Development of a micro biochip integrated traveling wave micropumps and surface plasmon resonance imaging sensors

Takaaki Suzuki · Yuji Teramura · Hidetoshi Hata · Koki Inokuma · Isaku Kanno · Hiroo Iwata · Hidetoshi Koter

Received: 30 June 2006 / Accepted: 3 January 2007 © Springer-Verlag 2007

Abstract Since most of miniaturized surface plasmon resonance (SPR) sensing systems need commercially available peristaltic or syringe pumps, it is difficult to reduce the system size, biosample volume, and the production cost. In this paper, a compact biochip for clinical diagnosis is presented. The proposed biochip is integrated traveling wave micropumps and SPR imaging sensors on one chip. The micropump is composed of flexible microchannel and piezoelectric bimorph actuator array, and achieved the maximum flow rate 336 µl/min. The SPR imaging biosensor can quantitatively measure biosamples with multi microchannels, i.e. one biosample and two reference flows to obtain an analytical curve. The SPR imaging measurements with bovine serum albumin solutions were carried out using the prototype of the proposed diagnostic system composed of a pair of the micropump and the sensor. Since the clear SPR signal curve was observed, it was confirmed that the proposed system can be applicable to the clinical diagnosis.

1 Introduction

Point of care testing (POCT), which is diagnostic testing carried out on site close to patients, significantly improves healthcare and healthcare delivery. Many miniaturized clinical diagnostic systems have been proposed on the basis of micro total analysis systems (µTAS), in which all processing steps are performed on a chip (Manz et al. 1990; Vikner et al. 2004). Miniaturization provides the means for reduction of sample and reagent volumes, high throughput screening, portability and easy-to-operate devices for a low-cost healthcare testing device.

In several types of biosensors proposed for µTAS, surface plasmon resonance (SPR) imaging is one of the most suitable biosensor for detecting the specific biosample with real-time and multisensing analysis (Rasooly and Jacobson 2006; Honola et al. 1999). Especially, miniaturized SPR sensors have been developed as an alternative to laboratory SPR systems to allow for the development of mobile, compact, and cost-effective sensing devices. However, most of the sensing systems need commercially available peristaltic or syringe pumps of normal size, and therefore it is not easy to reduce the whole size of the system and biosample volume as well as the production cost.

In this paper, we propose a compact POCT system integrated traveling wave micropumps and SPR
imaging sensors on one chip. The traveling wave micropump transports the fluid by the peristaltic motion induced on flexible microchannel by piezoelectric actuator array. The SPR imaging sensor measures the affinity binding of unlabeled biological molecules. In particular, we propose a double-sided molding process based on the soft lithography for low-cost and disposable biochip for POCT applications. After characterizing the performances of the micropump and the biosensor separately, we carried out the detection test in order to confirm the validity of the proposed diagnostic system.

2 Traveling wave micropump

A key component in μTAS is the micropump. A variety of micropumps have been proposed for fluid transportation and manipulation systems, but conventional diaphragm-type microumps that use mechanical valves or diffuser/nozzle elements have a complicated structure and high fluidic impedance (Laser and Santiago 2004; Nguyen et al. 2002). To address these limitations, we have proposed and developed a novel valveless traveling wave micropump (Suzuki et al. 2004, 2005). It uses piezoelectric actuator array to induce a traveling wave in a flexible microchannel made of silicon rubber. The valveless microchannels are rapidly and easily made from polydimethylsiloxane (PDMS) using UV lithography and molding (Sia and Whitesides 2003), thus allowing it to be used as a low-cost disposable device (piezoelectric actuators can be reused). In previous research literatures, whole microchannel was made of PDMS. In this paper, we proposed a flexible microchannel composed of PDMS microgrooves and a glass substrate with partially deposited gold thin film as a SPR excitation layer.

2.1 Principle of fluid flow

The traveling wave micropump used in the experiments is composed of flexible microchannel and piezoelectric bimorph actuator array as shown in Fig. 1. The fluid flow was controlled by changing the sine wave forms of the applied voltage. By changing the phases of the applied voltage to the piezoelectric actuators, the micropump can induce bi-directional fluid flow without using mechanical valves. When a wall surface of the microchannel oscillates in the form of the traveling sine waves, a trajectory of a fluid particle is close to the elliptic form. After a period of the wave, the fluid particle slightly moves from the initial position due to the fluid viscosity. The net fluid transport is achieved by repeating such motion.

