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CLOT GROWTH UNDER PERIODICALLY FLUCTUATING SHEAR RATE

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ABSTRACT **To control the morphology of a clot formed on an artificial flow path in pulsatile blood flow, the hydrodynamic effect of periodically fluctuating shear rate on clot growth has been quantitatively investigated *in vitro*. Uniform shear rates were applied to a sample of beagle blood in the concave-convex cones system. These shear rates were sinusoidally fluctuated between a maximum and a minimum in one direction at frequencies between 0.1 and 0.6 Hz. Evaluation of clot growth was derived from a clot ratio, which was experimentally determined from the rate of increase of frictional torque between the two cones. The results show that clot growth is controlled so as not to occupy a large space when the minimum shear rate is higher than 100 s^{-1}, or when the time of application of lower (< 100 s^{-1}) shear rates is modified by the intermittent application of higher (> 500 s^{-1}) shear rates as long as the frequency is less than 0.6 Hz.**

Introduction

Many factors control the dynamic balance between the formation and removal of clots. Through this control, the ruptured vessel wall is repaired and the flow path maintained. These factors are present in both the blood and the vessel wall. They include platelets, coagulants and anti-coagulants. Many studies have been performed in this field from the biochemical point of view, which includes the interaction between the blood and the surface materials (Salzman, 1981). On the other hand, the clot has been considered to grow up around a stagnation flow point. In a previous study (Hashimoto *et al.*, 1985), clot growth under steady shear flow was investigated with a cone and plate viscometer, and the results demonstrate that the morphology of the clot is controlled so as not to plug the flow path at shear rates of > 500 s^{-1}. In arteries or in the flow paths of extra-corporeal circulation powered by pulsatile pumps, on the other hand, periodically fluctuating shear rates are applied to the blood. Shear rates between zero and 500 s^{-1} are unavoidably applied to the blood in this case. If the peak shear rate of each pulse cycle interrupts the term of lower shear rates (t) and washes away the conditions for clot growth, both the maximum shear rate of each pulse and a shorter t for each pulse may be effective in preventing clot growth. To discover the conditions controlling the morphology of clots formed in the artificial flow path in pulsatile blood flow,

KEY WORDS: **Blood; clot; shear; Couette flow; torque; pulsatile flow**

this study investigates clot growth under sinusoidally fluctuating shear rate quantitatively *in vitro* using the modified concave-convex cones test system (Hashimoto, 1989).

Materials and Methods

Concave-convex Test System

In order to apply uniform shear rates to the blood, the concave-convex cones test system was created (Fig. 1). The principle used by the concave-convex cones system to create the uniform shear field is the same as that of the cone and plate system (Hashimoto *et al.*, 1985). The concave-convex cones system differs from the cone and plate system in that: (i) Concave and convex cones replace the cone and plate; (ii) the rotational speed of the cone fluctuates sinusoidally in one-direction and (iii) the frictional torque is measured continuously.

The combination of concave and convex cones has some advantages over that of cone and plate: (a) It neutralizes the tendency for erythrocytes to aggregate in the rim of the circular cone; (b) it equalizes the shear rate applied to the whole blood sample even at the rim of the circular cone; (c) it simplifies the removal of air from the space between the concave and convex cones; (d) it simplifies the collection of blood from the concave cone after the test; and (e) it minimizes secondary flow with the force of inertia by changing the rotating part from the inner to the outer cone. Among these advantages, both (b) and (c) have been pointed out as defects of the cone and plate system in a previous study (Monroe *et al.*, 1981).

Fig. 2(A) shows the velocity distribution (Couette-type flow) induced in a blood sample placed between a stationary convex cone (diameter, 4.8 cm) and a rotating (clockwise) concave cone. The shear rate, γ, calculated from the velocity distribution, is constant regardless of the value of x (the distance between P and S in Fig. 2), according to the following equation:

$$(1) \qquad \gamma = \frac{u}{\delta} = \frac{\omega\, x \sin\left(\frac{\beta}{2}\right)}{x\,\theta} = \frac{\omega \sin\left(\frac{\beta}{2}\right)}{\theta},$$

where u is the circumferential velocity at S on the concave cone, δ is the distance between S and Q (Fig. 2), ω is the angular velocity (rad s^{-1}) of the concave cone, θ is the angle (0.056 rad) between the convex and concave cones, and β is the apex angle of the cone (2.10 rad). Note that in deriving this equation and those to follow, θ is assumed to be very small.

