BLOOD COAGULATION TESTING METHOD BASED ON FLOW VELOCITY MEASUREMENT USING A SURFACE PLASMON RESONANCE (SPR)-BASED MICROFLUIDIC DEVICE

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ABSTRACT

We propose a blood coagulation testing method based on the measurement of the flow velocity near the bottom surface of a microchannel by using a surface plasmon resonance (SPR)-based microfluidic device. We were able to distinguish the coagulation ability in different human plasma samples by observing the flow velocity change caused by coagulation that occurred at an interface between a plasma sample and a coagulation trigger agent while flowing in a microchannel. Our blood coagulation testing method is simply carried out by placing a 2 µL plasma droplet at the inlet of a straight PDMS microchannel and introducing the sample into the microchannel with passive pumping.

KEYWORDS

Surface plasmon resonance (SPR), Blood coagulation testing, Flow velocity, Plasma, Coagulation trigger agent

INTRODUCTION

Blood coagulation testing is widely used to diagnose thrombotic diseases and to control the dosage rate in anticoagulation therapy. Blood coagulation results in changes in the physical parameters of plasma including its viscosity, light-scattering, and light-transmitting characteristics. Therefore, some blood coagulation testing methods have been developed based on the above physical parameters. A surface plasmon resonance (SPR) based biosensing technique is also widely used to measure the concentrations of biomarkers in biological fluids because it is label-free and highly sensitive. We have already developed a flow velocity measurement method based on the SPR measurement of a sample solution flowing in a microchannel [1]. A time-space refractive index (RI) map, which is constructed from the RI at stream-wise positions and its time change obtained with our SPR measurement system, gives us the flow velocity a few nanometers from the bottom surface of the microchannel. Our flow velocity measurement method also has the potential for use as a viscosity detection method for measuring coagulation time because the flow velocity of a sample solution is strongly related to its viscosity.

Here, we propose a new viscosity detection method for blood coagulation testing by using a surface plasmon resonance (SPR)-based microfluidic device. In the work reported here, we studied the degree of blood coagulation caused by agents used to determine prothrombin time (PT) that can be distinguished by a portable flow velocity measurement system.

EXPERIMENTS

SPR measurement setup: We used a portable SPR instrument, SMART SPR (NTT Advanced Technology, Tokyo, Japan). Figure 1(A) shows the SMART SPR and a schematic representation of an SPR optical system combined with a microfluidic chip. We used a wedge-shaped monochromatic beam whose maximum width was about 5 mm (480 pixels). This illuminated the gold thin film, and a 2D-CCD camera detected the light that it reflected. The RI distribution was obtained in the region where the SPR was observed in the microchannel. A sensing chip consisting of a BK7 glass chip covered with a gold thin film and equipped with a PDMS microchannel was mounted on the SPR instrument. The microchannel was 10 mm long and 1 mm wide, and the channel height ranged from 50 to 200 µm.

Flow velocity measurement principle: Figure 2 shows a schematic representation of a PDMS film with a microchannel and the SPR measurement area in the microchannel. As mentioned above, we have already reported the basic concept of flow velocity measurement using an SPR-based microfluidic device [1]. We can observe a liquid-liquid interface in a microchannel when injecting two solutions (RI: n1, and n2, respectively) separately. The flow velocity was

