Graduate School of Pure and Applied Sciences

Automatic processing of solutions for integrated microfluidic biosensing devices

（集積化マイクロフルイディック・バイオセンシングデバイスのための自動溶液処理）

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1. Introduction

Micro Total Analysis System (μTAS), in recent years, is showing its chief and true type potential for wide range of biological applications like cell analysis and manipulation, nucleic acid analysis, protein assay, organ-on-chip, organism-on-chip, high throughput drug screening, drug delivery and advanced therapeutic system, POC diagnostics, diversified biosensing, investigation of environmental cues and others on the platform of Lab-on-chip (LOC). The μTAS key configuration is in the order of sample input, sample pretreatment (if necessary), microfluidic system, detectors and output signal **(Berg et al., 1998)**. For operation unit, different trustworthy microfluidic components like microvalves, micropumps, mixer, separator or filter, heater etc. are becoming indispensable part for producing high performance integrated device depending on the purpose **(Berg et al., 1998; Kovarik et al., 2012; Culbertson et al., 2014; Reyes et al., 2002, Auroux et al., 2002**). For next generation throughput analysis system, integrated device with automatic or programmed operation regarding the flow control of solution is becoming critical. In majority cases, the solution flow is controlled by external pumps or pressure sources linked with bulky instrumental set-up. Therefore, in μTAS-LOC-Microfluidics platform, there remain huge room for the improvement of integrated system along with microfluidic component especially microvalves and micropumps for regulating and processing of fluid by cutting off the external signal.

In recent years, the autonomous operation of the integrated device are attempted to discard the bulky exterior setup. As for example, from our laboratory, **Siribunbandal et al. (2009)** reported on-device power generation from electrochemical cells for valve operation whereas **Obata et al. (2016)** demonstrated the on-chip bi-directional pump operation for on-device solution processing for sensing purpose. But, in terms of processing of many solutions in diversified microfluidic flow networks or chambers, the automatic system requires more advanced valve component and extraction unit for controlling the transport of wide range of solutions and removing the processed solutions respectively in a sequential manner.

In this study, a unique integrated microfluidic system for automatic processing of solutions especially controlled

handling and exchanging of multiple solutions for biosensing has been proposed. To realize the fruitful type device, the development and integration of a novel switchable hydrophobic valve and superabsorbent polymer based extraction pump have also been proposed. First, the protein sensing performance has been investigated by valveless simple microfluidic having the capability of sequential solution exchange. Later, an integrated system has been developed incorporating switchable valve, extraction pump and modified (superhydrophilic) substrate surface for realizing the automatic solutions processing for biosensing.

2. Materials and methods

2.1 Device fabrication

Normally, the test devices were fabricated by bonding a PDMS substrate having necessary flow patterns on a glass substrate (with or without electrode pattern). The thick-film photoresist (SU-8 25) was used as a template for patterning the flow channels, air vent and reaction chamber in PDMS by SU-8 replica molding process. The ports (injection and outlet ports) for sample solution injection and processing were formed by making though-holes in the PDMS layer using a disposable biopsy punch. The height of the flow channels and reaction chamber was 80 μm for all devices whereas the width varied according to the need. For the construction of valve electrode pattern, a sputter-coating of 300-nm thick platinum on glass substrate and subsequent lift-off process were followed. A chromium intermediate layer (50-nm thick) was used to promote adhesion of the platinum layer to the glass substrate. To regulate the movement of solutions in different flow channels, the PDMS with the patterns and glass substrates with the valve were carefully aligned and were bonded by applying slight hand pressure. To make strong bonding (wherever necessary), the patterned PDMS and glass substrate were exposed to oxygen plasma for 20 s at 20 W with 30 Pa for activating their surfaces and were bonded.

