Evaluation of Blood Cell Destruction by Measuring Occlusion Distance

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Abstract: - Roller pumps are commonly used for electric motor-driven blood purification. Even the optimal occlusion for a roller pump is stimulated in JIS (Japanese Industrial Standard) -T1603, the blood cells can be destroyed if an applied pressure is too strong on the tube. On the other hand, the perfused blood volume might decrease if the pressure becomes weaker. Therefore, skilled operation is required. However, as there are no techniques to automatically measure occlusion, a highly reproducible method is urgently required to obtain an optimal setting. In this study, we classified the occlusion specified in JIS-T1603 into five categories (3, 6, 9, 12, and 15 drops/min) and measured those using a laser sensor. The distance between each occlusion was only a few microns. Based on the microscopic observation of the blood cell morphology at each occlusion, the blood cells with normal outlines were classified as normal blood cells, while those with protrusions were labeled as acanthocytes. Further, we calculated the normalized milligram index of hemolysis (mgNIH) to confirm hemolysis for each occlusion. By classifying occlusion into five categories and converting them into distances, we derived a safe, easy, and highly reproducible method.

Key-Words: - occlusion, occlusion distance, mgNIH, acanthocytes, red blood cells, roller pump, laser sensor.

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1 Introduction

Currently, available medical pumps, especially infusion pumps, syringe pumps, and implantable axial flow pumps are indispensable for treatment. In particular, medical pumps used for extracorporeal circulation, broadly classified into centrifugal and roller pumps, are crucial for maintaining the body's physiological homeostasis, [1], [2], [3]. Roller pumps are inexpensive, easy to operate, and provide stable irrigation flow, [4].

According to Japanese Industrial Standards (JIS)-T1603, the optimal occlusion setting for a roller pump is obtained by clamping the inlet side of the circuit tube, opening the outlet toward the atmosphere, and connecting the infusion tube of the infusion circuit at a height of 1m, [5]. However, this adjustment method has a strong subjective element and low reproducibility because the falling speed is confirmed visually, [6], [7]. Moreover, changes in occlusion alter the perfusion volume and blood cell morphology, significantly impacting the therapy during extracorporeal circulation, [8], [9]. Therefore, there is a need for a highly reproducible and quantitative occlusal adjustment method, [10]. One method with high reproducibility is to use a laser

sensor to convert the tiny occlusion gap into distance. By quantifying the occlusion distance using a laser, a quantitative and highly reproducible method can be established.

In this study, to establish a highly reproducible occlusion setting method, we used a laser sensor to classify the occlusion into 3 drops/min, 6 drops/min, 9 drops/min, 12 drops/min, and 15 drops/min. The distance was measured. At this time, when quantified using mgNIH (Normalized Milligram Index of Homolysis), which indicates haemolysis, the percentage of acanthocytes was as high as 34.04% at the short distance of 3 drops/min, and the average of mgNIH the value was 178.83mg/L, which was a high percentage, [11]. On the other hand, at the longer distance of 15 drops/min, the rate of acanthocytes was low at 8.15%, and the average value of mgNIH was low at 37.22 mg/L.

2 Materials and Methods

2.1 Five Occlusion Categories Settings

Setting methods using static and dynamic pressures have been reported for roller pumps. Here, we adopted the static pressure occlusion method by clamping the tube outlet side, opening the inlet side to the atmosphere, and dropping 5-10 drops/min from a height of 1 m from the pump (6-13 drops/min due to changes in the standards of the infusion

