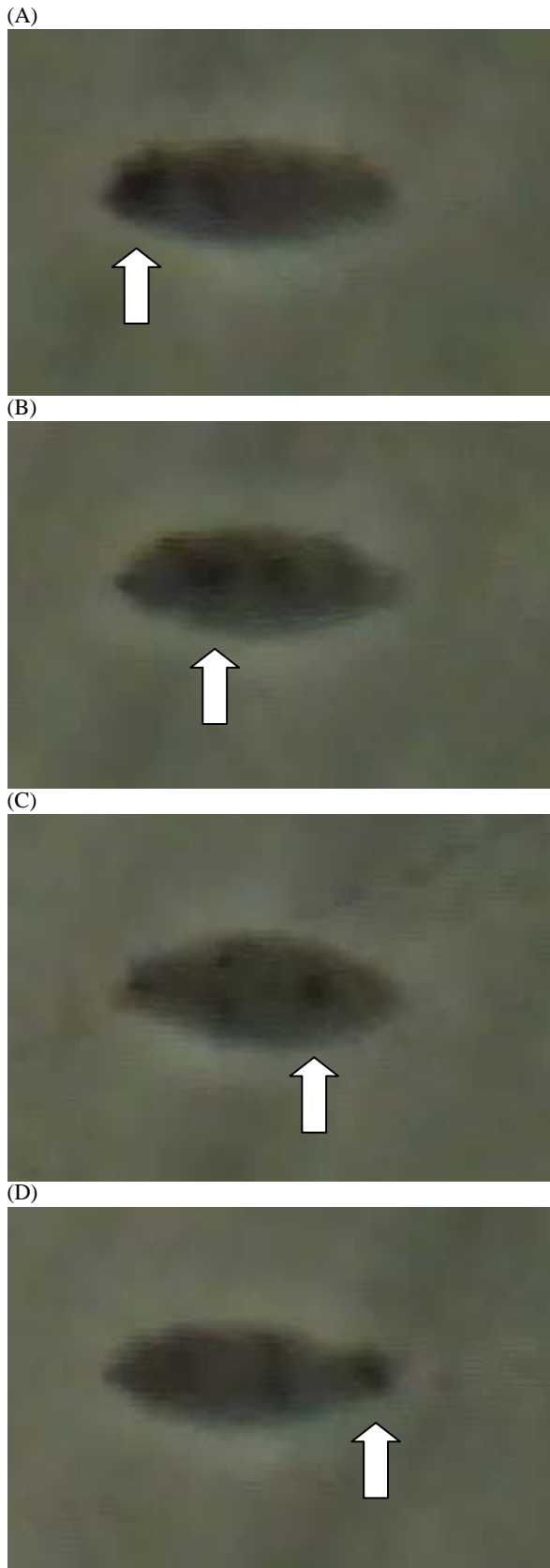
fragmented flasks adhere to the membrane of the erythrocyte. Fig. 3 exemplifies the tank tread motion of the membrane of erythrocyte at the shear rate of  $20 \text{ s}^{-1}$  with the viscosity of  $0.45$  Pa s. The adhered flask on the membrane is observed as a marker for the movement of the membrane. The marker of the membrane moves left to right on the front side, turns around to the rear side and moves from right to left on the rear side.

The tank tread motion of every ellipsoid with the same cycle confirms the uniform shear field of the Couette flow between the counter rotating disks, which are located at the front and the rear position of the observation window with the microscope. The rotational axis of the tank tread motion is perpendicular to the flow, and is parallel to the disk. The axis is from top to bottom in Figs. 3-4. The frequency of tank tread motion ( $>1$  Hz) is proportional to the shear rate ( $>20 \text{ s}^{-1}$ ) between the disks.