2.2 Construction of valve

For the construction of valve, a hydrophobic SAM layer was grown on the patterned platinum electrode by immersing the electrode in the thiol solution. Before that, the electrode with substrate was cleaned in a mixed solution containing water, 25% NH_3, 30% H_2O_2 in a 4:1:1 volumetric ratio tailed by additional cleaning process with potential cycling (10 times) using cyclic voltammogram in a potential range between −1.0 and +1.0 V (vs. Ag/AgCl/sat. KCl) in a 0.1 M KCl solution. The cleaned and washed electrode was dried by blow of nitrogen gas and was instantly dipped in a 1 mM 1-hexanethiol aqueous solution containing 0.1 M ethanol for 3 min. After the SAM formation, the surface was washed again with pure ethanol and water, and dried by nitrogen gas.

2.3 Preparation of absorbing polymer disc for pump system

The pump system was constructed by using the absorbing property of superabsorbent polymer (SAP). To absorb solutions, a disc of SAP was used as absorbing material in the pump chamber. To form the absorbing disc, an inert supporting material was mixed with the polymer gel. Then, this gel was put into the template of disc formed in PDMS and pressed with needle to make it compact. Finally, the usable polymer absorbing disc was obtained from the freeze drying process. The dried absorbing polymer discs were then preserved in sealed container to avoid contact with humid air.

2.4 Making of superhydrophilic flow pattern

To realize smooth flow of solutions in the flow channels and the reaction chamber, the glass surface was made more hydrophilic by increasing the surface roughness. At first, the exposed pattern of desired design was made by lithographic technique using dry film photoresist. In this case, the superhydrophilic flow pattern was generated by powder blasting process on exposed pattern of glass wafer. Finally, the photoresist was removed to get substrate with desired pattern. The substrate was then cleaned with the solution containing 25% NH_3, 30% H_2O_2 in a 4:1:1 volumetric ratio for 1 h.

3. Results and discussions

3.1 Simple valveless microfluidic device for protein detection

3.1.1 Exchange of solutions

Figure 1A shows the structure and setup of the simple microfluidic device for solution exchange. To realize proper exchange of multiple solution in the reaction chamber, a series of solutions were injected through separate inlet flow channels one by one and subsequently removed from reaction chamber using syringe pump **(Figure 1B)**. Each time, reaction chamber was filled without any air trapping inside the chamber and no solution moved to other flow channels connected to the reaction chamber. And exchange of solution was also performed smoothly without any splitting of solution.

3.1.2 Protein detection using simple microfluidic device

A sandwich fluorescence immunoassay process was followed for the detection of protein (Human IL-2) **(Figure 2.2)**. Instead of following the traditional immunoassay process, here a rapid protocol was performed based on APTES interacted antibody immobilization reported by our group **(Usuba et al., 2016)**. Stepwise, different inlet flow channels were used for injecting the reagent solutions and washing buffer required for the immunoassay. For immobilization, firstly, the mixture of coating buffer 1% (v/v) APTES and capture antibody was injected to the reaction chamber and incubated for about 30 min. Then, the blocking reagent (0.5% BSA) was delivered and kept for 30 min to avoid non-specific binding. Afterward, the mixture of FITC-labeled detection antibody and antigen (IL-2) was incubated for 15 min for cAb-Ag-FITC labeled dAb complex formation.

Depending on the formation of cAb-Ag-FITC labeled dAb complex in the measuring chamber, the concentration of sample antigen (IL-2) is normally determined. **Figure 2A** showed the on-device fluorescence signal. The variation of fluorescence signal from the protein complex (cAb-Ag-FITC labeled dAb) corresponds to each of the different sample concentrations. Fluorescence image taken from the device showed the variation in intensity due to change of sample concentration. The intensity increased as the concentration of sample concentration increased **(Figure 2B)**. This indicated the little bit higher background signal. By optimizing the each of step of immunoassay, the signal could be enhanced more.