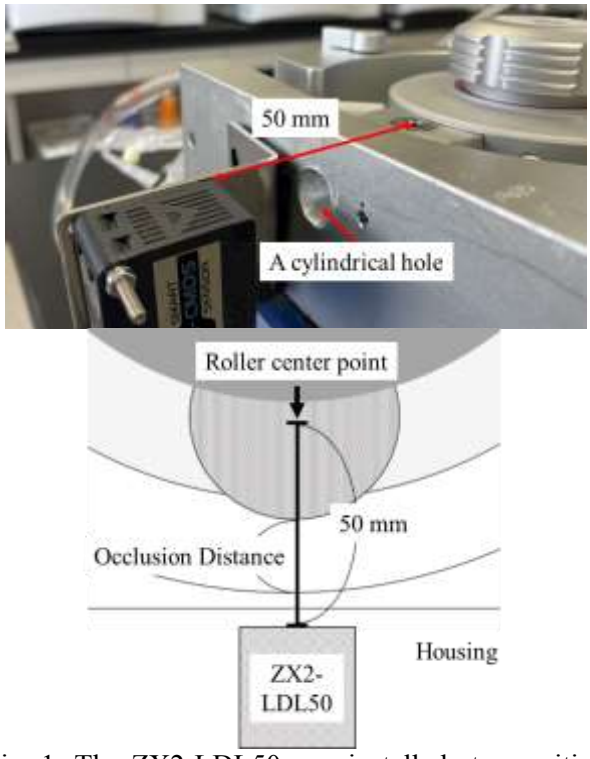

Fig. 1: The ZX2-LDL50 was installed at a position 50mm from Roller center point.

circuit), [12]. Based on JIS-T1603, which is considered to be the optimum occlusion, we used five occlusion categories: 3, 6, 9, 12, and 15 drops/min.

2.2 Sensor Performance

We fixed a ZX2-LDL50 device (Omron Corp., Kyoto, Japan) outside the housing 50 mm from the center of the roller and constructed a system for measuring the distance of occlusion. A cylindrical hole (20 mm in diameter) was made on the housing side to utilize the scattered light of the laser beam. The sensor utilizes a complementary metal oxide film semiconductor method with a triangular side distance method to detect the light intensity of the pixel and convert it into the distance.

The ZX2-LDL50 irradiated visible semiconductor laser of 660 nm was used to measure the occlusion distance. The measurement center distance was 10 mm out of 50 mm, the resolution was 1.5 μm, and the linearity was 0.05% full scale (measurement range). This sensor can also irradiate a linear beam and convert the amount of light in the pixel into a distance with a temperature characteristic of 0.02% full scale/°C.

The ZX2-LDL50 has a measurable range of 50 mm ± 10 mm, and it can measure the collapsed distance of the tube when the roller moves from the center point toward the housing. This device can also automatically measure the occlusion distance of up to 10 μ units (Figure 1), [12], [13].

2.3 Blood Circulation

Pig blood (Tokyo Shibaura Organ Co. Ltd, Tokyo, Japan), Meiron (115 mEq ; Fuso Pharmaceutical Industries, Ltd. Osaka, Japan), heparin (7.7 U/kg ; Nipuro Corp., Osaka, Japan) and MAP solution (D-Mannitol 14.57 g/L, Adenine 0.14 g/L, Sodium phosphate monobasic 0.94 g/L, sodium citrate 1.5 g/L, citric acid 0.2 g/L, dextrose 7.21 g/L, sodium chloride 4.97 g/L) were added to the circulation circuit to create a perfusate solution, which was circulated within the Excelline H3/8-inch tube (MERA Corp., Tokyo, Japan) using a HAS-150 roller pump (MERA Corp., Tokyo, Japan). Before starting the experiment, the distances for each of the five occlusion categories were measured. Thereafter, it was circulated for 180 minutes at a rotational speed of 150 rpm, and blood was collected every 30 minutes. At the same time, the volumetric flow rate was measured using a magnetic flowmeter MIM (Kobold Messring GmbH., Nordring Germany). Figure 2 shows the circuit diagram of the above experiment.

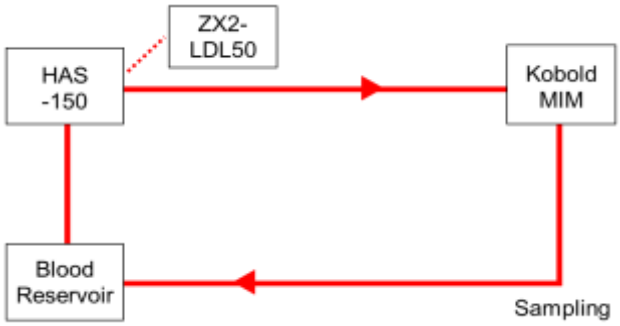

Fig. 2: Experimental closed-circuit diagram

2.4 Determination of Blood Cell Morphology

To confirm the blood cell morphology for all five occlusion categories, a 1 mL sample was collected from the blood circuit, and a smear specimen was prepared. The morphology of the prepared smears was observed using an optical microscope (Olympus Co. Ltd, Tokyo, Japan) at 1000x magnification and after 0 and 180 min. The red blood cells with round morphology were classified as normal, while those with protrusions were classified as acanthocytes. Further, we performed Wright-Giemsa staining following the manufacturer's protocol (Muto Pure Chemical Ltd., Tokyo, Japan) with the required staining reagents (Muto Pure Chemical Ltd.).