These shear rates (γ) were controlled in proportion to the angular velocity of the concave cone in one direction ($3.2 < \omega < 52$ rad s^{-1}; see Eq. *(1)*) by a function generator (Fig. 1), and were periodically fluctuated with variations in the following three independent factors in a sinusoidal mode (exemplified in Fig. 3): (i) The maximum ($\gamma_{max} < 800$ s^{-1}); (ii) the minimum ($\gamma_{min} > 25$ s^{-1}) of shear rates and (iii) the frequency of fluctuation ($0.1 < f < 0.6$ Hz). Both cones were made of transparent polymethylmethacrylate to enable the blood sample to be observed during tests.

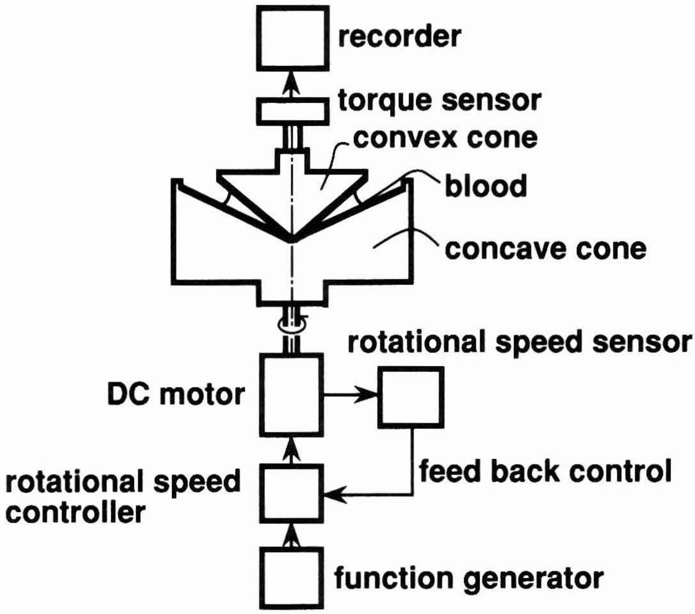

Fig. 1. Concave-convex cones test system.

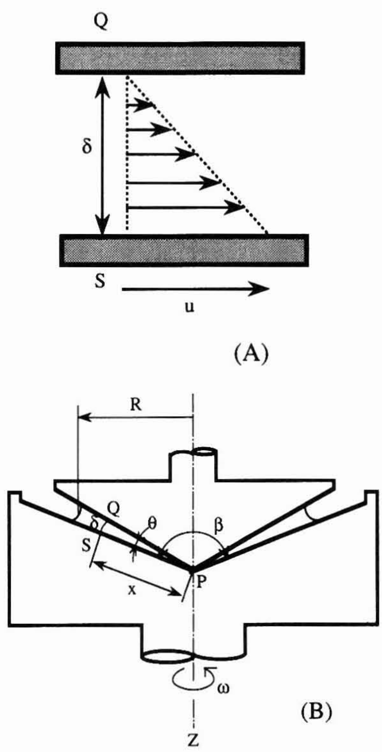

Fig. 2. Velocity distribution between S and Q (A), shear rate between a stationary convex cone and a rotating concave cone (B).

The frictional torque, T (dyn/cm), between the convex and concave cones is given by

$$(2) \qquad T = \int_0^R \frac{2\pi r^2 \eta \gamma}{\sin\left(\frac{\beta}{2}\right)} dr = \frac{\frac{2}{3}\pi\eta R^3 \gamma}{\sin\left(\frac{\beta}{2}\right)},$$

where η (dyn s cm^{-2}) is the apparent viscosity of the blood, and R (cm) is the radius of the shearing portion of which the blood sample is a part (Fig. 2). Combining Eqs. *(1)* and *(2)*, we obtain:

$$(3) \qquad T = \frac{\frac{2}{3}\pi\eta R^3 \omega}{\theta}.$$

Eqs. *(1)*, *(2)* and *(3)* are applied not only to steady flow but also to quasi-steady flow, such as sinusoidally fluctuated flow at the frequency of < 0.6 Hz (see Discussion).

The frictional torque (T), which fluctuates between the maximum (T_{max}) and the minimum (T_{min}) in proportion to the shear rate (γ), is continuously measured by the strain gauge.

