

# Thrombus Formation on Ultrasonically Vibrating Surface

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## ABSTRACT

The thrombus formation on ultrasonically vibrating surface was experimentally studied *in vitro*. A blood sample was contained on the glass plate attached to the piezoelectric oscillator, and erythrocytes aggregation was observed during clotting process with a phase contrast microscope. A drop of blood sample of 0.01 ml was spotted on the vibrating rock crystal, and the resonance frequency was continuously measured with an electric circuit. The results show that clotting time is shortened on the vibrating surface, and that the resonance frequency at oscillating crystal shifts when the contained blood starts forming clot.

**Keywords:** Bio-Measurement, Thrombus, Ultrasonic Vibration, Piezoelectric Oscillator, Crystal Oscillator, Resonance

## 1. INTRODUCTION

Many factors control the dynamic balance between the formation and removal of clots. This control repairs the ruptured vessel wall, and maintains the blood flow path. These factors include platelets, coagulants and anti-coagulants. Many studies have been performed in this research field from the biochemical point of view, which includes the interaction between the blood and the surface materials [1]. On the other hand, there is a hydrodynamic effect on clot growth in shear flow. The clotting time decreases in pulsatile flow [2]. The stirring effect of blood coagulants may accelerate the reaction for clot formation. Many instruments with ultrasonic wave are used in medical treatment. To design the application of ultrasonic oscillation to medical device in contact with blood, the effect of ultrasonic vibration on thrombus formation was investigated *in vitro*.

## 2. METHODS

### Erythrocyte aggregation

Human blood was collected into sodium citrate aqueous solution (1:6.6 sodium citrate/ blood; 9.8 mM final concentration in blood). The blood coagulation process

was initiated by adding calcium chloride (0.1:1 calcium chloride/ blood; 0.25 M aqueous solution) before the test. To observe erythrocytes aggregation during clotting process on ultrasonic vibrating surface, a piezoelectric oscillator (from 1 Hz to 1 MHz) was attached to the glass plate (Fig. 1). The blood sample was contained on the vibrating glass plate, and observed for ten minutes with a phase contrast microscope, a CCD (charge-coupled device) camera, a video tape recorder, a monitor, and a computer (Fig. 2).

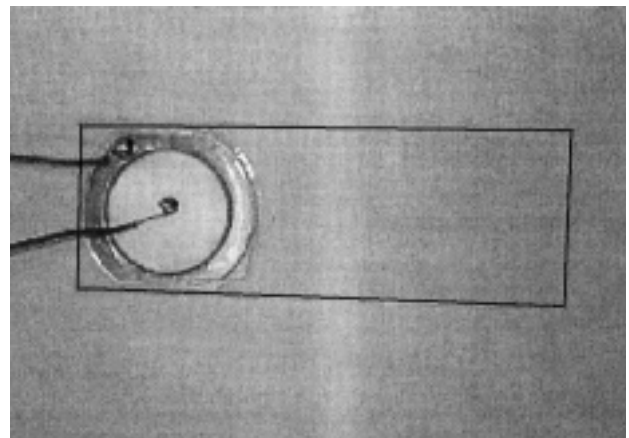


Fig. 1. Piezoelectric oscillator.

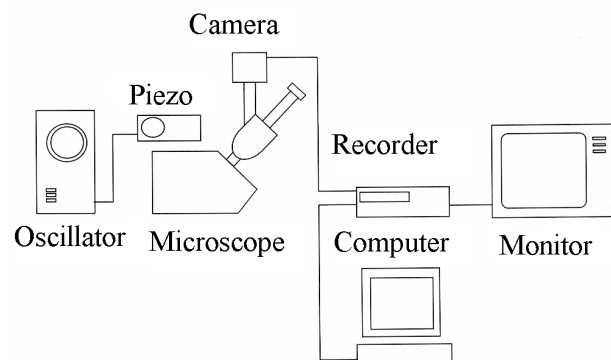


Fig. 2. Experimental system.

### Resonance frequency shift

A rock crystal (quartz) oscillator (Fig. 3) of 18 MHz resonance frequency was applied to detect blood clotting time. After removing the cover of the crystal oscillator, a drop of blood sample of 0.010 ml was spotted on the vibrating crystal, and the resonance frequency was continuously measured with an electric circuit (Figs. 4, 5).

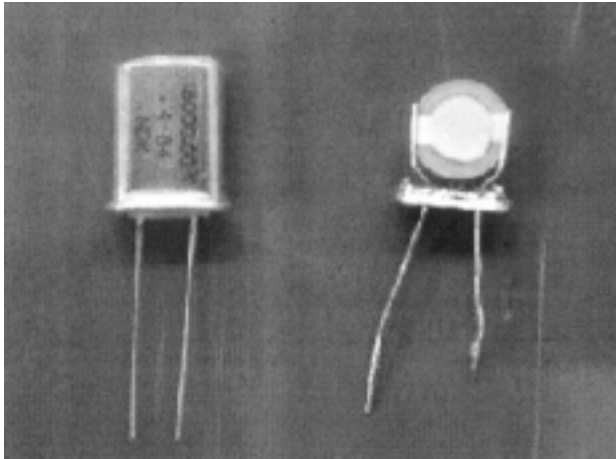


Fig. 3. Crystal oscillator. Left, with cover. Right, Oscillator disk (8.0 mm diameter) after removing the cover.