2.4.1 Calculation of mgNIH

To calculate the mgNIH, we collected the blood in the circulation circuit and centrifuged it at 1500 rpm for 10 minutes. We measured the free hemoglobin in the supernatant using Plasma HemoCue (HemoCue AB, AMCO Inc., Japan). Then, we measured the RBCs (Red blood cells), hemoglobin (Hb), and hematocrit (Hct) using the MEK-7300 Celltac Es hemocytometer (Nihon Kohden Co. Ltd, Tokyo, Japan). After extracting these items, the mgNIH was calculated as follows, [14].

$$mgNIH = \frac{\Delta pfHb \times v \times \left(\frac{100 - Hct}{100}\right)}{Q \times T}$$

$\Delta PfHb$: increment of plasma free hemoglobin concentration (mg/dL)
V : whole blood volume in flow loop (mL)
Hct : hematocrit%
Q : flow rate (L/min)
T : sampling period (min)

2.4.2 Evaluating the Relationship between the Occlusion Distance and mgNIH

To investigate the relationship between blood cell destruction and occlusion, we measured the occlusion distance and mgNIH at 3, 6, 9, 12, and 15 drops/min. We performed Welch's T-test at $p < 0.01$ using Excel statistics to identify the significant differences between occlusion distance and mgNIH.

3 Results

3.1 Occlusion Distance

Figure 1 shows occlusions at 3, 6, 9, 12, and 15 drops/min converted to distance. At 3 drops/min, the distance and dispersion are higher than other occlusions. Also, at 6 drops/min, the distance and dispersion were higher than at 9, 12, and 15 drops/min. The mean values and standard deviations of occlusion distances are shown in Table 1. From Table 1, it can be seen that 3 drops/min, and 6 drops/min are larger than the dispersion of 9 drops/min, 12 drops/min, and 15 drops/min.

Table 1. Mean value and standard deviations (SD) of occlusion distance when repeated 5 times

Drops rate	3	6	9	12	15
n	5	5	5	5	5
Mean (mm)	1.2051	1.191	1.1818	1.1771	1.1740
SD (mm)	0.0188	0.0097	0.0039	0.0038	0.0035

3.2 Relationship between Occlusion Distance at 0 and 180 min and Blood Cell Morphology

Figure 3 shows the morphology of blood cells in five occlusions. At the start time of 0 min, the percentage of acanthocytes in all occlusions was less than 10%. Figure 4 shows the morphology of each blood cell at five occlusion categories after 180 min. The number of acanthocytes was high at 3 drops/min and 6 drops/min, and there was no significant change at 9 drops/min, 12 drops/min, and 15 drops/min. Table 2 lists the respective occlusion and acanthocyte percentages at 0 min. Table 3 lists the respective occlusion and acanthocyte percentages at 180 min.