Clot Ratio

In each test, the blood sample (1.5 cm^3) was drained through a stainless steel needle from the jugular vein of an anesthetized beagle, and collected in a polypropylene syringe without anticoagulants. Immediately after the drainage (within 10 seconds), 1.0 cm^3 of the blood sample was placed between the convex and concave cones, and was sheared for 3–10 minutes, while the torque, T, was continuously measured (Fig. 4). When the torque, T, increased stepwise with clot formation, the rotation of the concave cone was stopped, and the clot was macroscopically observed. When the clot makes the angle, θ, narrow, the torque, T, increases, because the torque T is in inverse proportion to the angle, θ (see Fig. 5 and Eq. *(3)*). The remainder (0.5 cm^3) of the blood sample was poured into a glass tube and the coagulation time was measured without shear as a reference.

Evaluation of clot growth was derived from the clot ratio (Rc), which was experimentally determined from the rate of increase of torque, T, calculated in Eq. *(4)*.

$$(4) \qquad Rc = \frac{\left(1 - \frac{T_{max_0}}{T_{max_1}}\right) + \left(1 - \frac{T_{min_0}}{T_{min_1}}\right)}{2},$$

where T_{max_0} and T_{min_0} are torque before formation of the clot, and T_{max_1} and T_{min_1} are torque after formation of the clot. These torque values were read on torque tracings (Fig. 4). When the torque, T, does not increase ($T_{max_0} = T_{max_1}$ and $T_{min_0} = T_{min_1}$), Rc becomes zero. When the torque, T, increases markedly ($T_{max_0} \ll T_{max_1}$ and $T_{min_0} \ll T_{min_1}$), Rc approaches unity.

To minimize the scatter of data affected by clot capability fluctuation such as the fluctuation of coagulants-concentration in the circulating blood day by

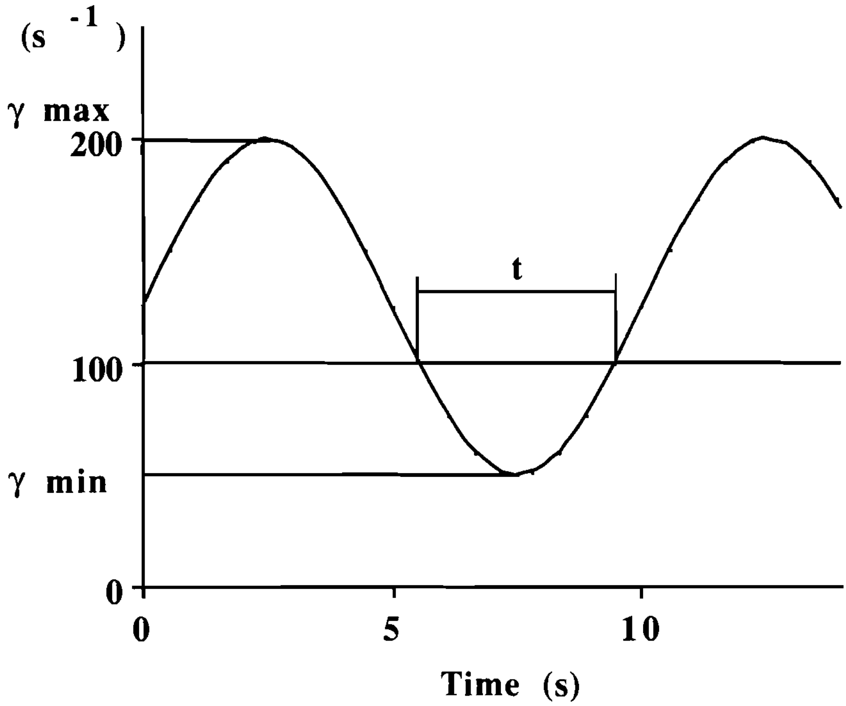

Fig. 3. Example of shear rate fluctuation in sinusoidal mode.