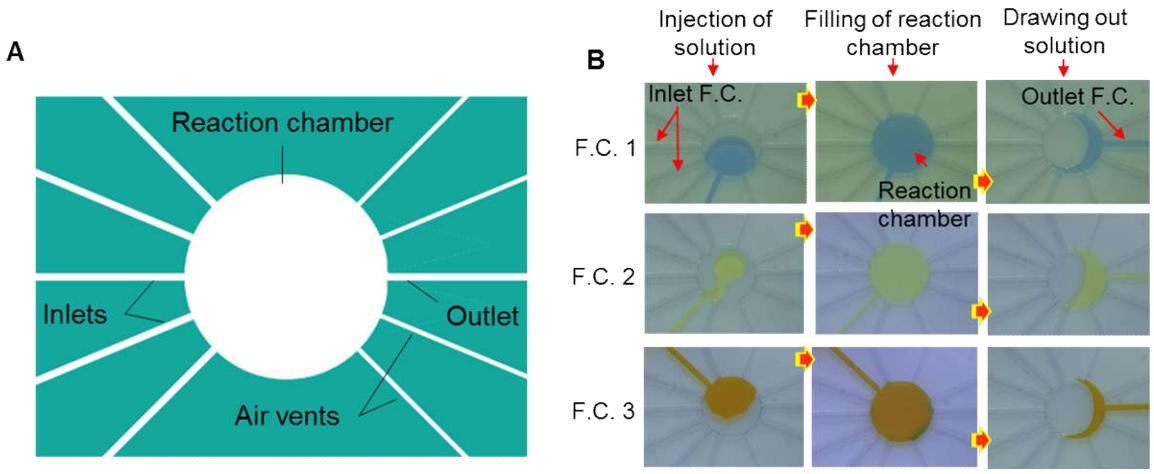

Figure 1 Solution exchange in simple microfluidic device. *(A) Schematic drawing of the simplified microfluidic device, (B) Sequential injection and extraction of solutions. (F.C. denotes the Flow Channel).*

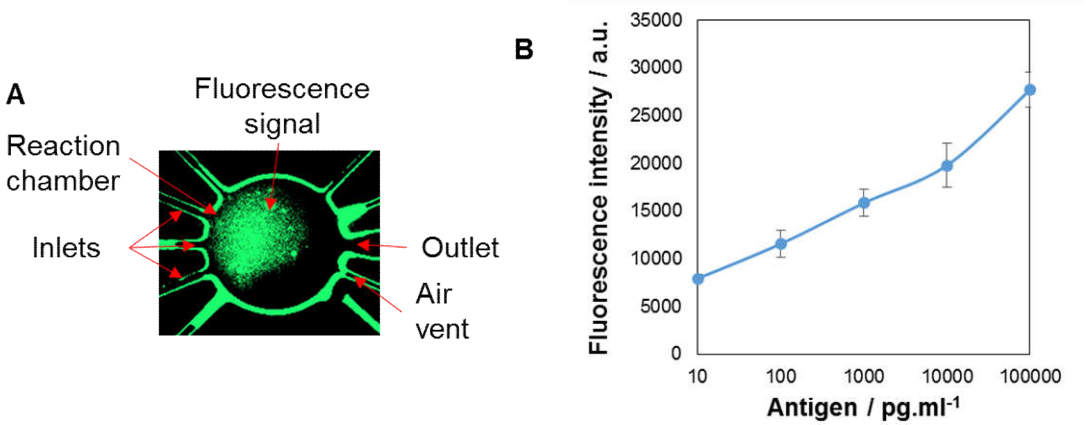

Figure 2 Fluorescence signal derived from the immunoassay. *(A) On-device fluorescence signal, (B) Fluorescence intensity at different concentration of antigen (IL-2) from simple microfluidic device.*

3.2 Controlled microfluidic processing using a switchable hydrophobic valve

3.2.1 Principle of the operation of the valve

The valves presented here were fabricated by the attachment of a poly-dimethylsiloxane (PDMS) substrate having the microchannels and a glass substrate containing thin-film platinum electrode on which the hydrophobic SAM was formed **(Figure 3A)**. The operation principle of the valve is briefed in **Figure 3B**. The hydrophobic SAM on the electrode offers the function of stopping for the valve (i). Application of an appropriated electric potential (-1.0 V) to the platinum electrode with respect to the reference electrode results in desorption of the SAM and the beginning of the solution movement (ii and iii). Once the solution moves across the valve electrode, the solution remains to flow in the microchannel (iv).