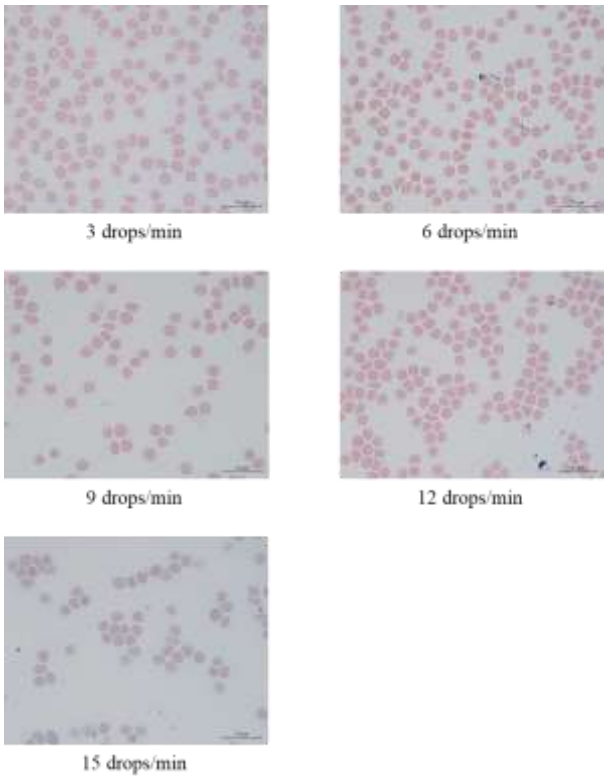

Fig. 3: Five occlusions' categories and blood cell morphology at 0 min

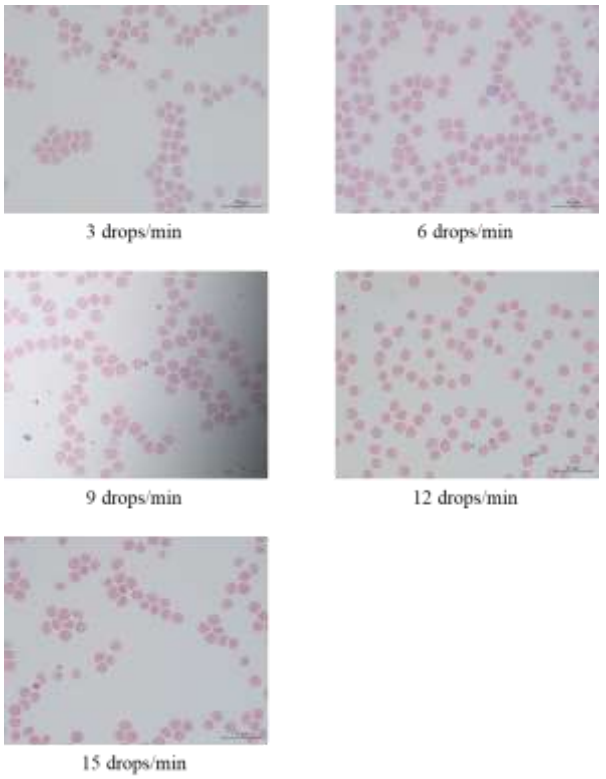

Fig. 4: Five occlusions' categories and blood cell morphology at 180 min

Table 2. Percentage of acanthocytes, mean and standard deviation (SD)

Percentage of acanthocyte at 0 min (%)					
Drops rate	3	6	9	12	15
1st	2.20	3.88	8.76	9.03	4.60
2nd	3.16	3.79	7.39	8.36	5.42
3rd	4.83	6.46	8.47	7.45	4.68
4th	4.25	5.32	6.87	5.13	8.12
5th	6.21	5.81	8.58	4.72	7.42
Mean	4.13	5.05	8.01	6.94	6.05
SD	1.38	1.06	0.75	1.72	1.45

Table 3. Percentage of acanthocytes, mean and standard deviation (SD)

Percentage of acanthocyte at 180 min (%)					
Drops rate	3	6	9	12	15
1st	35.14	22.83	9.14	7.88	8.31
2nd	29.92	25.94	11.77	11.12	7.81
3rd	31.22	24.27	10.2	9.57	7.40
4th	37.75	24.16	12.54	8.23	9.12
5th	36.17	21.92	12.23	9.08	8.09
Mean	34.04	23.82	11.18	9.18	8.15
SD	2.98	1.37	1.30	1.14	0.57

3.3 Relationship between Occlusion Distance and Blood Cell Destruction

We observed a large distribution for mgNIH at 3 drops/min, and the average value was 178.83 mg/L (Figure 5). Whereas the mgNIH was low at 15 drops/min, with an average value of 37.22 mg/L. Also, no significant difference could be confirmed for 3 and 6, but significant differences could be confirmed for the other 9, 12, and 15 drops/min ($p < 0.01$).

Further, although the initial values of mgNIH for the five occlusion categories are similar, the standard deviations for mgNIH at 3 and 6 drops/min were much higher than those at 9,12, and 15 drops/min. These values indicated that 3 and 6 drops/min had higher hemolytic indices than 9,12 and 15 drops/min.