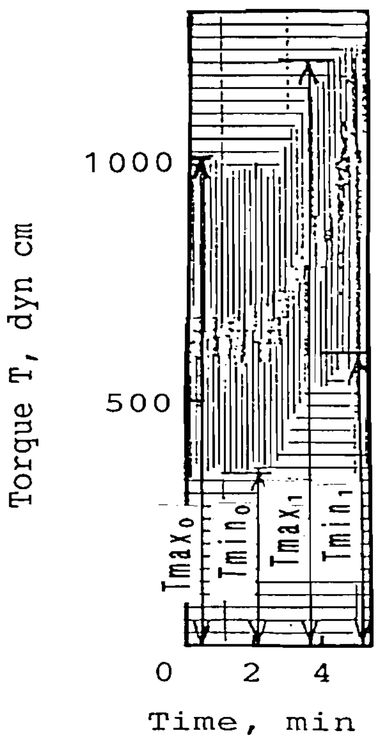

Fig. 4. Frictional torque tracings.

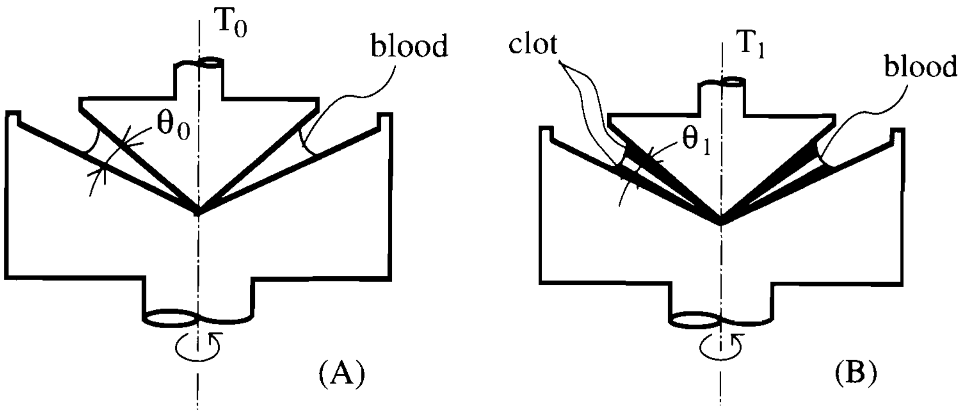

Fig. 5. Clot ratio (A) before clot formation; (B) after clot formation.

day, data arranged in each figure (Figs. 6–8) correspond to a set of data obtained from the same beagle within six hours, in each case. In the case of collecting data from a long-term test series (Fig. 9), values of the clot ratio in the same fluctuate condition of shear rate were compared as a reference.

Results

Fig. 6 presents clot ratio (Rc) as a function of maximum shear rate (γ_{max}), where γ_{min} is fixed at 50 s^{-1} and where frequency (f) is fixed at 0.1 Hz. The datum point at γ_{max} = 50 s^{-1} indicates Rc at the constant shear rate. The figure shows that Rc decreases as the maximum shear rate increases from 100 s^{-1} to 500 s^{-1}. Fig. 7A presents Rc as a function of minimum shear rate (γ_{min}), where γ_{max} is fixed at 750 s^{-1} and where f is fixed at 0.1 Hz. The datum point at γ_{min} = 750 s^{-1} indicates Rc at the constant shear rate. The figure shows that Rc is smaller than 0.5 and that Rc increases as the minimum shear rate decreases in the range of γ_{min} < 100 s^{-1}. Fig. 7B presents Rc as a function of minimum shear rate (γ_{min}), where γ_{max} is fixed at 650 s^{-1} and where f is fixed at 0.1 Hz. The datum point at γ_{min} = 650 s^{-1} indicates Rc at the constant shear rate. The figure shows that Rc increases as the minimum shear rate decreases and that Rc is bigger than 0.5 in the range of γ_{min} < 100 s^{-1}. Fig. 8 presents Rc as a function of the difference between maximum and minimum shear rate ($\gamma_{max} - \gamma_{min}$), where γmean is fixed at 300 s^{-1} and where f is fixed at 0.1 Hz. The datum point at $\gamma_{max} - \gamma_{min}$ = 0 s^{-1} indicates Rc at the constant shear rate of 300 s^{-1}. The figure shows that Rc is large when $\gamma_{max} - \gamma_{min}$ = 550 s^{-1}. Fig. 9 presents Rc as a function of frequency (f); where γ_{min} = 50 s^{-1} (A), γ_{max} = 650 s^{-1} (B), γ_{mean} = 300 s^{-1} (C), $\gamma_{max} - \gamma_{min}$ = 200 s^{-1} (D), respectively. The figure shows that Rc decreases as the frequency increases, as long as the frequency is less than 0.6 Hz. This tendency is marked when γ_{min} = 50 s^{-1} and

γ_{max} = 550 s^{-1}. On the other hand, Rc still has a small value even at 0.1 Hz, when γ_{min} = 500 s^{-1} and γ_{max} = 650 s^{-1}.

