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# The Measurement of Blood Coagulation Process in Extracorporeal Circuit Using LED Photoacoustic Imaging

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**Abstract:** Blood coagulation is measured by using a pressure sensor in a blood circuit, but it is not quick responsive because it is detected by pressure rise caused by coagulation. In this study, we have investigated a method to detect blood clotting at an early stage using photoacoustic imaging, which is thought to be more sensitive. The LED with a wavelength of 850 nm was used as a photoacoustic light source. An ultrasonic wave generated by thermal expansion of mouse blood sealed in a microtube was observed, and also many ripples were observed with time and the coagulation of blood progressed. It was also observed that the waveform considered to correspond to coagulation of blood broadens with time. It was found from the above that there is a possibility that the state of blood clotting can be observed from outside the circuit of the extracorporeal circulation device by using the LED as a light source.

**Keywords:** Blood coagulation, Photoacoustic imaging, LED, The circuit of the extracorporeal circulation device, Coagulation process.

## 1. Introduction

When patients undergo extracorporeal blood circulation using methods such as purification therapy, percutaneous cardiopulmonary support, granular leukocyte apheresis, leukapheresis, or auxiliary artificial hearts, blood comes into contact with foreign matter. This contact is known to cause phenomena such as the destruction of blood cells, blood coagulation, fibrinolytic system enhancement, or complementary activities (thrombus formation) [1-3]. Various attempts have been made to prevent blood clotting, and prevention methods using anticoagulants (such as heparin) are now in mainstream use [4-7]. It is known that interfacing negatively charged nonphysiological substances activates blood coagulation; a heparin coating is used in the extracorporeal circulation circuit to inhibit coagulation caused by the reaction between blood and foreign matter as it weakens this negative charge [8-9]. However, because heparin also acts as a foreign body, it is difficult to completely prevent blood from clotting on the surface of the circuit or in the dialyzer.

In extant extracorporeal circulation circuits, a pressure sensor is used for coagulation detection. This sensor reacts only when blood clotting causes clogs in the circuit or when the blood flow becomes difficult, both of which cause a rise in blood pressure. If the

response is delayed, the blood will continue to clot, putting the patient's health at risk. In addition, when blood coagulation is observed, the extracorporeal circulation circuit is changed to a new circuit. However, due to the irregularity of the response, the burden placed on healthcare professionals is also very large. Furthermore, the clogged circuit results in the patient's blood, which should have been returned to the patient's circulation, being discarded. However, if coagulation in the circuit can be detected in advance, it would be possible to carry out an early response to circuit blockage by reverse calculation.

Because of the importance of an early response, sensing blood coagulation as well as understanding the details of the process at an early stage is required. There are some published studies that have measured blood coagulation using a photoacoustic imaging technique that uses a laser [10-12]. Despite this, the measurement of the process of thrombus growth by blood coagulation has not yet been reported. In addition, a solid-state laser is only used as a light source in conventional photoacoustic imaging [13]. The conventional laser device was not suitable for use as the light source for this study due to its weight and size, considering that it was necessary to attach it to a blood circuit regularly to measure changes in the state of the blood. Compared with light emitting diodes (LEDs), a solid-state laser has a volume ratio 15000 times that of a conventional laser, with a power consumption 1000 times higher and a cost ratio of 10 times or more. Because of the benefits of the LED technology, photoacoustic imaging using LEDs or laser diodes (LDs) as light sources is much more suitable for monitoring blood properties in this way. An LD can emit at a specific wavelength and has a higher energy density than an LED; however, since a resonator structure is required, using an LD for measurement becomes expensive. Thus, we believed that an LED was the most suitable light source for measuring blood coagulation in this study. This paper presents a preliminary study to measure the process of blood coagulation in the extracorporeal circuit system, using a photoacoustic imaging method to measure the changes that occur during blood clotting.