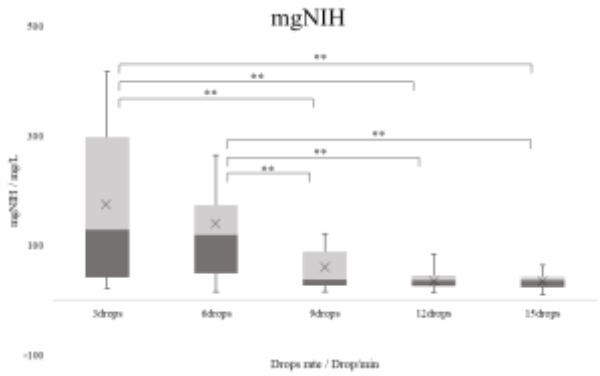

Fig. 5. Relationship with Plasma free Hb each occlusion

4 Discussion

4.1 Occlusion Distance for all Five Occlusion Categories

In extracorporeal circulation, a quantitative and highly reproducible setting method is required rather than setting the occlusion based on skill and experience. Here, we converted the five occlusion categories into distances using a laser sensor. Since the distance of the converted occlusion is extremely small, with a difference of several tens of microns, precise manipulation was required. Moreover, the reproducibility of the settings based on intuitive manipulation might be low. Based on these points, we evaluated the occlusion changes from two points of view, morphological and numerical.

4.2 Relationship between Occlusion Distance and Blood Cell Morphology

After 180 minutes, the rate of acanthocytes increased at 3, and 6, drops/min, but was similar at 9,12, and 15 drops/min. This might be because of the decrease in the amount of sialic acid due to the prolonged force on the blood cells, which might change the internal structure, [15]. The surface of red blood cells (RBCs) is negatively charged, and the amount of sialic acid determines the repulsive force between adjacent blood cells. In addition, the decrease in the amount of sialic acid and loss of other proteins decrease the repulsive force between blood cells and increase the rate of red blood cell aggregate formation. This reduction in the repulsive force might also reduce the viscosity of the plasma component of the Newtonian fluid.

When RBCs pass through narrow blood vessels, such as capillaries, their morphology changes due to the Tancred movement in the presence of certain conditions, including low Reynolds number, pressure, and velocity. Based on this theory, the morphology of the RBCs might change when they pass through the rollers of the pump. As a characteristic of Tancred movement, the biconcave shape can be changed vertically and horizontally to enable passage through blood vessels whose diameters are smaller than blood cells. Even though RBCs are subjected to uniform pressure as they pass through the narrow path, the membrane itself has a high restoring force and returns to its original biconcave shape. However, at 3 drops/min, the yield value limit of the RBCs' membranes might have exceeded as the pressure on the tube is higher than that at 15 drops/min. Originally, the RBCs' membranes were thought to undergo hemolysis due to high shear stress. However, reports have shown that the RBCs' membranes do not rupture immediately and are highly contractile over a wide range. In other words, when high pressure is applied to the RBCs, they do not immediately deform as they can withstand a few stretching cycles. After that, it can be assumed that the RBCs lose their shape and turn into acanthocytes.

4.3 Relationship between Occlusion Distance and mgNIH

Originally, when blood encounters the air, it undergoes anaerobic metabolism, which promotes the destruction of RBCs. Furthermore, the application of strong forces, such as shear stress, damages RBCs, which seems to be the case in this study.

From Figure 5, the average distance for 3, 6, 9, 12, and 15 drops/min is 1.2051, 1.191, 1.181, 1.1771, and 1.174 mm, respectively. There was a maximum difference of 0.031 mm between 3 and 15 drops/min, which is half the thickness of European and American hair. The occlusion distance of 3 drops/min against 15 drops/min is 0.03 mm when pushed into the housing, which might be due to the strong pressure applied at 3 drops/min that also increases the mgNIH.