The coagulation times without shear in the glass tube stayed around a control value of ten minutes throughout the tests.

Discussion

The data on clot ratio (Rc) shown in Figs. 6–9 are rearranged in Fig. 10 as a function of the interval, t, shown in Fig. 3, of the shear rate < 100 s^{-1}. The datum point at "whole term" indicates Rc when the shear rate is smaller than 100 s^{-1} at every time. The figure shows that Rc increases as the interval of 100 s^{-1} becomes longer and that Rc is smaller than 0.5 when the interval is shorter than two seconds.

Reciprocating concave-convex cones made of polytetrafluoroethylene were used to shear the blood in a previous study (Dintenfass, 1966), in which frictional torque tracings were recorded by thromboelastograph (Hartert, 1951). The results showed that the clot formed in a shorter term and the rising ratio of frictional torque is small at the higher shear rate. In the present study, the concave cone does not reciprocate, but rotates in one direction. This one-way motion was chosen for this study to imitate pulsatile flow. The results shown in the present study are qualitatively consistent with those of the previous study.

The angular velocity of the concave cone is controlled to apply periodically fluctuating shear rates in the present study. As a useful dimensionless parameter, the Womersley number (W) has been proposed to estimate the ratio of inertial to viscous forces in periodically fluctuating flows:

$$W = \frac{d}{2}\sqrt{\frac{2\pi f \rho}{\mu}}, \tag{5}$$

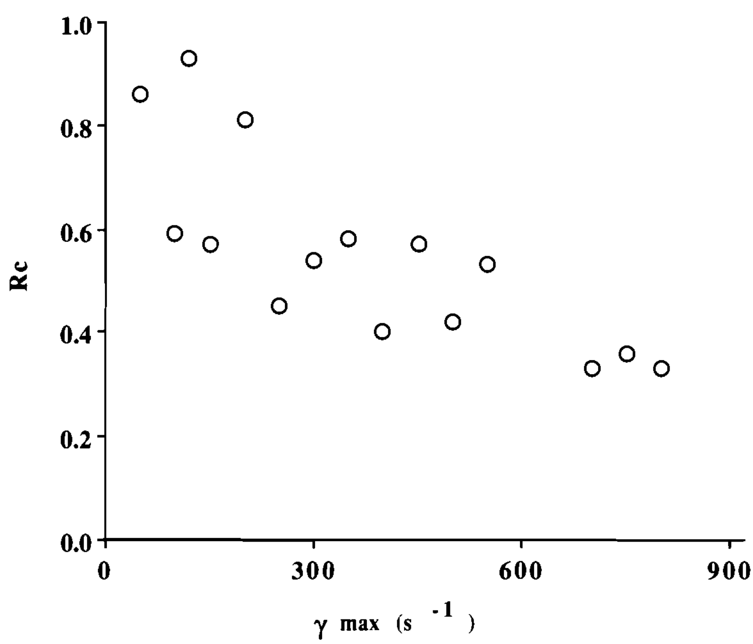

Fig. 6. Clot ratio, Rc, as a function of maximum shear rate.

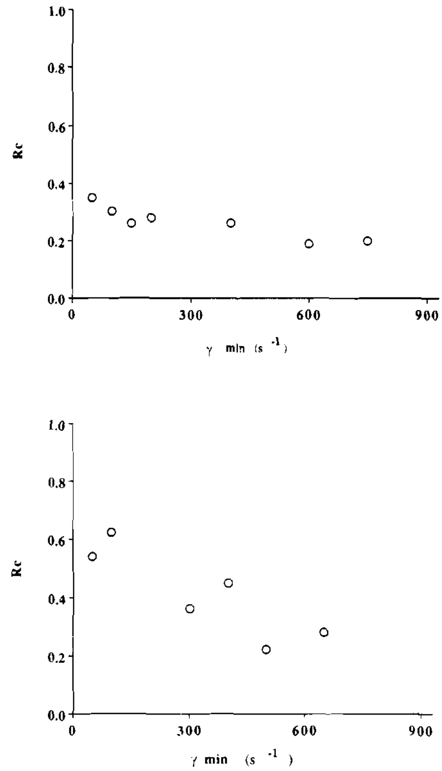

Fig. 7. Clot ratio, Rc, as a function of minimum shear rate: $\gamma_{max} = 750\ s^{-1}$ (A); $\gamma_{max} = 650\ s^{-1}$ (B).