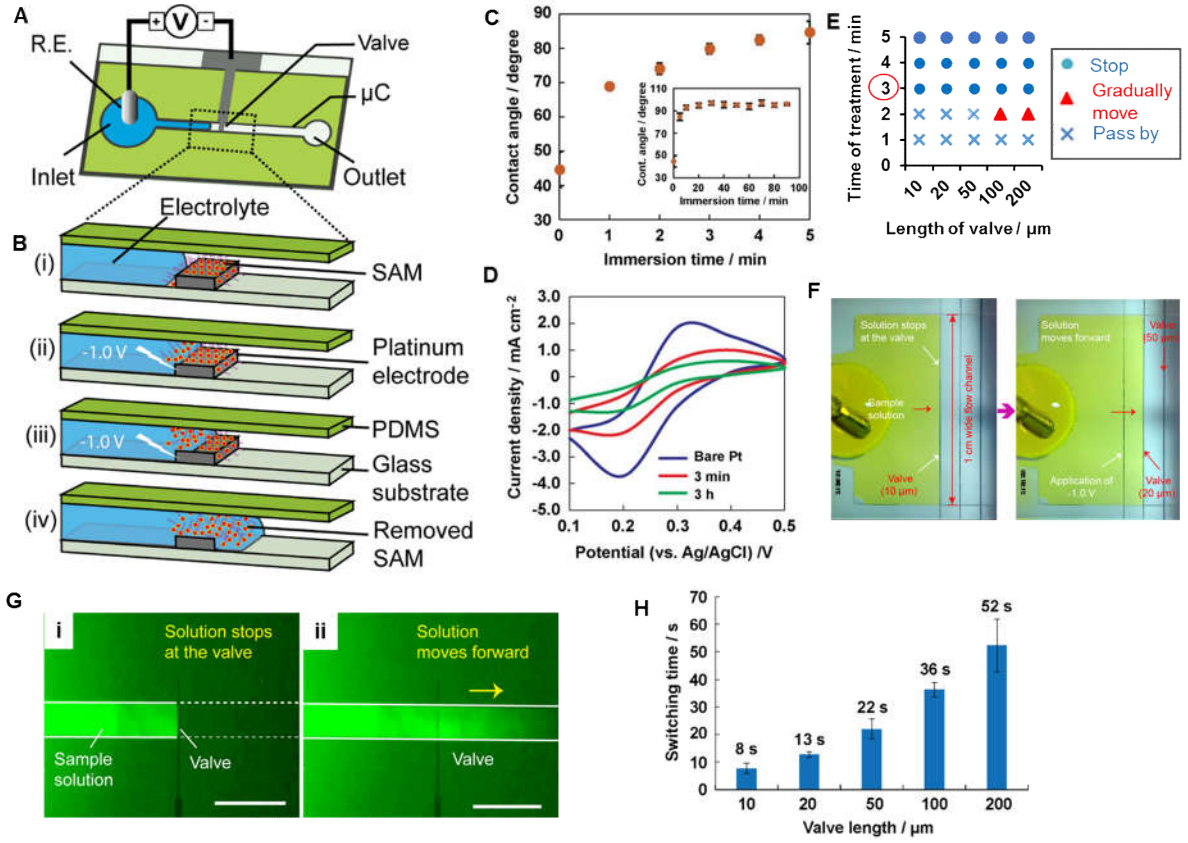

Figure 3 Principle of operation of valve, SAM formation strategy and trend of switching time of valve. *(A) Schematic of microfluidic system with single valve and a microchannel. R.E. denotes to reference electrode. μC denotes to microchannel. (B) Principle of the operation of the valve. (C) Contact angle scenario on platinum upon different SAM treatment time. (D) Electroinactive property of SAM layer on platinum. (E) Effect of the SAM treatment on the length of the valve. (F) Control of solution transport in 1 cm wide flow channel with 10 μm long valve. (G) Control of solution transport in 500 μm wide flow channel with 20 μm long valve. (H) Dependence of switching time on the length of the valve. Scale bar = 1 mm.*

