Analysis of Cyclic Deformation of Erythrocyte in Couette Type of Pulsatile Shear Field

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ABSTRACT

The cyclic deformation of an erythrocyte has been measured microscopically in the pulsatile shear field to detect dynamic deformability of an erythrocyte *in vitro*. A rheoscope system has been manufactured to observe deformation of 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. Erythrocytes are sheared in the Couette flow between the pair of counter rotating disks. The rotating speed varies sinusoidally to make the pulsatile shear field. Deformation of each erythrocyte was measured at the video image of the rheoscope. The experimental results show that the system is available to measure the following behavior of an erythrocyte. The ellipsoidal shape of each erythrocyte varies cyclically to follow the pulsatile cyclic shear field. The phase of deformation of each erythrocyte in the cycle delays from the sinusoidal fluctuation of the shear field according to its own dynamic deformability.

Keywords: Biomedical Engineering, Erythrocyte, Shear Flow, Deformation and Pulsatile Flow

1. INTRODUCTION

An erythrocyte has flexibility and deforms in the shear flow. It also passes through the micro-circulation system, 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 *in vivo*.

Deformation of the erythrocyte has been observed *in vivo* and *in vitro* with various methods: a micro-channel [1–6], a filter [7, 8], a micro slit [9, 10], and a rheoscope [11–13]. While erythrocytes are exposed to the shear flow, they show the tank tread motion at the membrane [11], and eject contents (hemolysis [13, 14]) through the crevasse of its own membrane, before fragmentation.

The repetitive stresses are applied to the erythrocyte during circulation *in vivo*: in the pulsatile flow, and through the microcirculation. The erythrocyte shows the viscoelastic property, when it recovers to the original shape [1, 3].

In the present study, the cyclic deformation of an erythrocyte has been measured microscopically in the pulsatile shear field to detect the dynamic deformability of an erythrocyte *in vitro*.

2. METHODS

Rheoscope System

A rheoscope system has been manufactured to observe 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) [12]. The erythrocytes are sheared in the Couette flow between a pair of counter rotating parallel disks, which are made of transparent silica glass. The velocity of the middle plane between the counter rotating disks is zero in the shear field of Couette type flow. The velocity of the erythrocyte floating near the middle plane in the fluid is low. The erythrocyte with the low velocity stays for a long time within the observation area of the objective lens of the microscope.

The radius (R) and thickness of the disk is 40 mm and 5 mm, respectively. The distance between the rotational axis and the observation point (r) is 27 mm. Both disks are supported at their rim by the ball bearing of 100 mm diameter. The lower disk has a wall at the rim to contain the fluid. The distance between two disks (d) is adjusted between 0.08 mm and 0.12 mm. The distance is confirmed by the volume of the suspension filled between the disks (between 0.3 cm^3 and 0.6 cm^3), and by the calibrated focus positions of the walls with the microscope.

The shear rate γ [s^{-1}] is calculated by the following equation.

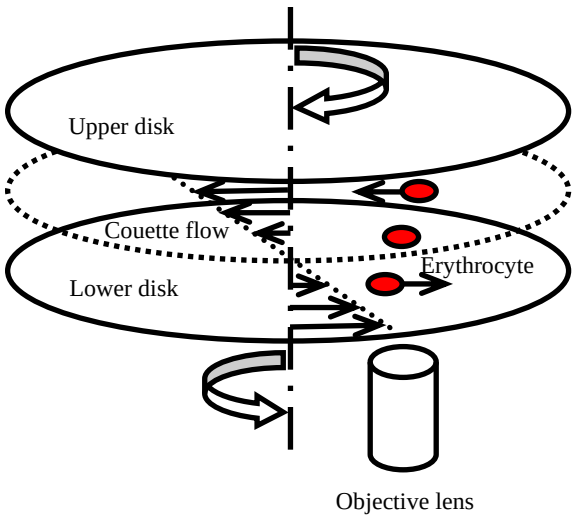

Fig. 1: Rheoscope system with counter rotating disks.

$$\gamma = \Delta V / d \quad (1)$$

In Eq. (1), d is distance [m] between two parallel disks, and ΔV is the circumferential velocity difference between the upper and the lower disks [m s^{-1}]. At the observation point with the same distance from the rotational axis, the shear rate γ is constant between two disks regardless of the distance from the surface of the disk in the Couette type of the flow.