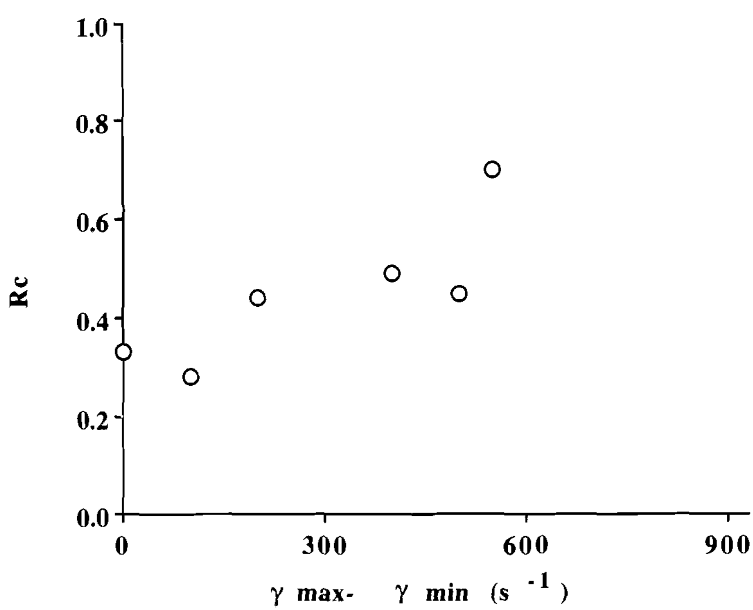

Fig. 8. Clot ratio, Rc, as a function of the difference between maximum and minimum shear rates.

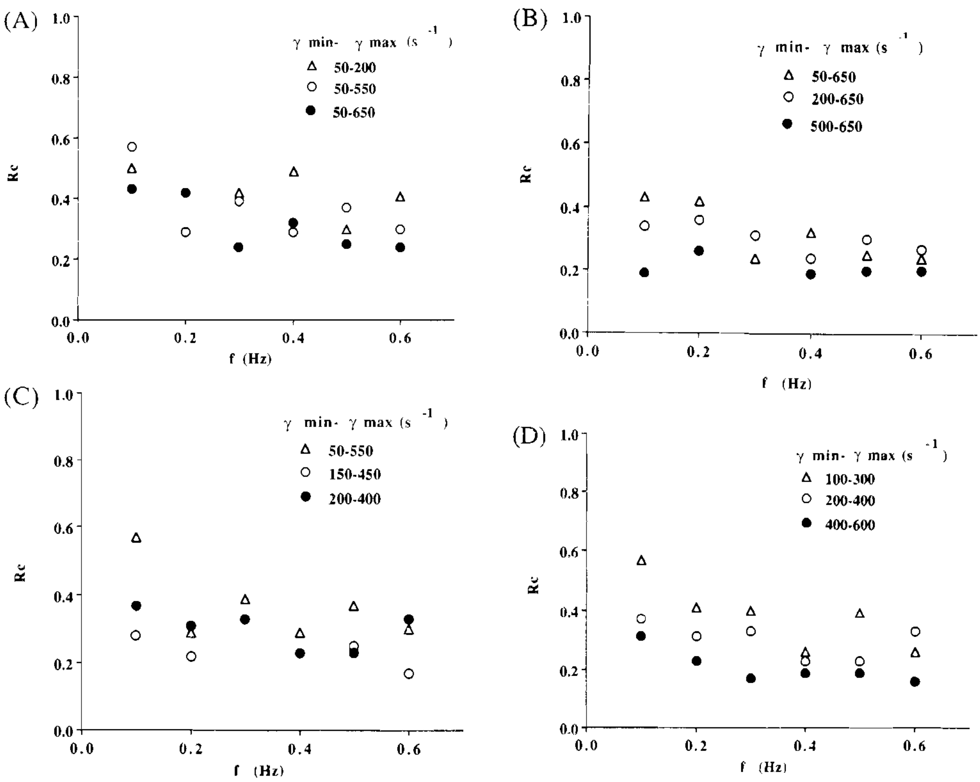

Fig. 9. Clot ratio, Rc, as a function of frequency: $\gamma_{min} = 50\ s^{-1}$ (A); $\gamma_{max} = 650\ s^{-1}$ (B); $\gamma_{mean} = 300\ s^{-1}$ (C); $\gamma_{max} - \gamma_{min} = 200\ s^{-1}$ (D).