3.2.2 Choosing of appropriate SAM treatment time for construction and proper switching of valve

Reflecting the SAM property, the length of time to remove or desorb the SAM from the platinum electrode increased as the SAM formation time increased. The minimum SAM immersion time was chosen by the solution stopping capability of the tiny valve (10 μm long) in 1 cm wide flow channel. The lower SAM treatment time shows enough hydrophobicity **(Li et al., 2003; Petrovykh et al., 2006)** along with imperfect SAM formation showing less electroinactive layer **(Addato et al., 2011) (Figure 3C & D)** which is critical for proper switching of valve. When the immersion time was less than 3 min, the solution moved slowly over the valve without applying a potential **(Figure 3E)**. Thus, 3 min immersion time was finally chosen for all upcoming experiments including characterization and demonstration.

3.2.3 Control of solution transport and switching time of valve

Single valves made-up in straight micro flow channels with platinum electrodes coated with SAM layers could consistently stop capillary movement of solution and permit flowing again upon switching of valve by desorption of SAM from the valve electrode by applying a negative potential of −1.0 V (vs. Ag/AgCl (sat. KCl)) **(Figure 3F & G)**. The 10-µm valves was capable of stopping the all test solutions in different dimension of flow channels, even for a 1 cm wide microchannel (**Figure 3F**). The switching time was quantified by recording the time needed for the solution to pass the total length of platinum valve electrode (from the front to rear edge) after the application of potential. The height and width of flow channel used for measuring the switching time were 80 µm and 500 µm respectively **(Figure 3G)**. As projected, the valve switching time is directly reliant on the length of the valve. The 10 µm valve took the switching time of about 8 s and the switching time of the valve shows increased trend as the length of valve increased **(Figure 3H)**.

3.2.4 Effect pH and partial SAM formation on the switching of the valve

In the case of the valve where the SAM fully covered the electrode, the switching time was shorter at higher pH up to 12. However, at pH 13, the valve functions in an uncontrolled manner and the alkaline solution spontaneously moved across the valve area without applying any potential **(Figure 4A)**. This activities proposes that the SAM is detached or desorbed in highly alkaline solutions, which was witnessed by **Kong et al. (2008)**. To make switching faster, the SAM could be formed only on the top surface and the front and back edges could be exposed to increase the local pH on electrode surface. In this case, valve switching time reduced to around 6 s **(Figure 4B)**. **Figure 4** shows time courses of current during operation of the both type of valves.

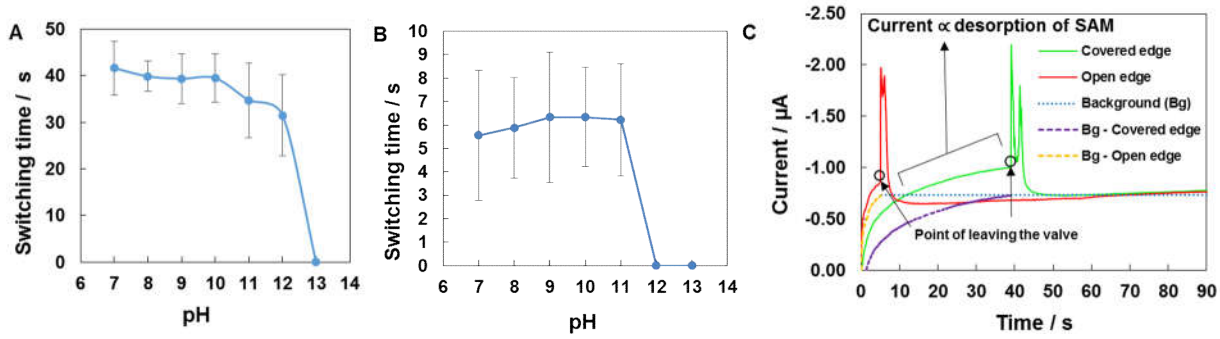

Figure 4 Effect of pH on the switching time of valve and current response during operation of valve. *(A) Effect of pH on the switching time of valves with fully covered SAM. (B) Effect of pH on the switching time for partially SAM covered (open edge) electrodes. (C) Time courses of current during and after SAM desorption from valve electrode.*