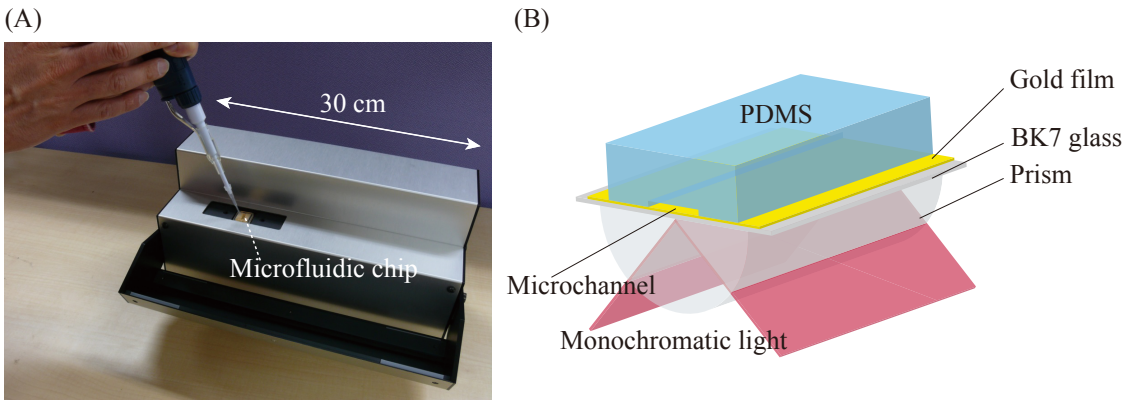

Figure 1 SMART SPR (A), SPR optical system with a PDMS microchannel for time-space refractive index (RI) measurement (B).

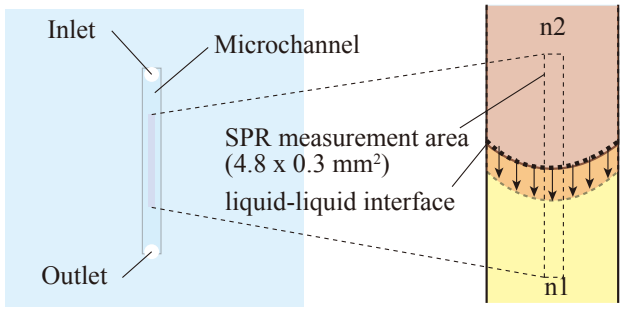

Figure 2 PDMS film with a microchannel and an SPR measurement area in the microchannel (B).

calculated from the migration distance (4.8 mm) and the migration time of the interface at the area where the SPR can be observed.

Blood coagulation testing protocol: Figure 3 shows the coagulation testing protocol that we propose. First, the microchannel was filled with a coagulation trigger agent (Siemens), and 10 µL of the agent was placed at the outlet of the microchannel. We did not modify the internal microchannel with heparin or any other biocompatible materials that inhibit coagulation. When starting the blood coagulation testing, 2 µL of standard human plasma (Siemens) was placed at the inlet of the microchannel (Fig. 3A), and an interface was formed between the plasma and the coagulation trigger agent (Fig. 3B). The temperature of the plasma and the trigger agent was adjusted to 37°C before the measurement. The plasma was introduced into the microchannel by a surface tension driven pump [2, 3] and mixed with the coagulation trigger agent at the interface while flowing in the microchannel (Fig. 3C). According to the Laplace Law, the smaller curvature radius of the smaller drop will induce a larger pressure and thus create a flow from the small drop towards the large drop. We observed the migration of the liquid-liquid interface in the microchannel with the SPR measurement system, and then calculated the flow velocity with software that we designed. With our method the coagulation occurs at the interface between the plasma and the trigger agent. Only a small amount of fibrin (a polymer formed from fibrinogen) is generated at the interface, and thus it is relatively easy to measure the flow velocity without experiencing a fibrin induced jam.

