ALL IN ONE LATERAL-FLOW CHIP FOR ARRAY IMMUNOASSAY
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ABSTRACT
We integrated an antibody array, a lateral flow channel and power-free pumping functions in one chip to realize a rapid and easy surface plasmon resonance (SPR) immunoassay technique. This one-time-use chip was composed of four injection-molded polymeric components. A continuous and constant liquid flow was realized by balancing flow resistance and capillary force. Simply by injecting a few microliters of sample solution onto the chip, we were able to detect kinetic reactions from 13 antibodies simultaneously within 10 min. even when the antigen was in real milk samples. This constitutes the first step towards fast screening for pathogenic toxigenicity.

KEYWORDS: SPR, Capillary-force, Injection molding, Immunoassay, On-site

INTRODUCTION
On-site immunoassay is expected to be useful in many fields and for many purposes including on-site clinical, food, and environmental analyses [1, 2]. The advantage of surface plasmon resonance (SPR) sensor technology is that can be used to monitor real-time and label-free antigen-antibody reactions [3, 4] with high sensitivity [5], and it is a promising candidate as a sensing approach for on-site immunoassay. We have already developed a portable SPR sensor, and have been working on overcoming certain major drawbacks, namely the complex protocols and relatively long measurement time needed for a conventional SPR immunoassay [6]. The development of a one-time-use and low-cost-chip is the key to making the SPR immunoassay a practical on-site technology.

Therefore our goal is to develop a disposable microfluidic chip optimized for performing SPR immunoassays that integrates a continuous liquid flow function and reagents (immobilized antibodies). The user needs only to install the sensor chip in an SPR sensor and supply a sample solution via the chip inlet.

Of the various liquid transportation principles, we consider capillary force to be suitable for our chip since it is induced by structures designed in the chip without the need for any other equipment.

Polymer or Si-based microstructures fabricated by photolithography and an etching technique [7-9] are known to induce a controlled capillary-driven flow. In this work, we designed a capillary-driven flow structure suitable for injection molding by using basic the design policy described in the theory section and by taking account of the characteristics of the constituent material.

THEORY
A continuous liquid flow is needed when monitoring an antigen-antibody reaction in real time. When we use capillary force as the driving force, the flow characteristics are determined by many factors including viscosity, surface tension, and contact angle and the geometry of the flow channel. But the dominant factor is flow resistance, $R_f$, and this means that the volume flow rate, $Q$, tends to decrease as the liquid travels a longer distance. With the simple straight flow channels (Models 1 and 2 in Fig. 1), $Q$ decreases exponentially as derived from the following equations;

$$Q = \frac{1}{\eta} \cdot \frac{\Delta P}{R_f}, \quad (1)$$

$$R_f = \int \frac{12(T(L) + W(L))^2}{T(L)^2 \left(1 + \frac{5T(L)}{6W(L)}\right)} dL, \quad (2)$$

where $\eta$ and $\Delta P$ are the liquid viscosity and the difference between the pressures inside and outside the liquid, and $W(L)$ and $T(L)$ are the arbitrary width and thickness of the flow channel, respectively, which are both functions of length.

However, a sudden widening of the flow channel can suppress any rapid decrease in the volume flow rate and also create a pseudo-plateau as shown in Model 3 in Fig. 1. Therefore, the basic design of our chip combines a channel with a narrow cross-section (corresponding to the SPR observation area) and a channel with a wide cross-section that becomes the pumping area. To realize a pumping function with an injection molding technique, we decided to prepare a pillar structure. To obtain a flat flow rate after the liquid had entered the wide fluidic channel, we adjusted the fluidic design to balance the flow resistance and capillary force by positioning a meander channel between the narrow observation area and the pumping area.
**EXPERIMENTAL**

The flow channels and pumping area are created by combining the flow channel part with a gold-deposited polymeric substrate as shown by the cross-sectional view in Fig. 2. Pillars were arrayed in the pumping area to increase the capillary (suction) force, and the narrow meander channel regulated the strong capillary force generated by the pumping area.

Antibodies were immobilized periodically as spots on the gold-deposited surface as shown in the perspective view in Fig. 2 (a). The chip was composed of four injection molded polymeric components that were easily assembled by inserting the substrate and flow channel part and snapping the cap part into the body part as shown in Fig. 2 (c). The channel part was made of a flexible polymer so that the flow channel could be configured without any liquid leakage.

A liquid sample was injected into the inlet and then traveled through a straight flow channel that was aligned with an array of antibody spots (Fig. 2 (a)). The sample reached the pumping area by being sucked continuously during the measurement. In our portable SPR equipment, 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 flow channel was aligned. This experimental setup enabled us to perform multi-spot measurements simultaneously. The chip was passively aligned with the chip mount area of our portable SPR equipment (Fig. 3) and contacted optically via a refractive index matching sheet attached to the rear of the gold-deposited substrate. Therefore the mounting/detaching operation was also very easy, thus shortening the measurement time.

![Figure 2 Perspective view (a) and cross-sectional view (b) (at dashed line AB) of the chip structure. The flow channel consists of an inlet, a straight flow channel, a meander flow channel and a pumping area. Flow channels are created by the flow channel part and the gold deposited polymeric substrate. Antibodies are immobilized periodically on the gold-deposited surface. Bottom perspective view (c) of snap-on cap, flow channel part, substrate and body part. The cross-sections of the straight channel and meander channel are 500 x 130 and 200 x 130 μm, respectively. The radius and distance between adjacent pillars are both 300 μm. All parts are designed to be aligned automatically with SPR equipment. The flow channel part and substrate assembly corresponds to the structure in Fig. 2 (a) and (b).](image)

**RESULTS and DISCUSSION**

We controlled the flow rate by changing the cross-sectional area of the meander (resistance) channel and the pumping area. We achieved a constant volume flow rate of around 2 μL/min for non-homogenized milk.

The chip we measured had 13 spots in which we immobilized anti-human IgG and anti-rabbit IgG. 1 μg/mL of human IgG was used as the sample solution. The multi-spot reactions were measured and recorded simultaneously. The response from an antigen spot was subtracted from that of a reference spot, which was treated with blocking solution and placed on both sides of the antigen spot, to eliminate the non-specific reaction. Figure 4 shows typical measured adsorption curves obtained from three different spots on the chip. Only the anti-human IgG spot exhibited a

![Figure 3 Photograph of portable SPR equipment.](image)
large SPR angle shift. Specific reactions were successfully obtained for all 13 spots with the multi-spot immunoassay.

We also measured the antigen-antibody reaction in non-homogenized milk as a practical sample. The prepared chip had three pairs of anti-human IgG and anti-protein A spots. We could clearly observe the antigen-binding reaction in the anti human IgG spot for human IgG diluted non-homogenized milk (1 μg/mL) as shown in Fig. 5. It is noteworthy that we were able to distinguish a positive reaction from a negative reaction within 250 sec without bound/free (B/F) separation.

**CONCLUSION**

We realized a lateral flow chip based on capillary force for array immunoassay. The chip was designed to provide a continuous flow at a constant flow rate during measurement without the need for an external pumping device. An array of antigens was immobilized in the chip in advance. The measurement only requires the mounting/detaching of the chip and the injection of a liquid sample. 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. Since the chip fabrication is very easy, simple and cost effective, this configuration provides us with a versatile method. If we select an appropriate antigen or other molecular recognition molecules, this chip can have many applications.

**REFERENCES**


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