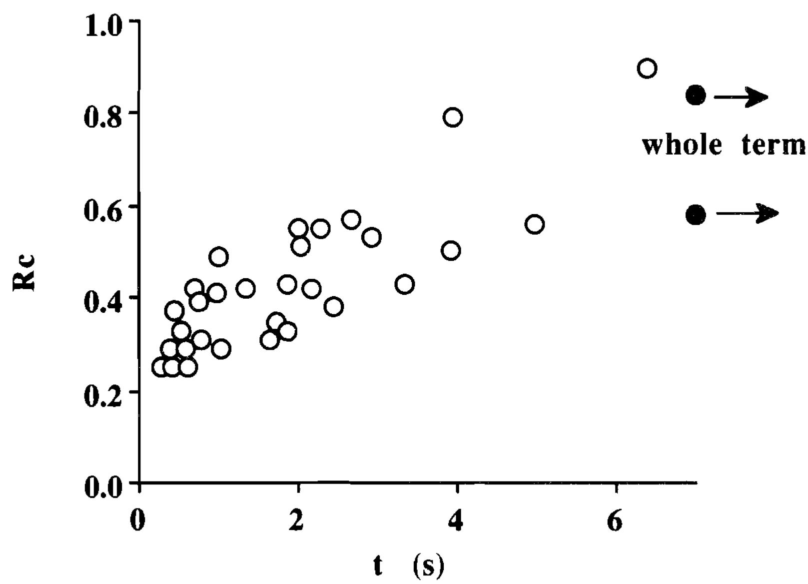

Fig. 10. Clot ratio, Rc, as a function of exposure time of shear rate of < 100 s^{-1}.

where d < 0.15 cm (distance between the convex and concave cones), $f < 0.6$ Hz (frequency), $\rho \approx 1$ g/cm^3 (density of blood), and $\mu > 0.05$ dyn s cm^{-2} (viscosity of blood at 24° C) in the present tests (shown in Figs. 6–9). Because W is calculated as a smaller value than unity in Eq. *(5)* (which indicates that inertial forces can be ignored (Caro *et al.*, 1978)), the flow between the concave and convex cones is estimated to follow the rotary motion of the concave cone. To limit the effect of inertial force, the frequency of fluctuation was controlled in the lower range (< 0.6 Hz) in the present study. If a more sophisticated system were developed to investigate the effect of fluctuating shear rate on clot growth at higher frequency, it might help indicate how long a term of $\gamma > 500$ s^{-1} is necessary to wash away the conditions for clot growth.

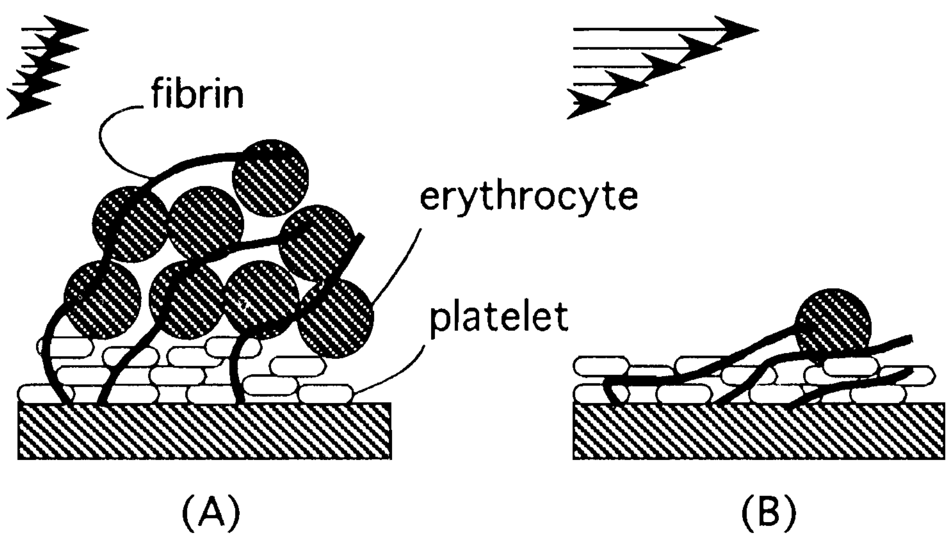

Fig. 11. Clot formed at low shear rate (A); at high shear rate (B).