## 2. Methods

### 2.1. Measuring Coagulation Process with Photoacoustic Image of LED Light Source

The extracorporeal circuit includes an air trap to prevent air contamination. In addition, a mesh filter is installed to prevent generated blood clots from reaching the inside of the body from the external circuit. The air trap and mesh filter are installed in the drip chamber. Fig. 1 and Fig. 2 show the overall layout of the system and the drip chamber, respectively. Blood flow in the drip chamber is almost stationary due to the presence of the air trap and mesh filter. The blood drip chamber plays an important role in the generation of coagulated blood, as blood is more likely to coagulate when flow is slow, as is the case in this component of the extracorporeal circuit. The low flow rates provide an advantage, however, in that they allow for measurement by photoacoustic technology. In this experiment, in order to reproduce the environment of a drip chamber with a very slow flow rate, blood was measured in a microtube.

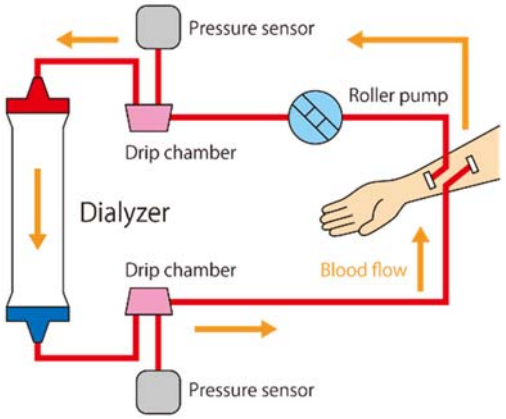


**Fig. 1.** Schematic diagram of the blood flow in the blood purification circuit.


**Fig. 2.** The drip chamber.

Fig. 3 shows the schematic of the experimental setup used in this study. Measurement of the blood coagulation process was explored using PreXionLED AcousticX (CYBERDYNE, INC.). Approximately 1.5 mL of blood was placed in the microtube, and care was taken to prevent air from entering into the microtube. Additionally, two LED arrays were attached to both sides of the conventional ultrasonic probe, fixed in the water at approximately 40° with a jig. The distance between the microtube and the ultrasonic probe with the LED was approximately 1 cm. For the setting of measuring equipment, we referred to previous research [14].

We referred to existing data for the light absorption of the liquid and the blood coagulation. [15] The light energy was approximately 200 μJ/pulse, whereas the wavelength was 850 nm.

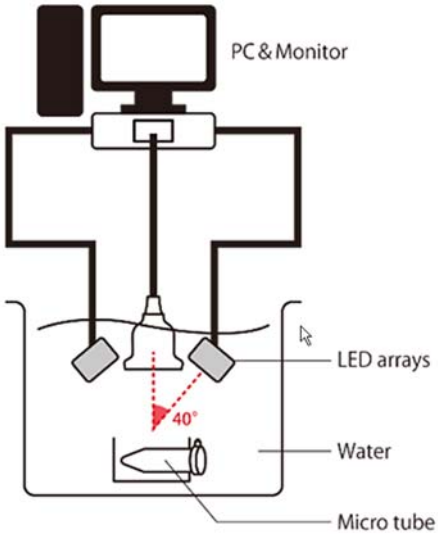


**Fig. 3.** Schematic diagram of the experimental apparatus.

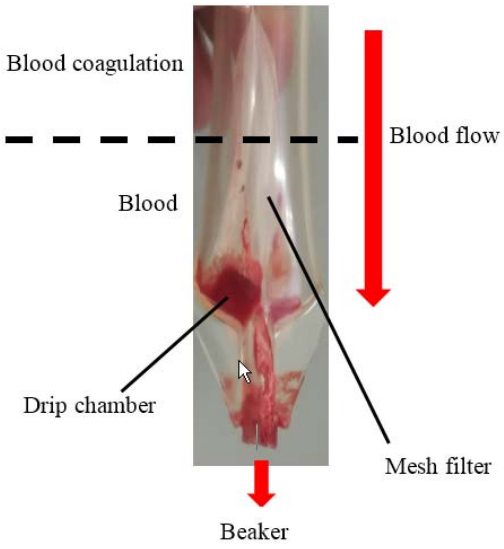


**Fig. 4.** The coagulation process and separation of liquid blood.

There were two types of ultrasonic probes: 10 and 7 MHz. At 10 MHz, the distance resolution improved to some extent, but the depth sensitivity increased 4 to 5 times at 7 MHz. Thus, the 7-MHz ultrasonic probe frequency was chosen.