Considering the difference in occlusion distance (mean value) between 3 drops/min and 15 drops/min, the momentum imparted to the RBCs when the roller is translated can be substituted into the following equation, where p is the power, m is the mass and v is the angular velocity calculate.

$$p = m \times v \tag{1}$$

It can be expressed by the formula of (1)

Let r be the position of the mass from the origin and the angular momentum vector measured from the origin can be expressed by the following equation.

$$L \equiv r \times p \tag{2}$$

Substitute the formula of (1) into (2).

$$L \equiv m \times r \times v \tag{3}$$

Based on angular momentum, v rotates n times per minute.

$$n = 1 / t = \omega / 2\pi$$

Therefore,

$$\omega = 2\pi n$$

It can represent.

$$L \equiv m \times r^2 \times 2\pi n \quad (4)$$

Also, the impulse generated when the roller encounters the RBCs through the tube from the moment of inertia has been considered (ignoring the elastic force of the tube).

$$I = F\Delta t \quad (5)$$

$$I = m \times R^2 \times \Delta t \quad (6)$$

p : Linear momentum
L : Angular momentum
v : angular velocity
r : Radius
t : Second
ω : Angular velocity
F : Impulse
I : Moment of inertia
R : Distance of roller movement from roller center to housing 0.00064 m
m: Roller mass 0.375 kg
t : 180 × 60 sec

At occlusion of 3 and 15 drops/min, the average distances to the housing side were 1.2051 mm and 1.1740 mm, respectively. Substituting these into equation (6), the difference in thrust between 3 and 15 drops/min is 1.54×10^{-9} kg・m. RBCs are deformed by shear stress. The limit value of membrane plasticity is based on the following equation, [16].

$$1.6 \times 10^{-2} \text{ dyn / cm} \leq \text{T0} \leq 8 \times 10^{-2} \text{ dyn / cm} \quad (7)$$

This surface tension unit can be expressed as N/m = dyn/cm = kgf/m, and to convert the obtained impulse to dyn/cm, it should be divided by the product of mass and time.

The moment of force on the RBCs is 116.25 dyn/cm, which is approximately 1400-7200 times the yield limit of the RBCs' membranes. However, considering the elastic resistance of the circuit tube, although the actual pressure applied to the blood is reduced, the long-term accumulation of force might cause blood cell destruction. Therefore, considering the moment of force, at 3 drops/min, the applied pressure is approximately 1400 to 7200 times higher than that of 15 drops/min, which might accelerate the rupture of the RBCs. Also, in terms of impulse, the RBCs go around a circuit of 1000 mm once every 2 seconds. Therefore, the blood damage might be massive under a 1400 to 7200 times higher pressure multiplied by 90 minutes x 60 seconds.

4.4 Clinical Indication

In our occlusion experiments, mgNIH values were lower at 9, 12, and 15 drops/min than at 3 and 6 drops/min. However, according to JIS-1603, occlusion at 6 to 13 drops/min is considered optimal. This is because as the occlusion loosens, the volume flow circulating in the circuit decreases, and the cannula tip may collide with arterial pressure, making it impossible to maintain the target perfusion rate. Considering the above, 6 to 12 drops/min is considered optimal.

5 Conclusion

When using a roller pump for extracorporeal circulation, too strong an occlusion pressure may promote blood cell destruction, and a weak occlusion pressure may reduce the perfused blood volume. Therefore, there is an urgent need for a method to achieve optimal and highly reproducible occlusion. In this study, we developed a safe, easy, and highly reproducible method by classifying occlusal conditions into five categories and converting them into distances using a laser sensor.

The main results are as follows:
(1) To establish a highly reproducible occlusion setting method, we used a laser sensor to convert five occlusion categories into 3 drops/min, 6 drops/min, 9 drops/min, 12 drops/min, and 15 drops/min.
(2) The laser sensor can also automatically measure the distance the roller moves from the center point toward the housing.
(3) The results suggested that occlusion at 3 drops/min caused higher blood cell destruction than that at 15 drops/min. However, considering the perfusion pressure of the human body at 13 drops/min, the perfusion amount may decrease.
(4) We concluded that the occlusion in the range of 6 drops/min to 12 drops/min is optimal.
(5) In the future, we would like to perform reflux at the same pulse pressure as in the human body and measure the amount of sialic acid at that time. Additionally, we would like to establish a roller pump that causes less blood cell destruction compared to centrifugal pumps.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

- Shota Kato conducted all of the experiments and wrote the submitted paper.
- Tadashi Handa performed the staining of the smear specimens.
- Jun Yoshioka created a blood cell preservation solution.
- Kazuhiko Nakadate prepared and analyzed the blood smears.
- Yasutomo Nomura performed the physical analysis of blood cell destruction and constructed the entire experiment.

The entire study was supervised by Hitoshi Kijima.

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Conflict of Interest
The authors have no conflicts of interest to declare.