The circumferential velocity difference at the observation point is calculated by Eq. (2).

$$\Delta V = r \Delta\omega \quad (2)$$

In Eq. (2), r is the distance between the rotational axis and the observation point [m], and $\Delta\omega$ is the angular velocity difference [rad s^{-1}] between the parallel disks.

With a DC (direct current) stepping motor, the angular velocity of each disk (ω < 0.1 rad s^{-1}) is controlled in one way. The rotating directions of two disks are counter direction (clockwise and counterclockwise) each other. The absolute values of the angular velocities of two disks are adjusted to the same value each other.

The angular velocity is periodically (period: between 0.67 s and 7 s) varied with the sinusoidal mode in one way. The cyclic fluctuation of the velocity of the disk makes the cyclic fluctuation of the shear rate (γ) between disks.

At the observation point (r = 0.027 m), the shear rate (γ) is lower than 6.8×10 s^{-1}, calculated with $\Delta\omega$ (< 0.2 rad s^{-1}) and with d (between 0.08 mm and 0.12 mm) in the present experiment.

Erythrocyte Deformation

To apply the high shear stress on each erythrocyte at the low shear rate, erythrocytes were sparsely suspended in dextran (molecular weight between 2×10^5 and 3×10^5) aqueous solution of high viscosity. The erythrocytes were sheared in the Couette flow between two counter-rotating parallel disks at 298 K. The human erythrocytes collected from the author were used in the experiment.

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

$$\tau = \eta \gamma \quad (3)$$

In Eq. (3), η is viscosity [Pa s] of the fluid, and γ is the shear rate [s^{-1}] between the disks.

Variation was made on the shear stress (< 4 Pa) with η (between 0.02 Pa s and 0.06 Pa s) and with γ (lower than 6.8×10 s^{-1}) in the present experiment. The viscosity of the dextran aqueous solution was measured with a cone and plate type of viscometer.

Deformation of each erythrocyte was observed with the microscope and recorded with a video camera system. The long-focus objective lens was used to observe erythrocytes suspended around the middle plane. The stationary images were captured from the video movie every 0.033 s.

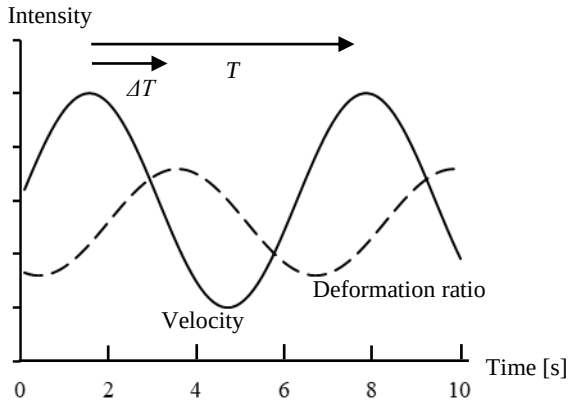

Fig. 2: Schematic tracings of deformation ratio (dotted line) and velocity (solid line): period (T), delay (ΔT).

Each erythrocyte deforms from the biconcave shape to the ellipsoid shape in the shear field. On the microscopic image, the outline of each erythrocyte was traced, and the contour of each erythrocyte was approximated to an ellipsoid. On the ellipsoid, the length of the major axis (a), and the minor axis (b) were measured. The ratio of axes is calculated as the deformation ratio (P) by Eq. (4).

$$P = 1 - b / a \quad (4)$$

At the circle, P = 0. As the ellipsoid elongates, P approaches to 1. As the shear rate fluctuates periodically, the deformation ratio of each erythrocyte fluctuates periodically. The cyclic fluctuation of the deformation ratio of each erythrocyte was approximated to the polynomial curve at the time course tracings.

The velocity of each erythrocyte was calculated by tracing of the same erythrocyte at the image every 0.033 s. The cyclic fluctuation of the velocity of each erythrocyte was approximated to the polynomial curve at the time course tracings. The period of the cyclic shear rate (T [s]) is confirmed by the center part of the curve in each figure. The delay (ΔT [s]) (Fig. 2) of the deformation of each erythrocyte is calculated by the phase difference between the approximated cyclic curve of the velocity and that of the deformation ratio. The delay ratio (S) was calculated by Eq. (5).

$$S = \Delta T / T \quad (5)$$

The delay ratio (S) is zero, when the cyclic deformation occurs synchronously to the periodically fluctuating velocity. The delay ratio (S) approaches to unity, when the delay of the deformation extends. When the cyclic deformation occurs at the counter phase of the cycle of the shear rate, the delay ratio becomes 0.5.