3.2.5 Microfluidic processing using a switchable hydrophobic valve

Figures 5 illustrates the controlled microfluidic processing using switchable hydrophobic valves. By sequential switching of the series of valves constructed in serpentine flow channel, a delayed delivery of solution by temporal stopping can be realized **(Figure 5A)**. Whereas, the different models of microfluidic multiplexers for

the solution distribution to several separate micro flow channels were also demonstrated. **Figure 5B** presents a 1-input to 6-output linear multiplexer having six separate and independent valves for distributing solutions to various selected microchannels. Each of microchannels has a valve for controlling the solution transport independently. The 1-input to 8-output circular or radial multiplexer, presented in **Figure 5C**, executes choosy distribution of the solutions to eight separate microchannels from a central inlet reservoir.

The valve can also be engaged for merging two different and head-to-head solutions. With the assistance of an air vent located at the position of the valve, two different moving solutions **(Figure 5D)** or liquid columns **(Figure 5E)** can be separated at head to head or sidewise respectively by tiny 10-µm valve and subsequently merged and mixed together via diffusion by valve switching operation.

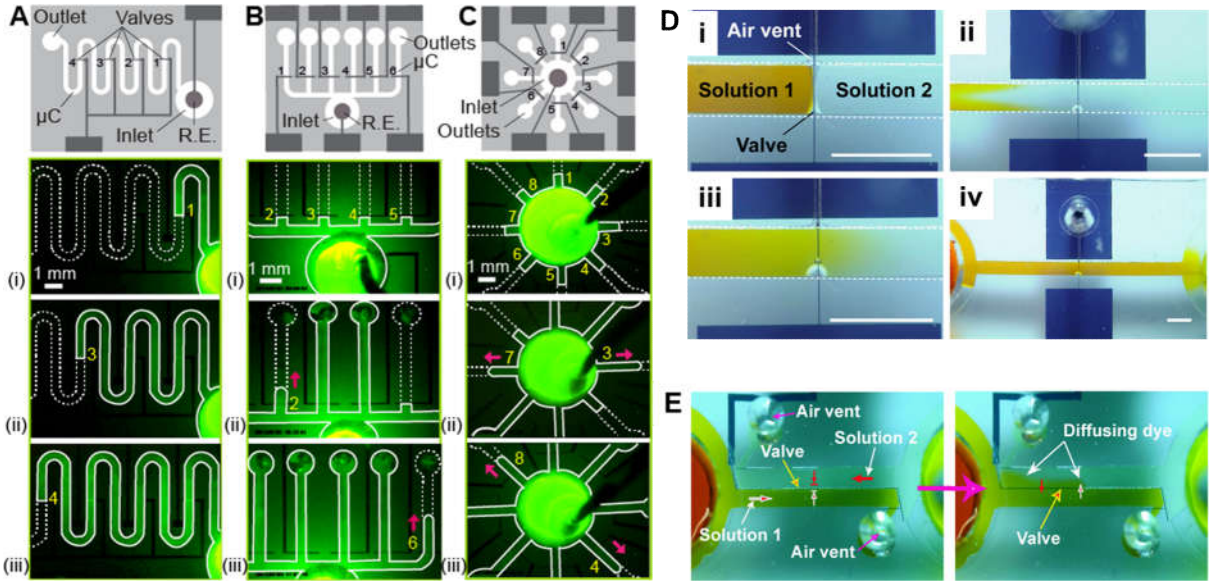

Figure 5 Controlled processing of solutions using switchable valve. *(A) Sequential operation of multi-valve in serpentine flow channel. (B) Operation of multi-valve in linear multiplexer system. (C) Operation of multi-valve in radial multiplexer system for selective distribution of solution. (D & E) Separating, merging and mixing of two different solutions and liquid columns. Scale bars: 1 mm. R.E.: reference electrode.*

