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Valve-less Microfluidic Device for Sequential Exchange of Solutions for Fluorescence Immunoassay

Shishir Kanti Pramanik and Hiroaki Suzuki

Graduate School of Pure and Applied Sciences, University of Tsukuba,
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan
Tel : +81-29-853-5598 E-mail: hsuzuki@ims.tsukuba.ac.jp

Abstract

A simple valve-less microfluidic device was developed for the detection of proteins. Solutions were introduced into and removed from a reaction chamber one by one. The influence of hydrophobicity of the reaction chamber and viscosity of a blocking solution on solution exchange was examined. Analytical performance of the device was demonstrated by detecting human interleukin 2 (IL-2) by sandwich immunoassay. Clear dependence of fluorescence intensity on IL-2 concentration was observed in a range between 125 pg/mL and 2.0 ng/mL.

1. Introduction

Point-of-care testing (POCT) is becoming indispensable for prompt treatment of acute diseases and for homecare diagnostics [1]. In POCT, diagnostic results rapidly obtained even by non-trained personnel enable patients to receive follow-up treatment [2]. Although highly sensitive detection of proteins is often required in POCT, currently available devices provide mainly qualitative results [3].

To detect proteins, enzyme-linked immunosorbent assay (ELISA) has been used [4]. However, conventional ELISA is labor intensive, consume unnegligible volumes of expensive reagents, and require long assay times, restricting their application in clinical diagnosis. As a solution to this problem, microfluidcs provides means for on-chip immunoassays [5]. Also, microfluidic platforms enable high throughput assay [6] and hold a great potential as point-of-care diagnostic tools.

In conducting immunoassay in a microfluidic system, exchange of solutions is required. For practical analytical devices, microfluidic systems that do not rely on external bulky pressure or power sources, moving parts, and other regulatory units are indispensable. In this respect, valve-less flow control will be one of the solutions. In this study, we actually realized such a device. Furthermore, to demonstrate the usefulness of this device, heterogonous immunoassay was conducted to detect IL-2.

2. Experimental section

Fabrication of the device

The device was constructed with glass and PDMS substrates with a microfluidic structure. A reaction chamber, eight flow channels, solution reservoirs, and air vents were formed (Fig. 1). The height of the reaction chamber and flow channels was 100 µm, and the diameter of the reaction chamber was 2 mm (volume: 310 µL). The flow channels were 200 µm wide, and the entrance of the flow channels to the reaction chamber was constricted to 50 µm to avoid entering of an injected solution into the other flow channels. The PDMS and glass substrates were bonded by oxygen plasma treatment for 15 s at 20 W and 25 Pa oxygen pressure.

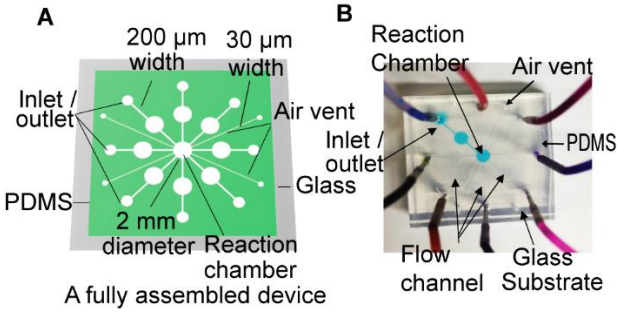

Fig. 1 Microfluidic device for solution exchange. (A) Top view. (B) Photograph of the device with silicone tubes.

Procedure for solution exchange

Silicone tubes were connected to syringes and were filled with dye solutions. The other end of the tubes was connected to a PDMS solution reservoir through a shortened syringe needle. Solutions were injected into the reaction chamber by applying air pressure gently and carefully by hand using a plastic syringe. After the reaction chamber was completely filled with a solution, it was withdrawn into the same solution reservoir by applying a negative pressure through the same syringe. The same steps were repeated sequentially for eight flow channels.

Procedure for on-chip immunoassay

Anti-IL-2 antibodies were labeled with FITC according to the manufacturer's instruction. To immobilize capture antibodies, solutions (1 µL) containing capture antibodies, APTES (1% v/v), and coating buffer were introduced into the reaction chamber and incubated for 30 min at 4°C. Then, 1 µL PBS containing 0.5% BSA and Tween 20 was introduced there and was incubated for 30 min at room temperature to reduce non-specific binding of proteins onto the surfaces. Then, a mixture containing IL-2 and FITC labeled detection antibodies was injected immediately into the reaction chamber and was incubated for 25 min.

After removing the solution, the reaction chamber was washed 3 times with PBS containing Tween 20, and fluorescence from the antigen-antibody complexes was detected using a fluorescence microscope (IX-73; Olympus, Japan) with a filter unit (U-FBNA, Olympus) and CMOS camera (ORCA-Flash 4.0; Hamamatsu Photonics, Japan).