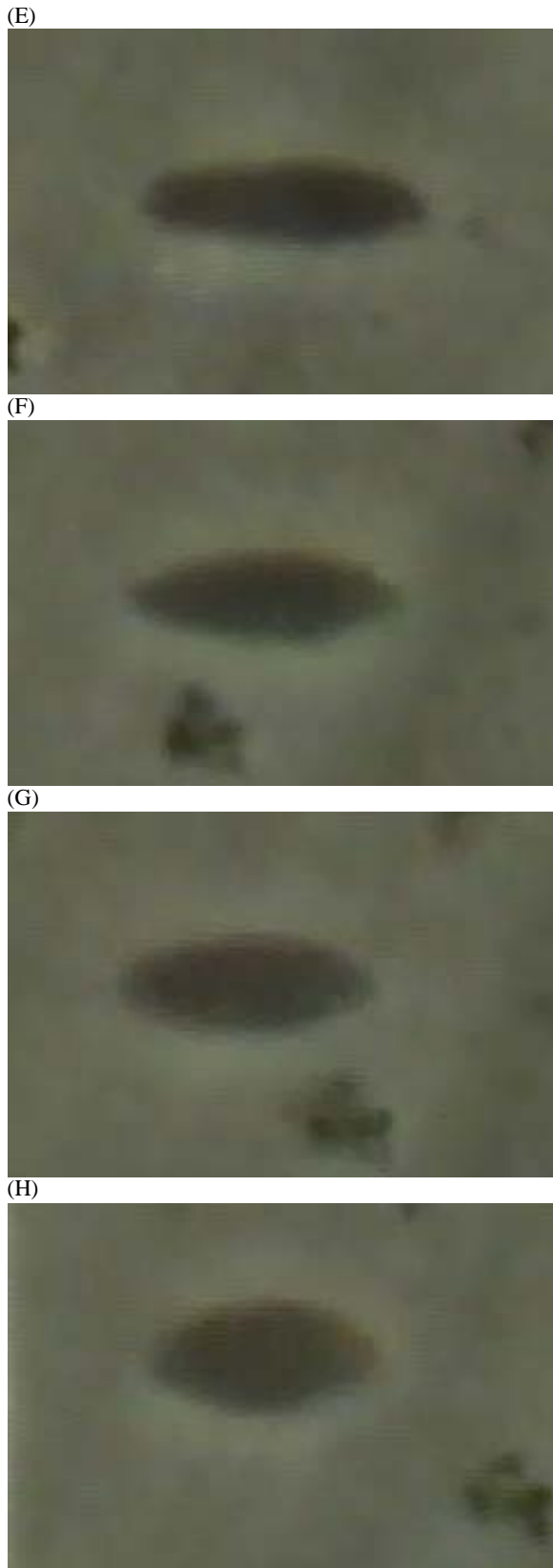
After exposure to the high shear stress field for several minutes, some of erythrocytes repeat the cyclic deformation. Fig. 4 exemplifies periodical deformation of an erythrocyte of the ellipsoidal shape in the shear field of Couette flow at the shear rate of  $25 \text{ s}^{-1}$  with the viscosity of  $0.45$  Pa s. The ellipsoid deforms from swollen to flat, while the membrane rotates with the quarter cycle of tank tread motion. The ellipsoid returns from flat to swollen, while the membrane rotates with the successive quarter cycle.



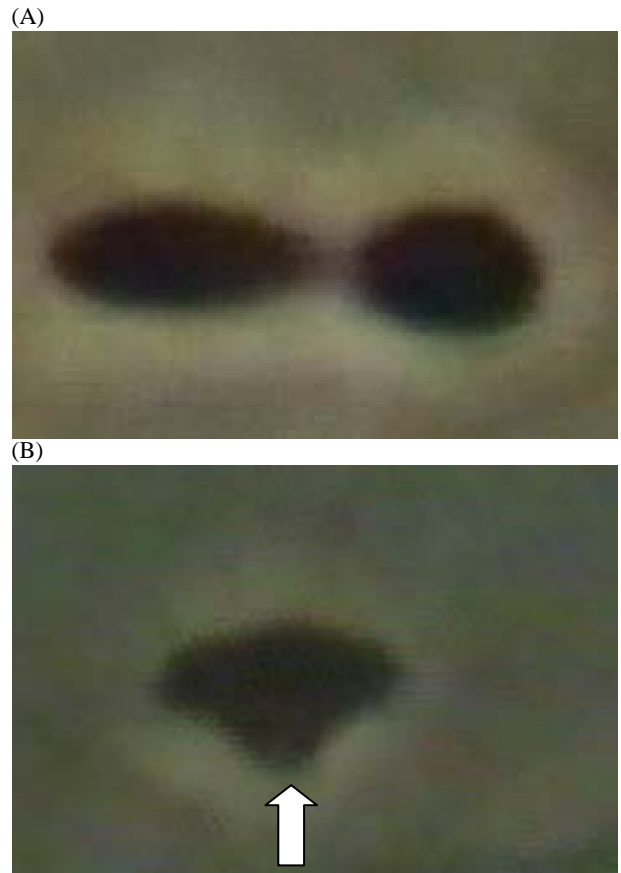
**Fig. 2:** Deformation of erythrocytes from biconcave (A) to ellipsoid (B). Dimension from left to right is  $1.5$  mm.



