MULTI-LAYERED APTAMER ARRAY INTEGRATED IN MICROFLUIDIC CHIP FOR ON-SITE BLOOD ANALYSIS

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ABSTRACT

We established a process for fabricating a multi-layered aptamer array integrated in a microfluidic channel using an inkjet spotter for on-site blood analysis. By the subsequent accumulation of pL droplets, aptamers that bind selectively with different sites of the blood coagulation marker, thrombin, were chemically immobilized on the substrate with high reproducibility and precision in the microfluidic channel. With the all-plastic capillary driven chips that we reported previously [1], we confirmed that thrombin could be measured simply by dropping the sample solution on the chip inlet using a multi-analysis SPR sensor instrument.

KEYWORDS: Surface plasmon resonance (SPR), Blood analysis, On-site, Aptamer, Inkjet spot, Capillary-force

INTRODUCTION

Surface plasmon resonance (SPR) sensor technology has a great advantage for on-site immunoassay since it can monitor real-time and label-free antigen-antibody reactions with high sensitivity. We have already developed a portable SPR sensor and all-plastic capillary driven chips that integrate a continuous liquid flow function and an antibody array. Using these technologies, the user needs only to install the sensor chip in an SPR sensor and supply a small volume sample solution (4 uL) via the chip inlet to monitor multi antigen-antibody reactions at a time.

To make our chip applicable for various kinds of detection targets, the immobilization technique for other capture molecules such as DNA, RNA, and enzyme, should be established. In this work, we attempted to fabricate a DNA aptamer array in the chip. DNA aptamers are very robust in dry conditions and easier to procure than antibodies. In contrast, DNA aptamers are difficult to immobilize directly on a gold sensor surface because of their lack of aggregability, which depends on their hydrophobic interaction, and may easily lose their binding activity. To form a highly flexible array, we established a process for fabricating a multi-layered aptamer array that employs a controlled chemical reaction in which droplets are stacked on a substrate with an inkjet spotter as shown in Fig. 1. Using this method, it is easy to arrange every spot of capture molecule and its volume. Moreover, the position and shape of the spot are adjustable even if the substrate is not flat, and modifications can be made by accurately controlling the inkjet spotter.

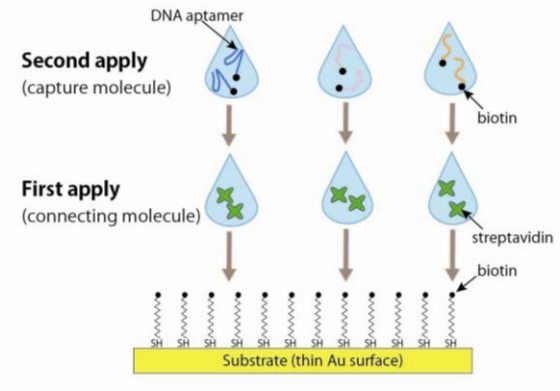

Figure 1. Fabrication of multi-layered aptamer array by chemical reaction control with droplet stacking.

EXPERIMENTAL

Array fabrication. We used two thrombin aptamers that bind with either the fibrinogen-binding site (15-mer) or the heparin-binding site (29-mer) [2, 3]. 20-mer thymine was also used as negative control (NC), (Table 1). First, biotinylated self-assembled polyethylene glycol with an SH end group was prepared on a thin gold film substrate. Then, streptavidin solution and biotinylated aptamer solution were applied to the substrate as pL droplets with the inkjet spotter. Since trace amounts of these solutions begin drying immediately after spotting, we optimized the concentration and amount of the spotted solutions and also the reaction time to complete streptavidin-biotin binding with an optimized ratio. We deposited six multi-layered aptamer spots and seven reference spots alternately (300 x 800 μm ellipse for each) with a 350 μm interval in the detection area (800 x 4500 μm rectangle) of the multi-analysis SPR sensor. Reference spots were made to cancel out the background-noise in the adsorption analysis.

Measurement of sample with capillary flow chip and SPR instrument. A liquid sample was injected into the inlet and then traveled through a straight flow channel that was aligned with an array of aptamer spots (Fig. 2A). The sample reached the pumping area by being drawn continuously during the measurement. In our portable SPR equipment (Fig. 2B), light from the LED was linearly focused through a cylindrical lens, and so the SPR detection area was 4.5 mm along the focused line with which the antibody array immobilized in the flow channel was aligned. We activated a standard human plasma sample by

mixing it with ellagic acid reagent. The sample was then incubated at 37 °C for 4 min and finally $CaCl_2$ was added before sample was supplied via the chip inlet.