### 2.2. Confirmation of Blood Coagulation by Change in the Blood Volume

Blood coagulation process in the microtube was observed under the same conditions as in 2-1. Activated clotting time (ACT) is an important factor that can be used as a strict control for the administration of anticoagulants to the extracorporeal circuit. The measurement principle is activating blood coagulation by mixing an activator with the blood. ACT represents the time taken for clot formation [16]. However, there is no way to measure the coagulation process required for this experiment. Therefore, the index indicating the solidification process is scheduled. A schematic diagram of the experiment is shown in Fig. 4. The experimental procedure was as follows.

1. Blood was added into the transparent plastic microtube and incubated for a certain period.
2. A mesh filter was used to separate the liquid and coagulated parts of the blood.
3. The liquid part of the blood was transferred into a beaker, and the liquid weight was measured.

As clotting progresses, it can be expected that the weight of liquid blood will decrease.

## 3. Result

As shown in Fig. 5, the vicinity of the wall surface of the microtube, where blood coagulation reacts with the foreign material, was measured. The thick black line indicates the side wall of the microtube.

The lower side is the side in contact with the blood and the upper side is the underwater side, with an arrow showing the boundary between the two. The red arrow is the A-mode measurement point described in 3.1.

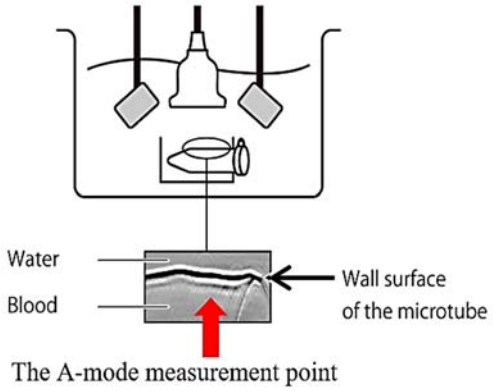


**Fig. 5.** Correlation of experimental apparatus schematic diagram and imaging chart.

### 3.1. Measurement of Photoacoustic Phenomena Using A-mode Ultrasonic Imaging

First, the photoacoustic phenomenon generated from the microtube was measured with an ultrasonic probe to show the intensity of the phenomenon (A-mode). The vertical axis represents the magnitude of vibration due to the photoacoustic phenomenon, whereas the horizontal axis represents the distance from the ultrasonic probe. To measure the time course of the blood clotting process, the photoacoustic response of the blood was measured just after blood was injected into the microtube (Fig. 6 (a)) and at 2 (Fig. 6 (b)), 5 (Fig. 6 (c)), and 10 min (Fig. 6 (d)).