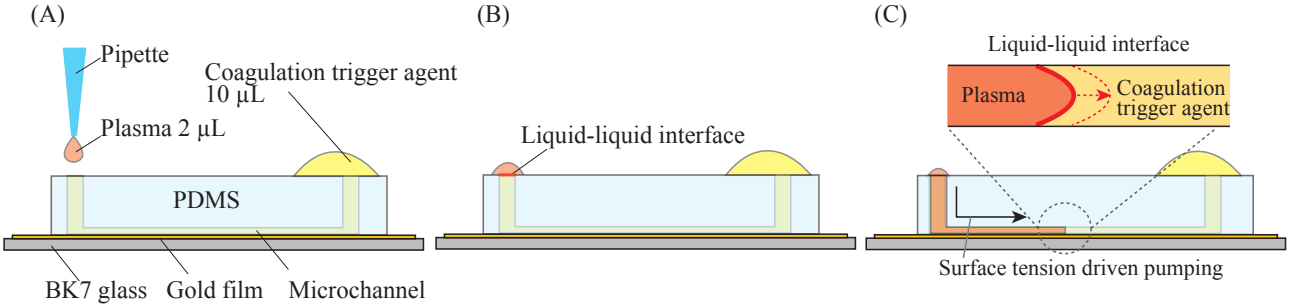

Figure 3 Blood coagulation testing protocol. A microchannel is filled with a coagulation trigger agent. 10 µL of the coagulation trigger agent is placed at the outlet of the microchannel. 2 µL of plasma sample solution is placed at the inlet of the microchannel. A liquid-liquid interface is formed at this time (A), (B). The plasma sample solution is introduced into the microchannels by a surface tension driven pump, and the liquid-liquid interface also migrates in the microchannel (C).

RESULTS AND DISCUSSION

Blood coagulation testing with flow velocity measurement: Figure 4A shows the relationship between the microchannel height, which was set at 50, 75, 100, and 200 µm and the flow velocity when standard human plasma was introduced into a microchannel filled with the coagulation trigger agent. RI maps obtained at each channel height are also shown in Fig. 4B. The vertical axis of the RI maps is the migration distance of the liquid-liquid interface, and the horizontal axis is time. For channel heights of 50 and 75 µm, the liquid-liquid interface migrated to the lower right in the corresponding RI maps and the flow velocity was calculated as shown in Fig. 4A. The RI became higher at each pixel as time passed (the color changed to yellow and then red). The flow velocity increased as the channel height increased. The red region probably indicates the area where fibrin was generated. On the other hand, for channel heights of 100 and 200 µm, the liquid-liquid interface stopped migrating upstream of the microchannel and the flow velocity was not calculated. In addition, it seems that the liquid-liquid interface obtained with the 200 µm high microchannel stopped further upstream than that obtained by the 100 µm high microchannel. These results suggest that the flow velocity strongly affects the coagulation efficiency. It is well known that a pressure-driven flow has a parabolic profile in a microchannel. As the volume flow rate increases, the parabolic profile becomes longer in the flow direction. To confirm the relationship between the parabolic profile and flow velocity, we used a microchannel in which the flow had stopped (channel height: 100 µm) and varied the flow velocity by changing the size of the droplet placed at the inlet port. We found that control of the flow velocity is one of the most important factors as regards blood coagulation testing in our measurement system (data not shown).

Relationship between activation rate of standard human plasma and flow velocity: In the PT measurement, we prepared a calibration curve using plasma sample solutions with different activation rates, and then examined the patient's plasma by matching it with the calibration curve. We also tried preparing a calibration curve using a 75 µm high microchannel and diluted standard human plasma sample solutions. The activation rates of the sample solutions were 10,