3. RESULTS

Fig. 3 exemplifies the image of erythrocytes in the shear field of 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 near the middle layer are able to be observed for a

long time within the focused area of the microscope, while the erythrocytes are sheared in the Couette flow. Each erythrocyte deforms from a biconcave to an ellipsoid in the shear stress field. The ellipsoid is elongated, as the shear stress increases. The direction of the major axis of each ellipsoid is parallel to the flow direction.

Figs. 4a-4j exemplify the tracings of the deformation ratio (triangle) and the velocity (circle) of each erythrocyte. Some erythrocytes deform to the deformation ratio of 0.65 under the shear stress field lower than 4 Pa. Data scatter in Fig. 4: the deformation ratio between 0.3 and 0.65, and the velocity between −40 μm/s and 150 μm/s. Data were able to be approximated to the cyclic curve (red lines in Fig. 4). From the approximate curve, the period and the delay ratio were calculated at each erythrocyte. Each period was in the range between 0.67 s and 7 s. Each delay ratio was in the range between 0.09 and 0.58.

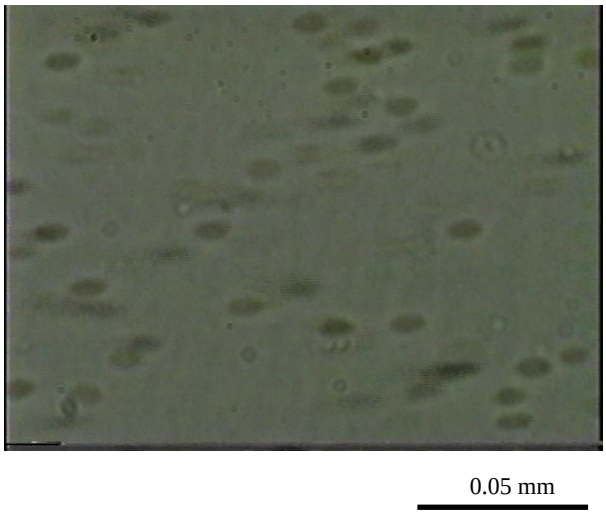

Fig. 3: Deformed erythrocytes in shear field.

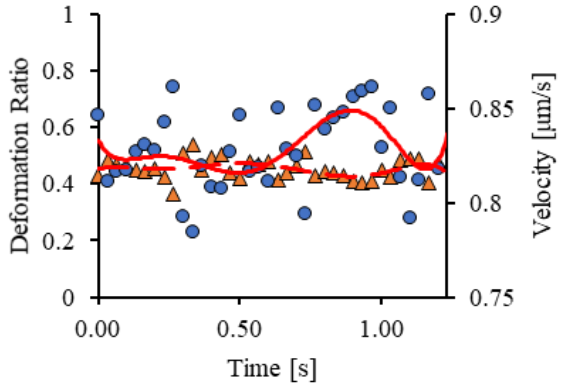

Fig. 4a: Tracings of deformation ratio (triangle) and velocity (circle): approximate curve (deformation ratio (dotted line) and velocity (solid line)): period 0.67 s, delay ratio 0.58.

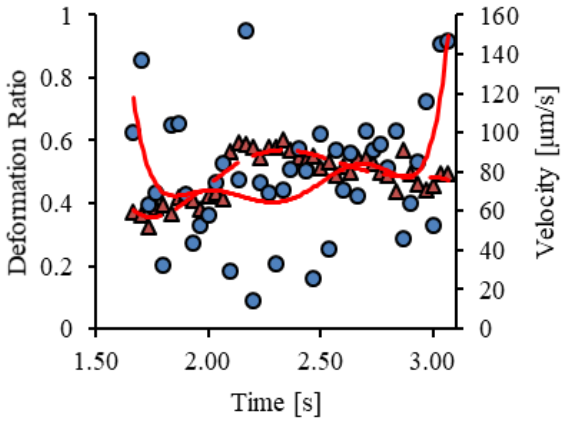

Fig. 4b: Tracings of deformation ratio and velocity: period 0.67 s, delay ratio 0.48.