Yin and Fung (1971) theoretically analyzed the two-dimensional peristaltic pump velocity profile using the perturbation method. When the traveling wave is applied to the fluid, the time-averaged flow velocity of the flow-direction $\bar{u}$ is

$$\bar{u} = \frac{1}{2}a^2\Psi(z)$$

where $\Psi(z)$ is a function of the height of the microchannel, and $a$ the amplitude of the traveling wave. From Eq. 1, the time-averaged flow velocity in the flow-direction is proportional to the square of the amplitude of the traveling wave on the microchannel wall.

![Fig. 1 Schematic of traveling wave micropump](image1)

![Fig. 2 Double-sided molding process](image2)
2.2 Fabrication process

To efficiently transfer the deflection of the actuators to the fluid transport in the microchannel, we prepared the flexible thin wall made from PDMS using the double-sided molding process as shown in Fig. 2. First, we prepared two SU-8 molds for groove and bump. The thick photoresist SU-8 was first spin coated on a glass substrate. The masters of the microgrooves and the bumps were fabricated on the glass substrate using photolithography procedure. Next, liquid PDMS was infused between two molds using the vacuum-forming technique. After heating solidification, a set of PDMS and the bump mold was peeled off from the groove mold. On the other hand, gold thin film was deposited on the glass substrates using a vacuum evaporator as a SPR excitation layer. Then, the PDMS/bump mold and the Au/glass substrates were bonded using O₂ plasma bonding technique (Bhattacharya et al. 2005). Finally, PDMS/glass microchannel was peeled off from the bump mold. The order of the peeling molds can be controlled by the exfoliative coating area and O₂ plasma bonding force. Figure 3 shows SEM image of the prepared microchannels at the micropump. The dimensions of the fabricated microchannel at the area of the micropump were 200 μm wide and 50 μm height. The parallel bumps aligned along the flow direction of the microchannel as shown in Fig. 3. For easy alignment of the microchannels and the bumps, we prepared the mold composed of parallel lines of bumps on larger area than the top wall of the microchannel. It is confirmed that the microchannel having groove and bump structures on both sides of the thin wall can be simultaneously fabricated by the proposed process.

2.3 Pumping performance

As pumping performance of the prototype of the traveling wave micropump, we measured the flow rate and the back pressure. Flow rate was calculated by velocity profiles obtained experimentally with the micro-particle image velocimetry (micro-PIV). In the micro-PIV system, the velocity vector fields can be measured by image-analyzing the motion of the fluorescent microbeads in the fluid by a digital high-speed camera (Santiago et al. 1998). The back pressure was derived from the height difference of the water between inlet and outlet. The flow rate and the back pressure as a function of the applied voltage are shown in Fig. 4. From the experimental results, the flow rate and the back pressure are proportional to the square of the amplitude of the traveling wave as well as the applied voltage. The flow rate is also proportional to the square of the amplitude of the traveling wave in 2D theoretical analysis based on the perturbation method (Yin and Fung 1971) and 3D numerical calculation based on the finite volume method (Suzuki et al. 2004). Therefore, the experimental result has a similar tendency to the theoretical and numerical results. Due to an upper flow velocity limit of the micro-PIV system, a sinusoidal voltage in range of 0–40 Vpp was applied in the experiments. When it can be applied up to voltages of 100 Vpp and 2.4 kHz near the resonance frequency to the fabricated piezoelectric actuator array, the prototype of the micropump would generate a flow rate of approximately 336 μl/min and back pressure of 87 kPa.

3 SPR imaging sensor

Miniaturized SPR imaging sensors with multi micro-channels have been proposed to achieve high
throughput screening capability with parallel analyses (Berger et al. 1998; Lee et al. 2001). However, the detection sensitivity of the systems in which microchannels and substrates are not bonded decrease due to cross contamination and dehydration of immobilized materials. In this paper, the sensing microchannels based on the SPR imaging is made from PDMS using the double-sided molding process, and PDMS microchannels and a glass substrate are irreversibly bonded using O2 plasma bonding technique in order to increase the detection sensitivity.