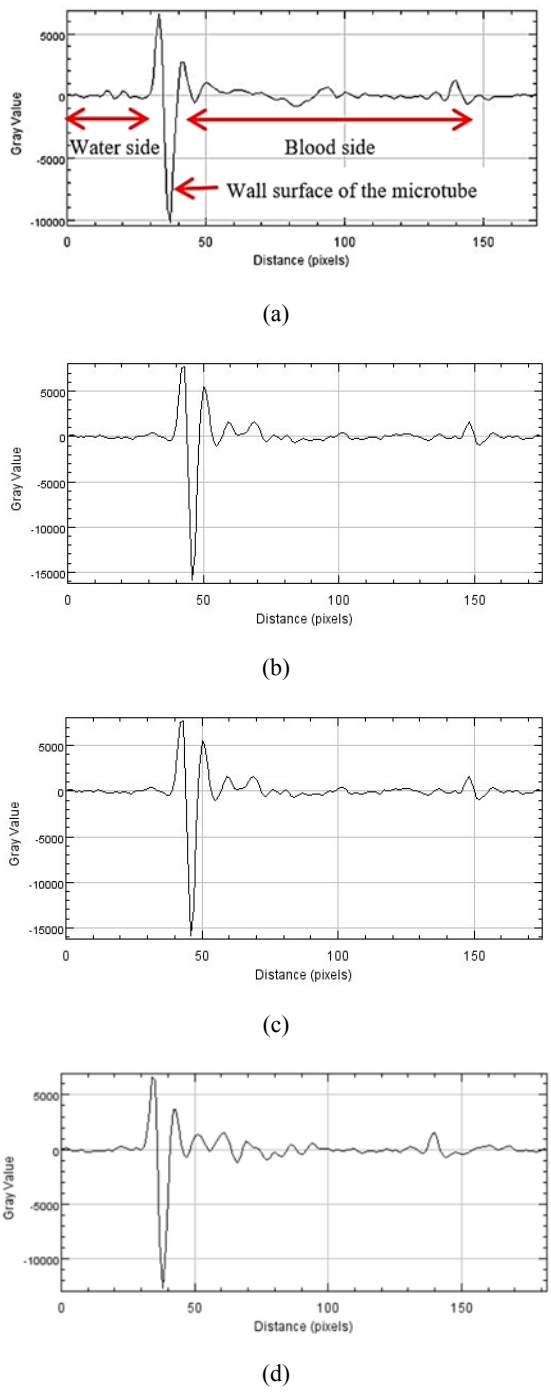


**Fig. 6.** Measurement of photoacoustic phenomena using ultrasonic images (A-mode): (a) just after blood injection (0 min); (b) 2 min; (c) 5 min; and (d) 10 min.

## 3.2. Measurement of Photoacoustic Phenomena Using B-mode Ultrasonic Imaging

The ultrasonic probe is in the form of an array, and the location where the photoacoustic phenomenon occurs can be identified. Fig. 7 shows the state of the elastic wave inside the microtube by synthesizing the waveform of the A-mode wave observed along the tube's longer axis. The measurement timing was the same as in Fig. 6. Fig. 7 is rotated 90° from the actual state shown in Fig. 5 for the convenience of subsequent graphing. The microtube shown in Fig. 7 is vertically oriented so that the wall surface is on the left side. As time elapsed, the number of white parabolic streaks increased on the side of the microtube in contact with the blood. The white part (high brightness) of the image represents the elastic wave from the absorber obtaining light energy.

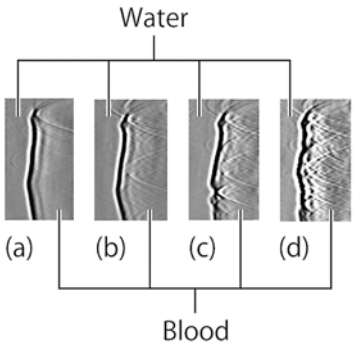


**Fig. 7.** Photograph by photoacoustic imaging: (a) just after blood injection (0 min); (b) 2 min; (c) 5 min; and (d) 10 min.

The pixel luminance of Fig. 7 is shown in Fig. 8 as a 3D color scale graph+ converted by free software (ImageJ) [17-18].

The x-axis represents the horizontal distance (5 mm), the y-axis represents the vertical distance (12 mm), and the z-axis is the value obtained by calculating the pixel luminance (256 scales).

It was unclear whether the origin of the photoacoustic phenomenon seen in Fig. 8 was the coagulation activity of the blood or not. Therefore, measurement was performed while blood coagulation in the tube was suppressed by introduction of heparin. Heparin was added to the blood and incubated for 10 min before measurement, in the same manner as the coagulated blood test in Fig. 8d. The results of this test are shown in Fig. 9.

## 3.3. Confirmation of Blood Coagulation by Change in the Blood Volume

The blood volume passed through the mesh filter was found to decrease as time elapsed, dropping down to 79% after 10 min, as indicated in Table 1.