**Fig. 3:** Tank treading motion of the membrane of erythrocyte. Adhered fragment on the membrane (arrow) is moving from left (A) to right (D). Dimension from left to right is 0.03 mm.



**Fig. 4:** Repetitive cyclic deformation of erythrocyte, which has sublethal damage. The ellipsoid deforms from swollen (A) to flat (E), and return to swollen (H). Dimension from left to right is 0.03 mm. Two cycles of deformation for one cycle of tank tread motion.



**Fig. 5:** Damaged erythrocyte. Separated to small spheres (A). Ejected contents (arrow) through the crevasse of the membrane (B). Dimension from left to right is 0.03 mm.

The deformation repeats two cycles at each tank tread cycle. The cycle of the tank tread motion is the same as another erythrocyte, which does not demonstrate cyclic deformation.

Fig. 5 exemplifies damaged erythrocytes, after exposure to the high shear stress field for several minutes. Some cells eject the contents through the crevasse of the membrane. When the direction of the ejection is parallel to the rotating axis of tank tread motion, repetitive cyclic deformation is not observed. In that case, the tank tread motion is observed. A cell is divided into two parts in Fig. 5(A).

#### 4. DISCUSSION

In Fig. 2(B), every cell in two-dimensional projection shows ellipse, which means ellipsoidal shape. The disk should show circle or ellipse in two-dimensional projection according to the direction.

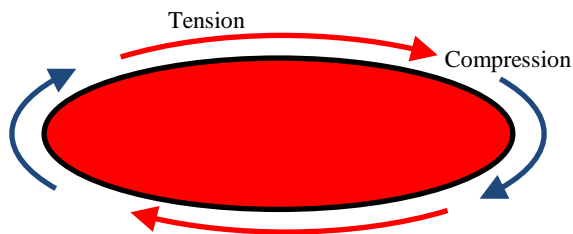
The shear rate between two parallel disks increases in proportion to the distance from the rotational axis. The shear rate varies with less than 5 percent in the observation area of 1 mm square, of which distance from the rotational axis is 20 mm. The shear rate in the observation area is approximately mean value in the whole volume of the suspension between two disks.

Direction of a rotational movement of the marker follows the shear field. In the shear field, the difference between speeds of two layers ( $V$  [ $\text{m s}^{-1}$ ]) is calculated by Eq. 4.

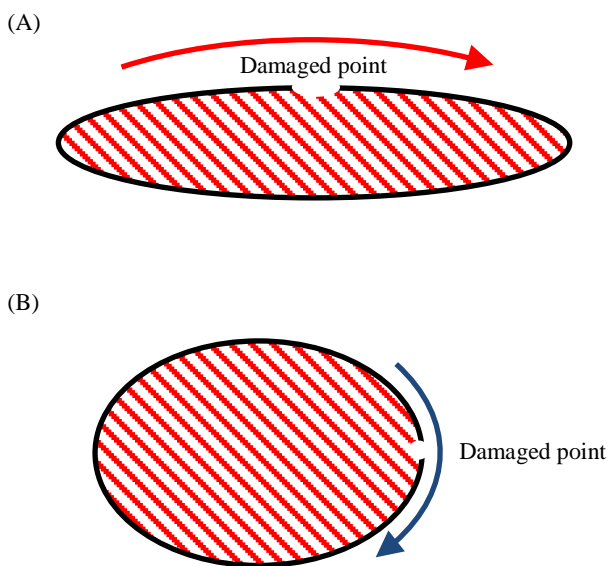
$$V = G x \quad (4)$$

In Eq. 4,  $G$  is shear rate [ $\text{s}^{-1}$ ], and  $x$  is the distance of the two layers [m].  $V$  is  $0.02 \text{ mm s}^{-1}$  at  $G$  of  $20 \text{ s}^{-1}$  with  $x$  of  $0.001 \text{ mm}$ . The speed of tank tread motion of the membrane depends on the difference of the speed of circumferential flow. The speeds observed in the present experiment are in the same order of  $V$  calculated by Eq. 4, so that the frequency of the cyclic tank tread motion is higher than 1 Hz.