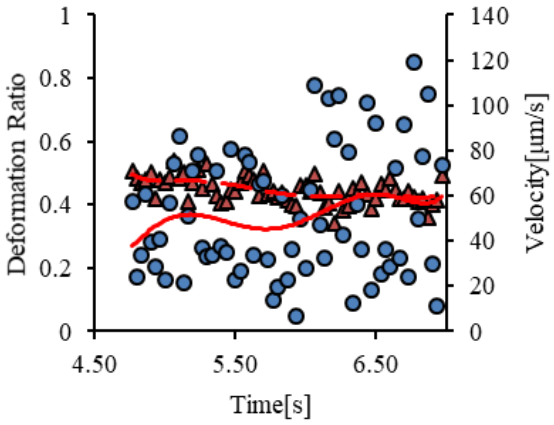

Fig. 4c: Tracings of deformation ratio and velocity: period 1.28 s, delay ratio 0.16.

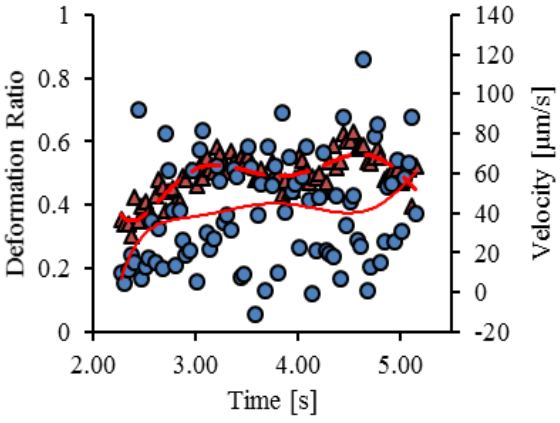

Fig. 4d: Tracings of deformation ratio and velocity: period 1.46 s, delay ratio 0.51.

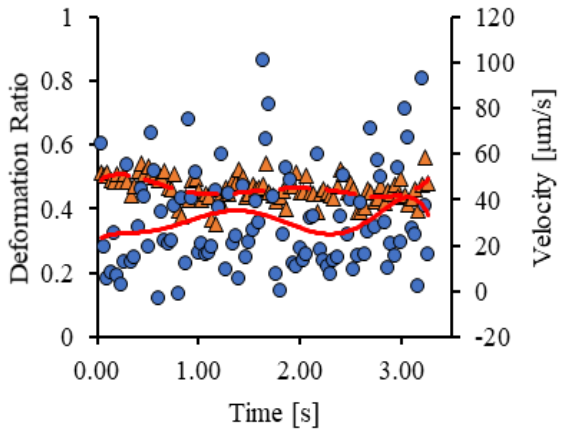

Fig. 4e: Tracings of deformation ratio and velocity: period 1.67 s, delay ratio 0.35.

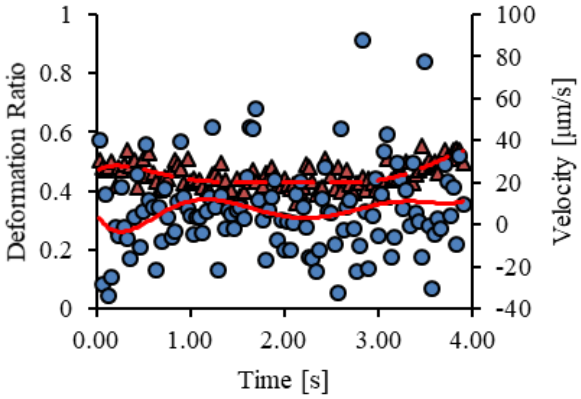

Fig. 4f: Tracings of deformation ratio and velocity: period 2 s, delay ratio 0.42.

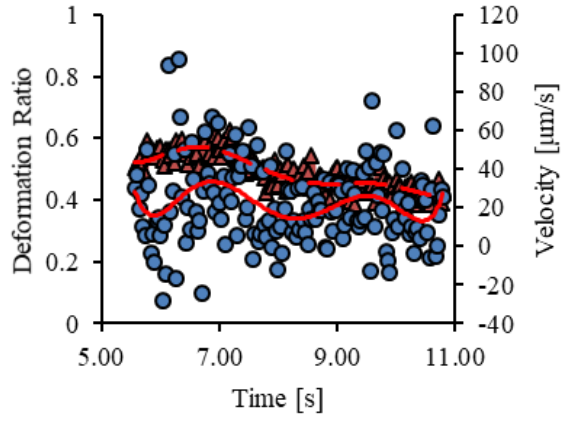

Fig. 4g: Tracings of deformation ratio and velocity: period 2.7 s, delay ratio 0.09.