Table 1. DNA sequence of aptamers

Aptamer	DNA sequence with modification
15-mer	5′-B-TTTTTGGTTGGTGTGGTTGG-3′
29-mer	5′-B-TTTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3′
20-mer thymine (NC)	5′-B-TTTTTTTTTTTTTTTTTTTT-3′

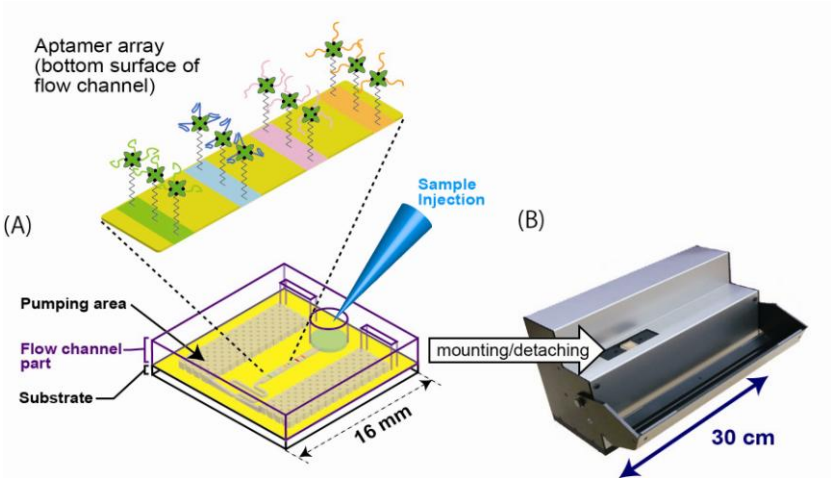

Figure 2. (A) View of capillary-driven chip. Flow channels are created by the flow channel part and the gold deposited polymeric substrate. The aptamer array is immobilized periodically on the gold surface. (B) SPR measurement instrument (SmartSPR, NTT Advanced Technology, Japan).

RESULTS AND DISCUSSION

We first confirmed the reaction of the thrombin aptamer in the array by employing a mechanical pump flow. As shown in Fig. 3A, the SPR angle shift of the aptamer spots started to increase when 1 μg/mL of thrombin solution was injected into the chip inlet. By fitting the absorption curve with the equation derived from Langmuir's adsorption isotherm [4], we extracted the first absorption rate (slope) and used it as a response parameter. Figure 3B is the calibration curve of a 15-mer thrombin aptamer that indicates the good correlation between the absorption rate and the thrombin concentration in the range above 50 ng/mL. Next, we measured the thrombin by using a capillary-driven chip with the aptamer array built in. The observed thrombin absorption in PBS corresponded to the positive responses obtained with the pump flow (Fig. 4A).