The repetitive cyclic deformation reveals the local defect of the membrane of the erythrocyte, because the cycle is synchronized with the tank tread motion of the membrane (Fig. 4). During the tank tread motion, the membrane is subjected to tension and compression alternately according to the curvature (Fig. 6). During the tank tread motion of the membrane, the damaged point is moving along the circumference of the ellipsoid, which has cyclic variation of curvature. The cyclic variation of curvature makes cyclic deformation of the ellipsoid.



**Fig. 6:** Two cycles of tension and compression of the membrane of ellipsoid during every tank-tread motion.



**Fig. 7:** Two cycles of deformation of ellipsoid with damaged membrane during every tank-tread motion.

When the locally damaged point moves along the area of the large curvature, the area might be extended. The extension might deform the ellipsoid to flatten shape (Fig. 7). When the damaged point moves along the area of the small curvature, on the other hand, the extended area might return to the former shape. The shrinkage might deform the ellipsoid to swollen shape.

When the locally damaged point cannot support the moment, the pattern of deformation might change to the reverse pattern. At the small curvature, the membrane receives the larger moment. The ellipsoid might be flattened, when the local defect moves along the area of the small curvature.

The repetitive cyclic deformation might occur, when the damaged point of the membrane locates near the equator of the rotating movement. It might not occur, on the other hand, when the damaged point of the membrane is near the rotational axis. When the damage of the membrane extends, the damaged part might approach to the rotational axis, and the repetitive deformation might be stopped.

The repetitive cyclic deformation may not depend on the turbulence of the flow. Some erythrocytes repeat the cyclic deformation, but others do not. The erythrocytes repeat the cyclic deformation even in the low shear field, after exposure to the high shear stress field. In this case, the damage is irreversible.

The dextran solution was applied to the suspension to inhibit turbulence in the flow with increase of viscosity. Reynolds number ( $Re$ ) is a useful index for estimation of the turbulent flow.

$$Re = d D v / N \quad (5)$$

In Eq. 5,  $d$  is density [ $\text{kg m}^{-3}$ ] of the fluid,  $D$  is distance [m] between two parallel disks,  $N$  is viscosity [ $\text{Pa s}$ ] of the fluid, and  $v$  is the circumferential velocity of the disk [ $\text{m s}^{-1}$ ].

$Re$  is smaller than 0.006 at  $d$  ( $10^3 \text{ kg m}^{-3}$ ),  $N$  ( $>0.1 \text{ Pa s}$ ),  $D$  ( $<10^{-4} \text{ m}$ ), and  $v$  ( $<6 \times 10^{-3} \text{ m s}^{-1}$ ) in the present experiment. The number is small enough to inhibit the turbulent flow.

The cone and plate type instrument is used to make uniform Couette type flow [3, 11]. There are several reasons why the parallel disks type is chosen for the rheoscope in the present study. It is easier to make transparent disk with a flat surface. The distance between two disks is uniform, so that it is easier to maintain the distance between disks constant.

The single erythrocyte in the blood is hardly distinguished with microscope, because the volume ratio of erythrocyte in the blood is higher than 0.3. In the present study, erythrocytes are dispersed in the dextran solution to make it easy to be observed as each single cell.

The density of content in cells increases with aging *in vivo*. In the previous study, 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 mechanism of erythrocytes deformability was a target for previous studies [2]. Deformability of erythrocytes might be an

index for diagnostics [6]. Both the shear rate and the shear stress govern destruction of erythrocytes in the shear flow [11].

A micro channel could simulate the microcirculation system. The effects of shear flow on cells were observed in the previous studies [12]. The micro-slit is also useful for observation of deformation of a cell.

In the present study, the deformation of erythrocyte is observed in the shear flow to detect the sublethal damage of an erythrocyte with the rheoscope.

## 5. CONCLUSION

The deformation of an erythrocyte has been observed microscopically in the shear flow to detect the sublethal damage of the erythrocyte *in vitro*. A rheoscope system has been manufactured to observe the deformation of the suspended erythrocytes in the shear flow. The experimental results show that the erythrocytes deform from a biconcave to an ellipsoidal shape and that the erythrocytes with the sublethal damage repeat cyclic deformation at the double frequency of tank tread motion of the membrane.

## 6. ACKNOWLEDGMENT

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