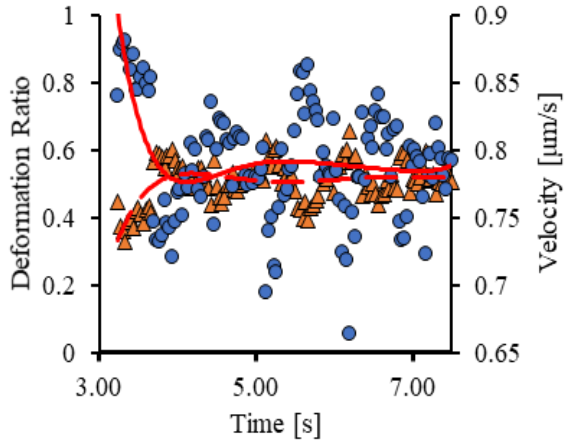

Fig. 4h: Tracings of deformation ratio and velocity: period 3 s, delay ratio 0.43.

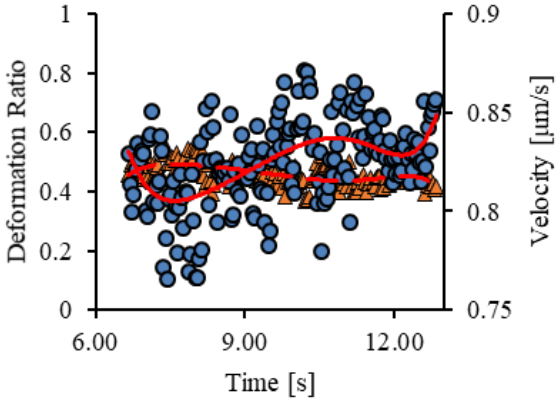

Fig. 4i: Tracings of deformation ratio and velocity: period 4.9 s, delay ratio 0.35.

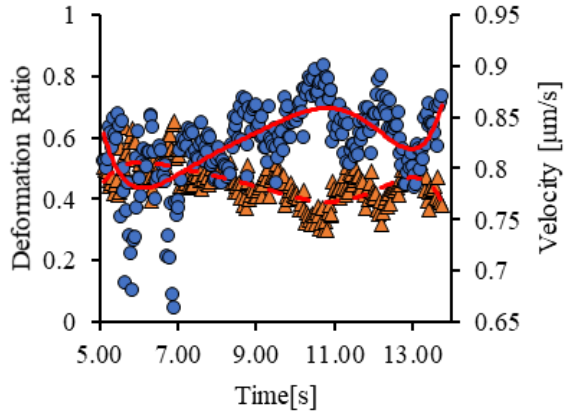

Fig. 4j: Tracings of deformation ratio and velocity: period 7 s, delay ratio 0.34.

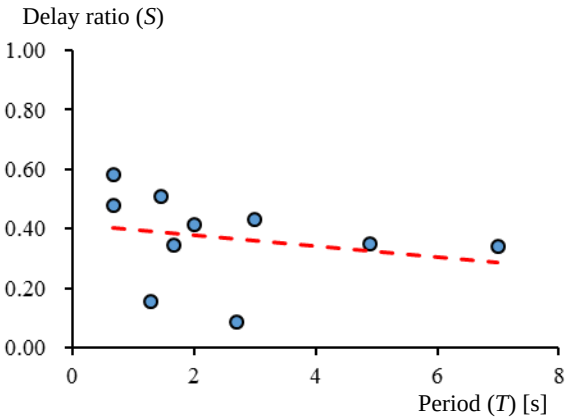

Fig. 5: Delay ratio (S) vs. period (T) [s]: dotted line shows regression line ($S = 0.42 – 0.019\ T$, $r^2 = – 0.25$).

Fig. 5 shows the relationship between the delay ratio and the period of cyclic fluctuation of the shear stress on ten erythrocytes. The dotted line shows the regression line on data (r^2 is the correlation coefficient). The delay ratio scatters, which can show the property depends on individuality of each erythrocyte. Some erythrocytes deform within the short delay. Even in the cyclic flow with the long period (with the low frequency) of seven seconds, deformation of the erythrocyte occurs with a certain ratio of the delay. In the cyclic flow with the period shorter than one second (with the frequency higher than 1 Hz), deformation of the erythrocyte tends to be delayed with the higher ratio.