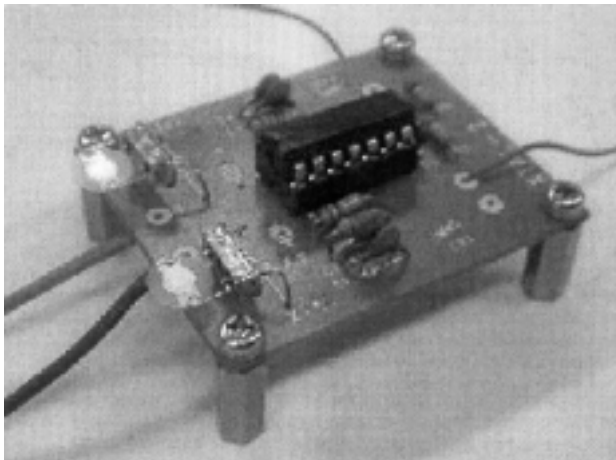


Fig. 4. Electric circuit

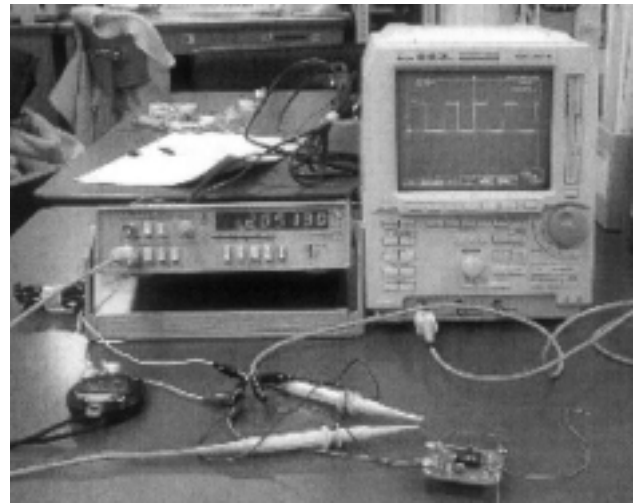


Fig. 5. Measurement system. Left, Frequency counter. Right, Oscilloscope.

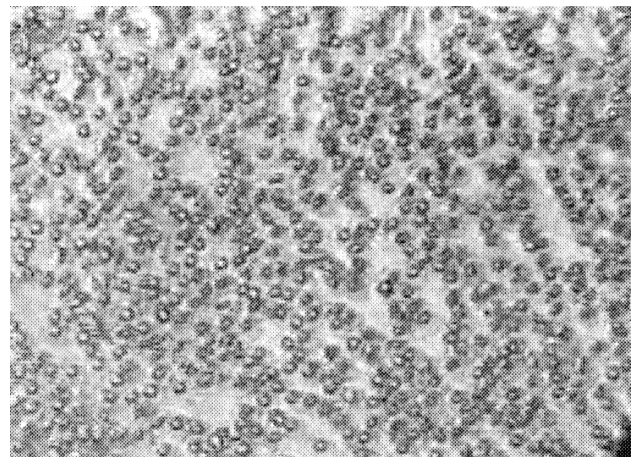


Fig. 6a. Erythrocytes before aggregation (0 min).

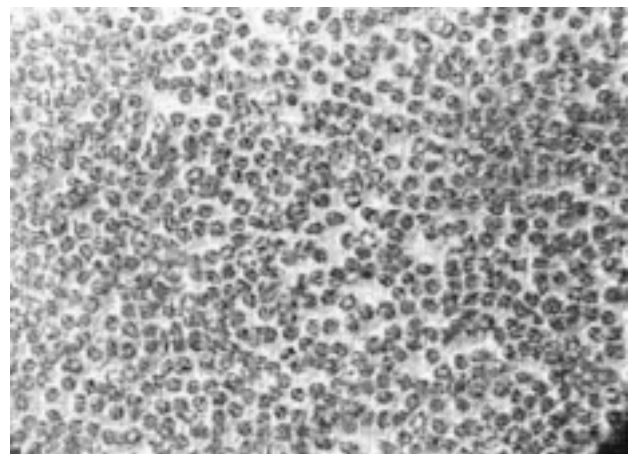


Fig. 6b. Erythrocytes aggregation during clotting process (2 min).