Table 1

Several Effects of Shear Flow on Clot Formation

(a) Damage endothelium	+
(b) Activate platelets	+
(c) Promote fibril linkage with molecular orientation	+
(d) Activate precursor of coagulation factors	+
(e) Destroy coagulation factors	–
(f) Inhibit fibril linkage	–
(g) Inhibit adhesion of factors to walls	–
(h) Deform fibrin network and decrease the number of captured erythrocytes	–

+ = Promotion of clot formation
– = Inhibition of clot formation

Minimizing inertial force with the frequency of the fluctuating flow restricts imitating pulsatile flows in arteries; the flow rate in pulsatile flows does not fluctuate in a simple sinusoidal mode, but in a more complicated periodical mode which includes harmonic contents of higher frequency (Hashimoto, 1988). The present study relates to the periodically fluctuating flow rate in the simple sinusoidal mode.

There are some limitations to the test system in the present study. Clot ratio satisfactorily corresponds to the amount of optically observed clots, but the data reveal scatter (see Figs. 6–9). One of the reasons for data scatter is that clots did not take uniform shape, shown in Fig. 5(B), but took irregular shapes. Blood is a non-Newtonian fluid, and the apparent viscosity cannot be treated as a constant value at shear rates of $< 25\ s^{-1}$. This factor must be considered when the clot ratio evaluation is extended to the low shear rate range. Shear rates applied in the present study were chosen in the range of shear rates of blood flow in the canine cardiovascular system ($< 800\ s^{-1}$). This range is the same level as that in the human cardiovascular system (Whitmore, 1968).

Several effects of shear flow on clot formation have been pointed out by previous studies (Dintenfass, 1964; Hartert, 1974; Hashimoto, 1985; Spaeth, 1973). These effects are listed in Table 1. Effect (a) is excluded in the present experiment, because the test system does not include endothelium. The main effect of shear flow in the present experiment might be (h), which is schematically illustrated in Fig. 11.

References

CARO, C. G., PEDLEY, T. J., SCHRÖTER, R. C., and SEED, W. A. (1978). *The Mechanics of the Circulation.* Oxford University Press, Oxford, pp. 57–60.

DINTENFASS, L. (1964). Viscosity and clotting of blood in venous thrombosis and coronary occlusions. *Circ. Res. 14,* 1–16.

DINTENFASS, L. (1966). Effect of velocity gradient on the clotting time of blood and on the consistency of clots formed *in vitro. Circ. Res. 18,* 349–356.

HARTERT, H. (1951). Die Thrombelastographie. Eine Methode zur physikalishen Analyse des Blutgerinnungsvorganges. *Zeitschrift f. d. gesamte experimentelle Medizin 117,* 189–203.

HARTERT, H. (1974). Rheo-simulation: A new method for the assay of clotting process and factor XIII. *Biorheology 11,* 355–360.

HASHIMOTO, S., MAEDA, H. and SASADA, T. (1985). Effect of shear rate on clot growth at foreign surfaces. *Artif. Organs 9,* 345–350.

HASHIMOTO, S. and MORIYA, H. (1988). Effect of right ventricular bypass peak flow-rate on intrapulmonary shunt ratio. *Artif. Organs 12,* 67–77.

HASHIMOTO, S. (1989). Erythrocyte destruction under periodically fluctuated shear rate: Comparative study with constant shear rate. *Artif. Organs 13,* 458–463.

MONROE, J. M., TRUE, D. E. and WILLIAMS, M. C. (1981). Surface roughness and edge geometries in hemolysis with rotating disk flow. *J. Biomed. Mater. Res. 15,* 923–939.

SALZMAN, E. W. (1981). *Interaction of the Blood with Natural and Artificial Surfaces.* Marcel Dekker, New York.

SPAETH, E. E., ROBERTS, G. W., VADWADKAR, S. R., NG, P. K., and JACKSON, C. M. (1973). The influence of fluid shear on the kinetics of blood coagulation reactions. *Trans. Am. Soc. Artif. Intern. Organs 19,* 179–187.

WHITMORE, R. L. (1968). *Rheology of the Circulation.* Pergamon Press, Oxford, pp. 93–96.

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