4. DISCUSSION

The mechanism of erythrocytes deformability was a target for previous studies [2]. Deformability of erythrocytes might be an index for diagnostics [8]. Both the shear rate and the shear stress govern destruction of erythrocytes in the shear flow [14].

A micro flow channel could simulate the microcirculation system [1–6]. The effects of the shear flow on cells were observed in the previous studies [1–6]. The micro-slit is also useful for observation of deformation of a cell [15].

The cone and plate type instruments are used to make uniform Couette type flow [12, 14]. 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. Because the hardness of the disks supports the preciseness of the gap distance between two disks, the glass is selected for the material of disks in the present study.

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 27 mm. The shear rate in the observation area is approximately mean value in the whole volume of the suspension between two disks.

In many cases, each erythrocyte keeps own position in the same plane, so that the velocity of each erythrocyte shows the velocity of the layer, where each erythrocyte is floating. In some cases, on the other hand, erythrocyte moves over the center stationary plane, and moves to the counter direction (Figs. 4f & 4g).

In the shear field, the difference between speeds of two layers (Δv [m s^{-1}]) is calculated by Eq. (6).

$$\Delta v = \gamma\, \Delta x \tag{6}$$

In Eq. (6), γ is shear rate [s^{-1}], and Δx is the distance between two layers [m]: Δv is 68 μm s^{-1} at γ of 6.8×10 s^{-1} with Δx of 1 μm. When the erythrocyte moves to the neighbor layer of the shear flow, the velocity of the erythrocyte varies. The variation might cause scattering of the velocity of the erythrocyte at the tracings (Fig. 4).

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.

$$Re = \rho\, d\, V / \eta \tag{7}$$

In Eq. (7), ρ is density [kg m^{-3}] of the fluid, d is distance [m] between two parallel disks, η is viscosity [Pa s] of the fluid, and V is the circumferential velocity of the disk [m s^{-1}].

Re is smaller than 0.02 at ρ (10^3 kg m^{-3}), η (> 0.02 Pa s), d (< 1.2×10^{-4} m), and V (< 2.7×10^{-3} m s^{-1}) in the present experiment. The number is smaller than 1, so that the turbulent flow hardly occurs. The inertial effect is very small around the middle plane, where the velocity is low.

In the present study, the shear rate is periodically varied to simulate the pulsatile flow. Womersley number (α) is a useful index for estimation of the viscous effects on the pulsatile flow.

$$\alpha^2 = d^2\, (2\pi f\, \rho / \eta) \tag{8}$$

In Eq. (8), ρ is density [kg m^{-3}] of the fluid, d is distance [m] between two parallel disks, η is viscosity [Pa s] of the fluid, and f is the frequency of the oscillations of the disk [s^{-1}].

Womersley number (α) is smaller than 0.01 at ρ (10^3 kg m^{-3}), η (> 0.02 Pa s), d (< 1.2×10^{-4} m), and f (< 2 s^{-1}) in the present experiment. The number is smaller than 1, so that the inertial effect is very small [16].

Each 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 an erythrocyte 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.

In Fig. 3 in the present study, every cell in two-dimensional projection shows ellipse, which means ellipsoidal shape. The

disk, on the other hand, would show circle or ellipse in two-dimensional projection according to the angle. The deformation ratio of 0.65 shows that the erythrocyte is exposed to the shear stress around 3 Pa in Couette flow in the present study. The erythrocyte with high flexibility would make the cyclic deformation with very short delay to follow the periodically fluctuated shear stress. The periodical velocity fluctuation was used to trace the phase of cyclic shear stress fluctuation in Couette flow in the present study.

5. CONCLUSION

The cyclic deformation of an erythrocyte has been measured microscopically in the pulsatile shear field to detect the dynamic deformability of an erythrocyte *in vitro*. A rheoscope system with a pair of counter rotating disks has been manufactured to observe deformation of the erythrocytes suspended in the dextran aqueous solution. The experimental results show that the system is available to measure the cyclic deformation of the ellipsoidal shape of each erythrocyte delayed from the sinusoidally fluctuated shear field.

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