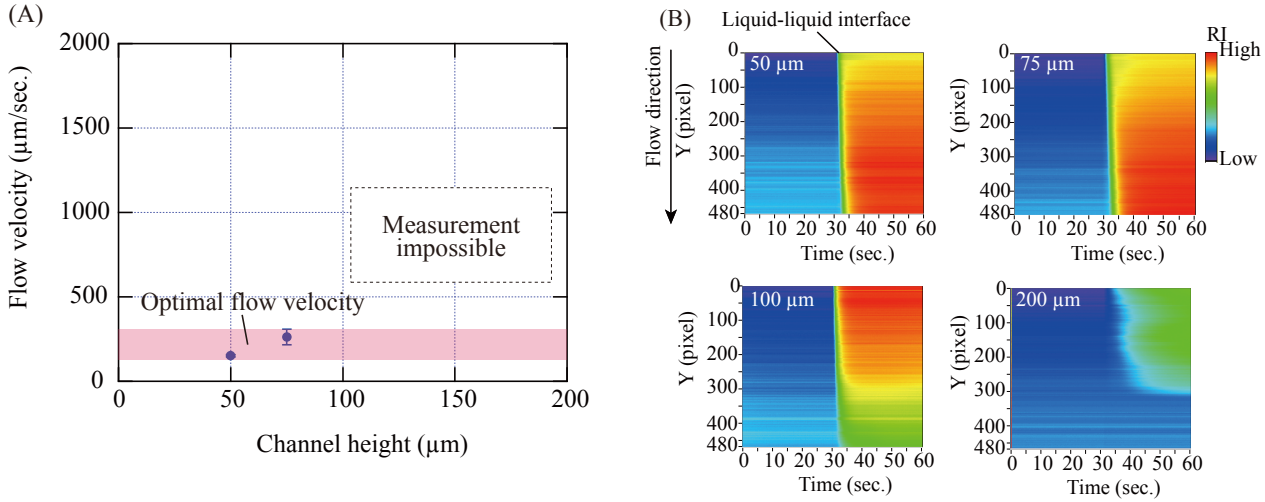

Figure 4 Relationship between channel height and flow velocity when 2 μL of standard human plasma was introduced into a microchannel filled with coagulation trigger agent (A). RI maps obtained at channel heights of 50 μm, 75 μm, 100 μm, and 200 μm (B).

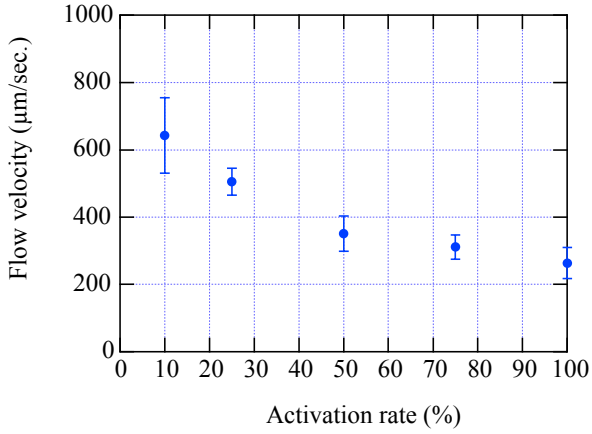

Figure 5 Flow velocity when varying the activation rate of a plasma sample solution. The activation rate of each sample solution was adjusted with Owren's Veronal Buffer. The microchannel we used was 10 mm long, 1 mm wide and 75 μm high.

25, 50, and 75 %. Owren's Veronal Buffer (Siemens) was used to prepare and dilute these sample solutions. Figure 5 shows the relationship between the activation rate of the plasma sample solution and the flow velocity. As the activation rate increased, the flow velocity decreased. This is because the viscosity of the plasma increased owing to coagulation and the generation of fibrin as the activation ratio increased. When the plasma activation rate was varied from 10 to 100%, the flow velocity also varied from 250 to 650 μm/sec. These results show that we succeeded in differentiating between the activation rates of each sample solution. Our method simply needs a rectangular microchannel with two ports, and does not require complex microfluidic systems to mix the plasma with the coagulation trigger agent. Our approach is very simple and easy. To apply our method to blood coagulation testing, we will need to convert flow velocity into PT and APTT (activated partial thromboplastin time). However, the conversion will be easy to accomplish by matching the calculated flow velocity with the PT and APTT examined using a conventional testing method.

CONCLUSION

We have proposed a new method for performing a blood coagulation testing by means of SPR measurement, and demonstrated its potential for clinical use. A parabolic profile of the liquid-liquid interface between standard human plasma and a coagulation trigger agent worked as a coagulation location, and we found that we could control the coagulation efficiency by controlling the flow velocity. In this study, we used a surface tension driven pump, which is a very powerful tool for microfluidic devices. However, various pumps can be used with our method. The accuracy of the measured data will be improved by using an automatic pipette. Only one microchannel with two ports is required for our method, and no mixers or reactors are needed to initiate coagulation. This approach is very simple, easy and quick to use. In this study, we used agents to determine the PT, however our method can also be employed to determine the APTT and fibrinogen. Our method, which we achieved with a portable SPR measurement instrument, will be a powerful tool for daily blood coagulation testing.

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