**Table 1.** Confirmation of blood coagulation process in microtube.

| Elapsed time after injection of blood into the microtube (min) | Blood volume passed through the mesh filter (mL) |
|---|---|
| 0 | 1.35 |
| 2 | 1.30 |
| 5 | 1.25 |
| 10 | 1.07 |

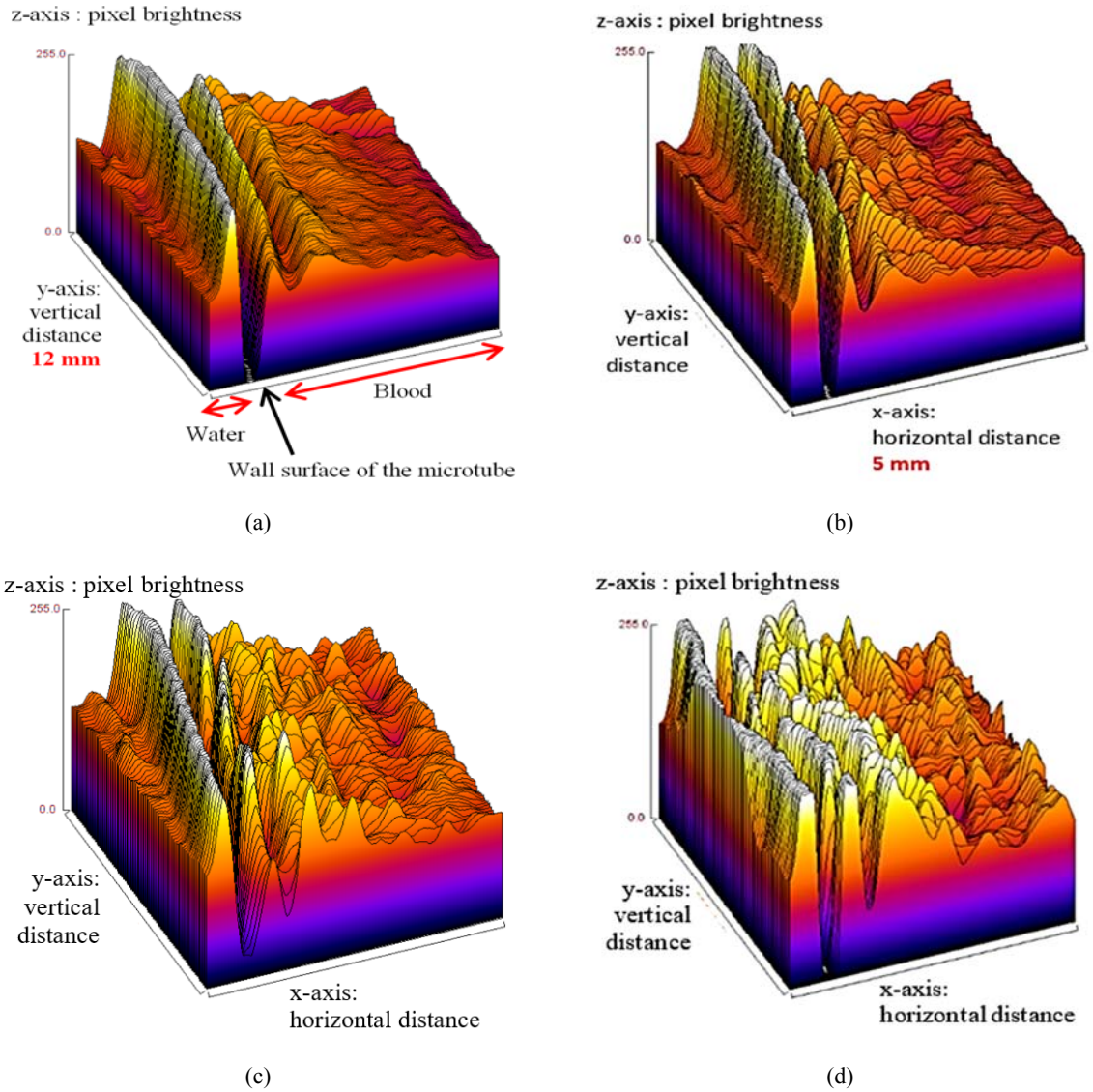


**Fig. 8.** 3D diagram of luminance part by ImageJ:
(a) just after blood injection (0 min); (b) 2 min; (c) 5 min; and (d) 10 min.