3.3 Integrated microfluidic device with switchable hydrophobic valve and polymer extraction pump for automatic solution exchange

3.3.1 Principle of pump operation

A passive pump was constructed based on the solution absorbing and locking property of superabsorbent polymer. When a solution comes in contact with the preplaced absorbing polymer disc in the pump chamber, the total solution in the flow channel is absorbed and locked by the polymer disc **(Figure 6)**. As the polymer swells upward

by absorbing the solution, it provides the platform for solution exchange by extracting multiple solutions in a sequential manner.

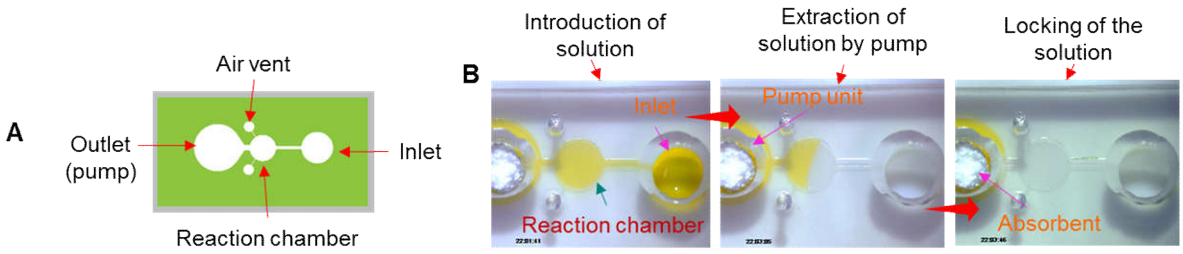

Figure 6 Controlled transport and extraction of solution. *(A) Simple device layout, (B) Performance of pump for extraction of solution – introduction, extraction and locking of solution.*

3.3.2 Powder blasting on glass substrate for the superhydrophilic pattern

For producing useful superhydrophilic pattern, along with lower contact angle, a minimum depth on blasted surface is essential. Otherwise, the solution flow could be disturbed or unexpectedly stopped by the deep ridge alongside valve or other electrode structures. It was found that, powder blasting at a constant pressure of 75 kPa for 15 s showed superhydrophilic surface by decreasing the contact angle below 5º. And the depth of the pattern was compatible with the flow system.

3.3.3 Operation of the integrated device for solution exchange

First, an integrated device with four inlet reservoirs, reaction chamber and pump unit was fabricated on glass surface having the pattern of valve electrode and superhydrophilic surface for the purpose of solution exchange **(Figure 7A & B)**. Before the operation of the device, solutions were loaded in the reservoirs. The capillary motion of the solutions were stopped by the hydrophobic valves constructed in each flow channel before the entrance of reaction chamber. For the performance of solution exchange, each of the solution from inlet reservoirs were allowed to fill the reaction chamber sequentially by switching the hydrophobic valve. The solution from the reaction chamber was extracted by the polymer pump unit when the processing valve was opened **(Figure 7C)**. The polymer disc absorbed the total solution coming from reaction compartment and locked to facilitate the entry of the next solution. After complete removal of previous solution, next one was introduced by opening the valve and subsequently again extracted by pump. Thus, a sequential exchange of multiple solutions was accomplished **(Figure 7D)**.

3.3.4 Protein sensing on integrated device

Protein (IL-2) sensing by fluorescence immunoassay described in the section 3.1.2 was performed on the integrated device with switchable hydrophobic valve, polymer extraction pump and superhydrophilic surface. The device responded by showing signal variation with the change of protein concentrations **(Figure 8)**.