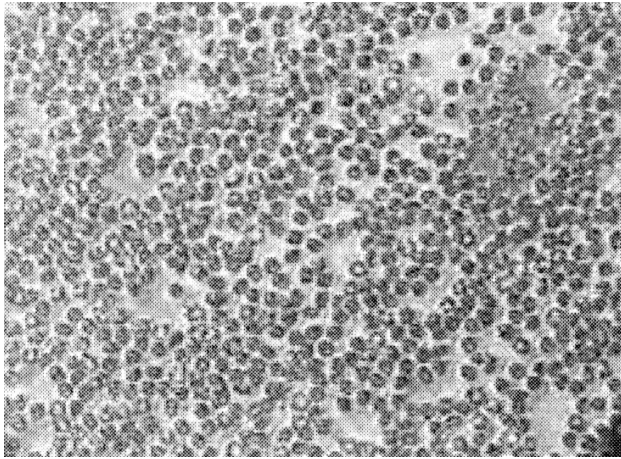


Fig. 6c. Erythrocytes aggregation during clotting process (4 min).

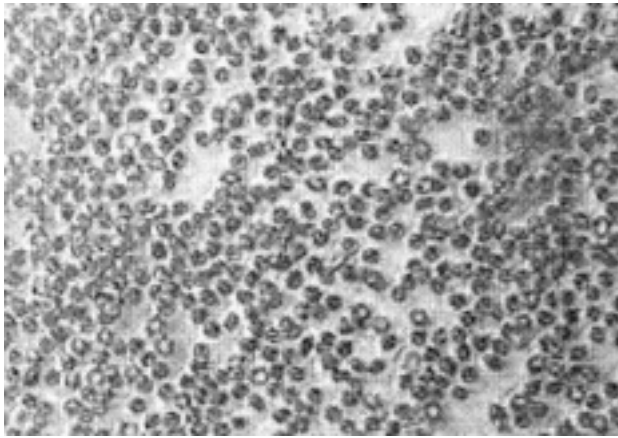


Fig. 6d. Erythrocytes aggregation during clotting process (6 min).

### 3. RESULTS

Fig. 6 shows erythrocytes aggregation during clotting process at 1 MHz. Density of erythrocytes was uniform, before formation of clot (Fig. 6a). After a few minutes, erythrocytes started to aggregate and form some colonies (Figs. 6b-6d). This aggregating time was three minutes shorter on the vibrating glass surface than on the static glass surface.

Fig. 7 exemplifies the relation between resonance frequency and time. The resonance frequency decreased by 0.1 % ( $F_0$ ) after a drop of blood spotted on the vibrating crystal. Frequency shift ( $F_s$ ) was calculated by

$$F_s = F_t - F_0 \quad (1)$$

where  $F_t$  is the resonance frequency at each time ( $t$ ), and where  $F_0$  is resonance frequency at fifteen seconds after spotting the blood. The frequency shifted by step in a few minutes (3.5 minutes in Fig. 7), when the blood started

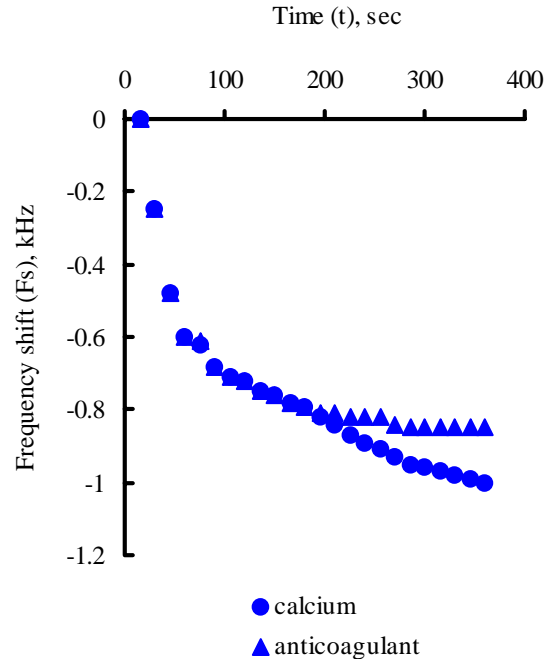


Fig. 7. Relation between frequency and time.

forming clot. After forming clot (“calcium” in Fig. 7), the frequency shift was different from that of blood with “anticoagulant”.

### 4. DISCUSSION

Many instruments with ultrasonic wave are used in medical treatment. To design the application of ultrasonic oscillation to medical device in contact with blood, the effect of ultrasonic vibration on thrombus formation was investigated *in vitro*.

The previous study shows that the clotting time decreases in the pulsatile flow by the stirring effect of blood coagulants, while clot does not grow in the pulsatile flow by the wash out effect [2]. The present study shows that there is an analogy between clot formation on the ultrasonically vibrating surface and that in the pulsatile flow. The ultrasonically vibrating surface shortens the clotting time and may prevent clot growth.

The crystal oscillator has stable resonance frequency. A shift of resonance frequency occurs with molecular adsorption, and it has been applied to the gas molecule sensor [3]. In the present study, the crystal oscillator was used to apply ultrasonic vibration to the blood, and to detect clot formation. The present study shows that the shift of resonance frequency is able to be applied to measurement of clot formation.

## 5. CONCLUSION

The present study shows that the clotting time decreases on the ultrasonically vibrating surface. The shift of resonance frequency is able to be applied to measurement of clotting time.

## 6. ACKNOWLEDGMENT

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## 7. REFERENCES

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