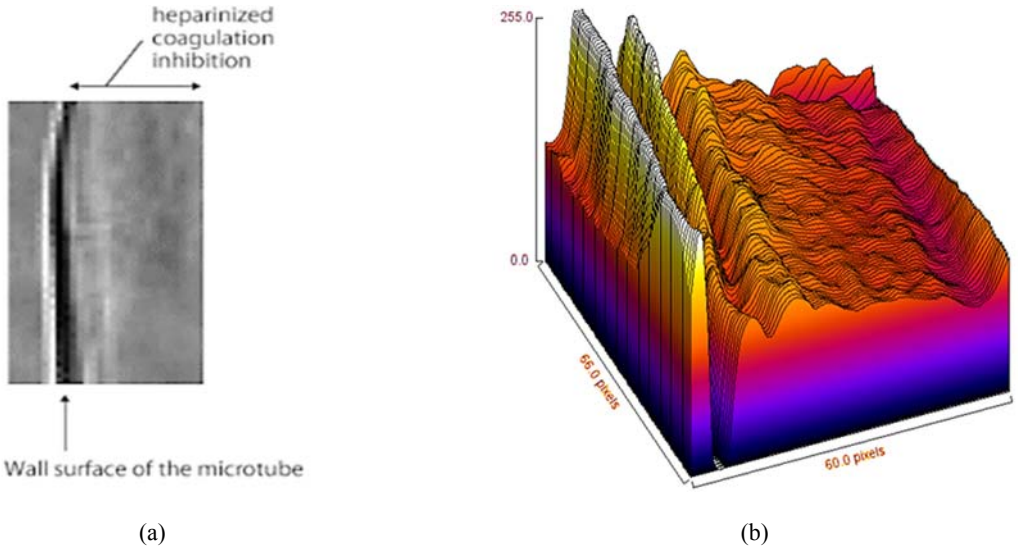


**Fig. 9.** Photoacoustic imaging of heparinized coagulation inhibition:
(a) Photograph; (b) Image; 3D diagram of luminance.

## 4. Discussion

In this study, changes in blood coagulation through contact with transparent plastics were measured by using photoacoustic methods. As shown in Fig. 7, it can be seen that ripples (indicated by white streaks) were generated from the center of the microtube, moving out toward the walls. The blood coagulation process was found to occur at the side of the tube in contact with blood, whereas no change was observed in the water outside the microtube. Additionally, in all measurements, as the light energy value irradiated to the microtube did not change, the elastic wave increased. Fig. 8 shows that the luminance of the blood portion of the microtube increased with time, indicating that blood coagulation was increasing from the inner wall of the microtube where the foreign matter reacted with the blood. Fig. 9 does not show the same elastic wave because of the inhibition of coagulation by heparin; the changes in photoacoustics over time do not occur in blood which does not coagulate. Fig. 7 shows that the elastic waves are generated inside the whole microtube, and that they grow over time. The results of the photoacoustic tests were found to correlate with the change in blood flow volume observed.

## 5. Conclusion

In this study, it was difficult to measure each the blood clot that formed in the microtube as was originally intended. However, the photoacoustic imaging using an LED light source confirmed the temporal change of blood coagulation in the microtube. Thus, it is possible to measure the temporal change of the blood coagulation process generated in an extracorporeal circuit using photoacoustic imaging with an LED light source. The optimization of wavelength energy and ultrasonic probe frequency in order to better measure blood coagulation through blood circuits should be studied further.

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