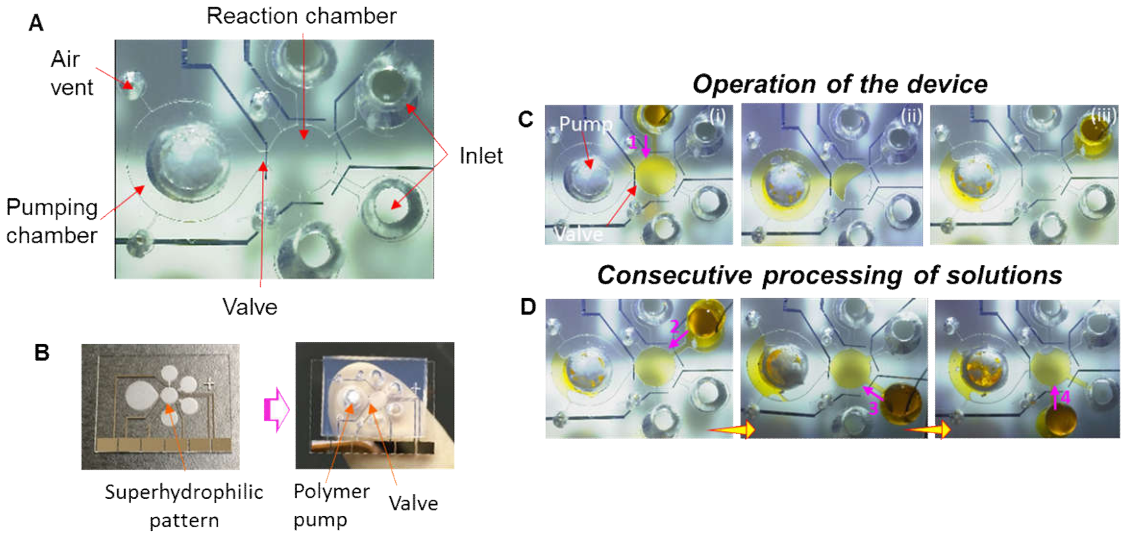

Figure 7 Automatic processing of solutions in integrated device. *(A) Integrated device, (B) Device fabrication, (C) Operation of the device for solution exchange (processing) – (i) filling, (ii) absorbing and (iii) locking of solution, (D) Consecutive processing of solutions.*

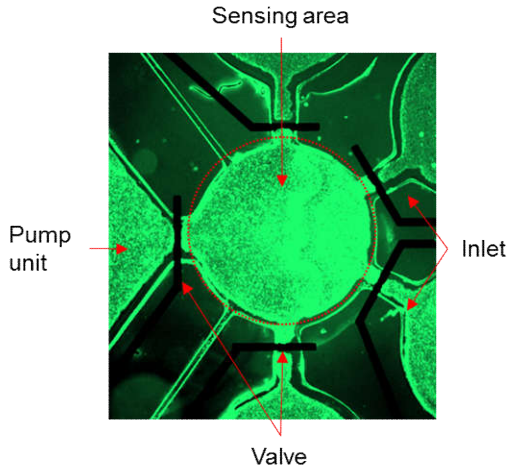

Figure 8 On-chip protein sensing. *Fluorescence signal on the integrated device with switchable hydrophobic valve, polymer extraction pump and superhydrophilic surface.*

4. Conclusion

A simple microfluidic device without active component was successfully developed for sequential exchange of solutions. The device also showed the efficient performance for protein sensing by immunoassay. On the other hand, a novel and simple switchable hydrophobic valve without having any internal or external moving parts has

been developed for the controlled processing of solutions using a low-power electric signal. The simplified structure, fabrication technique, and easy going electrical operation present this valve as an essential phase forward in achieving an integrated systems for controlled microfluidic processing along with the automatic micro-analytical systems. Therefore, an integrated device with switchable hydrophobic valve, superabsorbent polymer based passive pump and superhydrophilic pattern surface was successfully developed which could be used to realize automatic solutions processing. The integrated device bears a resemblance to the applicability of microfluidic immunoassay by performing exchange of series of solutions required for producing assay signal. Therefore, this integrated device could be very useful tool for bio-chemical analysis for centralized laboratory as well as on-site diagnostic system like POC.

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