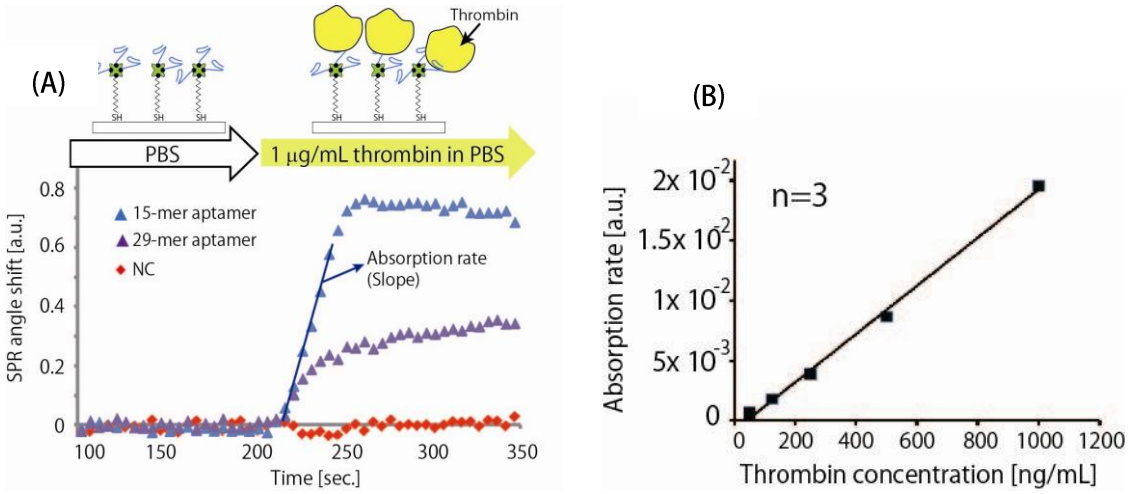

Figure 3. (A) Absorption curve measured using mechanical pump flow. Typical response of 15-mer aptamer spot, 29-mer aptamer spot and NC are plotted. Thrombin diluted by 1 mg/mL with buffer solution was used as the sample solution. Reactions of aptamer spots are larger than that of the NC spot. (B) Calibration curve of 15-mer aptamer spot. Each measurement was performed three times and the average results plotted. Error bars are too short to include.

We also tested standard human plasma activated by ellagic acid reagent (Fig. 4B). Ellagic acid is used as the trigger of the intrinsic pathway in blood coagulation. Normal human plasma has generated thrombin for hematosis explosively when activated, for example, by an injury. It indicated that both 15-mer and 29-mer aptamers exhibit similar reaction tendency in a PBS spiked measurement. The SPR angle shifts was much larger (10-fold) than maximum thombin absorption that was confirmed in a PBS spiked measurement. Therefore, our multi-layered aptamer array may capture thrombin and thrombin-related molecules (ex. aggregating fibrinogen or heparin). So our chip could detect molecules in human plasma real sample before the coagulation process was completed, the established aptamer array fabrication process is considered to be effective for detecting molecules in blood.

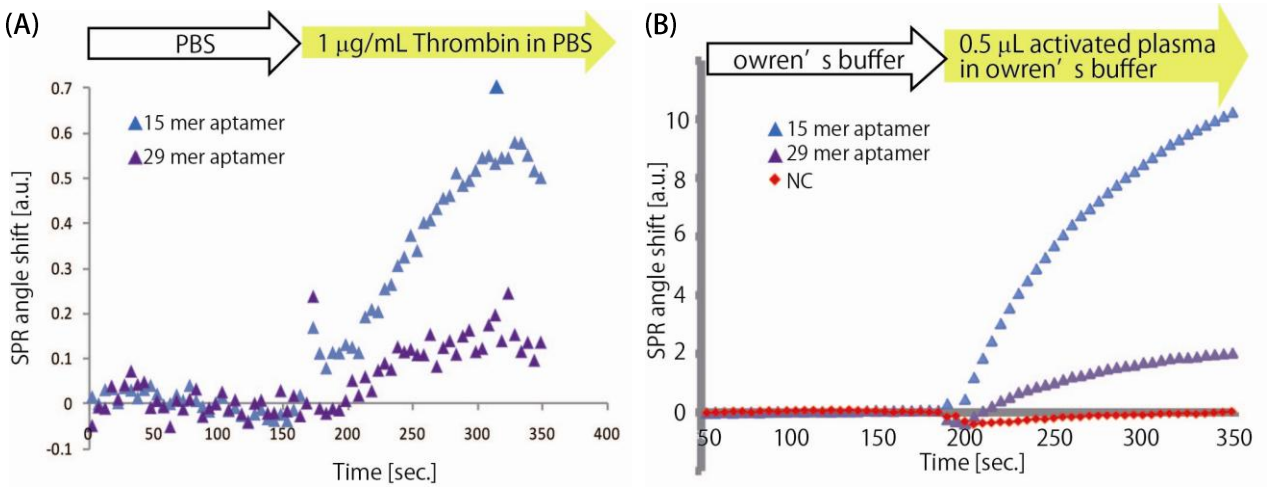

Figure 4. (A) Measured SPR angle shift for thrombin spiked in PBS buffer. Compared with the pumping flow measurement (fig. 3A), both 15-mer aptamer and 29-aptamer spots exhibit similar shift. The reaction can be detected simply by supplying a 10 μL sample solution via the chip inlet. (B) Measured SPR angle shift for thrombin in activated blood plasma with a capillary flow chip. This chip detected thrombin related signals before coagulation process was complete.

CONCLUSION

We realized a process for fabricating a multi-layered aptamer array integrated using an inkjet spotter. Using this aptamer array construction, we also fabricated a capillary flow chip that was designed to provide a continuous flow during measurement without the need for an external pumping device. The measurement only requires the mounting/detaching of the chip and the injection of a blood plasma sample, and completion before a thrombus is completely generated. The combination of this chip and SPR equipment reduces both the operator's work and the measurement time, and enables us to perform an on-site immunoassay.

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