3.1 Principle of SPR imaging

The SPR phenomenon occurs at gold surfaces subjected to the incident light at a particular angle (Davies 1996). Depending on the thickness of a molecular layer on the gold surface, the intensity of the reflected light is gradually changed by the SPR phenomenon. The SPR imaging is a variation of the SPR technique in which multiple adsorptions can be monitored in an array chip under identical conditions. The presence of the biosample in the fluid can be detected by analyzing the time-lapse SPR images of the reflected light from the substrate. The PDMS/glass microchannels at areas of the SPR imaging biosensor and the micropump were simultaneously fabricated by the double-sided molding process. Since the PDMS microgrooves and the Au/glass substrates were bonded using O2 plasma bonding technique, high detection sensitivity of the SPR imaging sensor can be obtained without cross contamination and dehydration of immobilized materials. The dimensions of the fabricated multi microchannels at the area of the SPR imaging were 500 μm wide, 80 μm height, and 2 mm intervals.

3.2 Evaluation of quantitative measurement

To demonstrate sensing characteristics of the proposed chip, the adsorption of bovine serum albumin (BSA) onto the gold surface was evaluated by putting the prepared chip on an optical apparatus composed of a halogen lamp, a prism, filters, optical lens and a CCD camera as shown in Fig. 5. Three samples with different concentrations 0.1, 0.5 and 1.0 wt% of BSA/water were simultaneously infused to the microchannels by syringe pumps connected to inlets of the microchannels by Teflon microtubes. The flow velocity driven by the syringe pump was 4.2 mm/s. Time lapse SPR images were clearly observed by the CCD camera as shown in Fig. 6. Three bright lines in the figure indicate three independent microchannels, and the dark lines in the bright lines SPR measurement areas with partially deposited gold thin films. It can be seen that the light intensity in the SPR measurement area was gradually increasing with the concentration of the BSA/water after the solutions flow. The light intensity at the yellow points in the images is plotted as a function of the time as shown in Fig. 7. From the SPR sensorgram derived by the images, it is seen that the quantity and the rate of the adsorption depend on the concentration of the sample in accordance with kinetics as shown in Fig. 8. Therefore, the proposed system is applicable for characterizing and quantifying biomolecular interactions, e.g. the antigen–antibody reaction.

4 Compact diagnostic device

To demonstrate basic sensing characteristics of the proposed diagnostic device, we prepared a prototype of the system integrated a pair of a traveling wave micropump and a SPR imaging sensor with a microchannel on a glass substrate as shown in Fig. 9. The affinity binding of unlabeled BSA onto the gold surface was evaluated by putting the prepared chip on the conventional optical apparatus for the SPR imaging as shown in Fig. 10. Since the prototype is a microfluidic system on one chip, it can be achieved that miniaturization of a device, reduction of sample and reagent volumes, and high disposability of a low-cost chip.
BSA solution of the concentration 1.0 wt% was flowed using the traveling wave micropump and the SPR sensogram derived by the CCD camera images. Flow velocity generated by the micropump was 0.55 mm/s with applied voltage 120 V_{pp} and 1.0 kHz. Since the clear SPR signal curve was obtained as shown in Fig. 11, it is confirmed that the proposed system fabricated by the double-sided molding process is
applicable for characterizing and quantifying the affinity binding of biomolecular interactions as well as unlabeled biological molecules onto the gold surface. For clinical diagnosis, the antigen–antibody reaction, e.g. detection of the tumor marker, can be quantitatively analyzed by the proposed system with the multiple microchannels.

5 Conclusions

In this study, we proposed a compact and low-cost disposable system for clinical diagnosis, which is composed of surface plasmon resonance imaging biosensors and traveling wave micropumps fabricated by the double-sided molding process. Since the clear SPR signal curve was observed using the prototype of a pair of the traveling wave micropump and the SPR imaging sensor, the proposed diagnostic system is applicable for Point of care testing.

Acknowledgments This study is a part of Kyoto City Collaboration of Regional Entities for the Advancement of Technological Excellence of JST on the basis of research results supported in part by grant-in-aid for Scientific Researches (A-No.15201033) and Center of Excellence for Research and Education on Complex Functional Mechanical Systems (COE program) of MEXT, Japan.

References