3. Results

Exchange of solutions

Fig. 2 shows how solutions in different reservoirs were injected into the reaction chamber and returned to each reservoir one by one. Solutions did not split during the process and no residues were left in the reaction chamber after flushing of the solutions. To understand the influence of hydrophobicity on solution exchange, we made the bottom of the reaction chamber hydrophobic by patterning a hydrophobic negative photoresist (OMR-83, Tokyo Ohka Kogyo, Kawasaki, Japan). No adverse influence was observed in solution exchange. We also investigated the influence of solution exchange with bovine serum albumin (BSA) solutions of different concentrations. With a 1% BSA solution, air bubbles were sometimes trapped in the reaction chamber due to its viscosity. Therefore, in this study, 0.5% BSA solution was used for blocking.

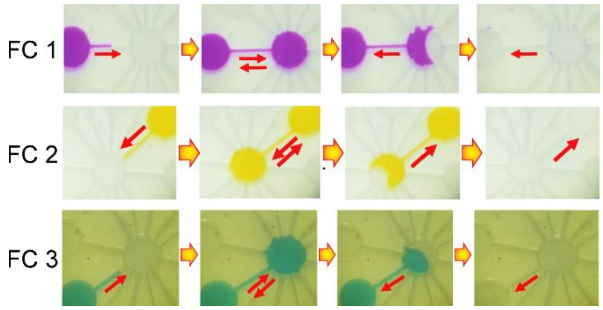

Fig. 2.Images that show sequential exchange of solutions in the microfluidic device. F.C.: Flow Channel.

Immunosensing performance

Fluorescence immunoassay was conducted for the detection of human IL-2 [7]. Fluorescence intensity depends on the number of antigens bound to the immobilized capture antibodies. Fig. 3A shows fluorescence from the reaction chamber. Fig. 3B shows the deependence of fluorescence intensity on IL-2 concentration. Fluorescence intensity increased with the increase in IL-2 concentration. A sharp increase in fluorescence intensity was observed in a range of concentration between 125 pg/mL and 2.0 ng/mL. Fluorescence intensity tended to saturate at concentrations higher than 4 ng/mL. The calculated relative standard deviation was 5.0%, demonstrating the reproducibility of the assay.

3. Conclusions

A simple valve-less microfluidic device consisting of a reaction chamber and eight flow channels was developed to exchange multiple solutions for biochemical analyses. Solutions can be exchanged by only injecting a solution from reservoirs into the reaction chamber and returning the solution in each reservoir. The device is applicable to the detection of proteins. IL-2 could actually be detected within 25 min. Our device may be a useful tool for POCT.

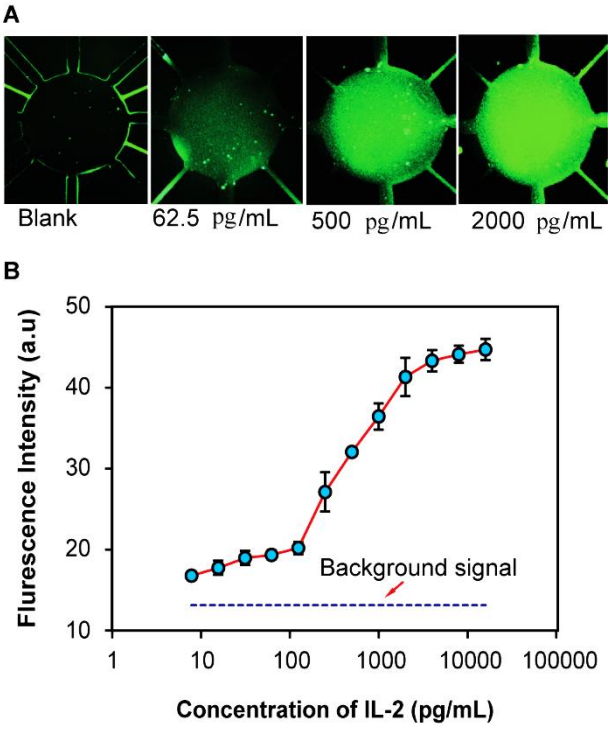

Fig. 3 (A) Optical microscope images of fluorescence from the reaction chamber observed with IL-2 of different concentrations. (B) Dependence of fluorescence intensity on IL-2 concentration. Error bars represent means ± standard deviation for 3 replicates.

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References

[1] D. Huckle, Expert Rev. Mol. Diagn. **8** (2008) 679.

[2] V. Gubala, L.F. Harris, A.J. Ricco, M.X. Tan, D.E. Williams, Anal. Chem. **84** (2012) 487.

[3] V. Linder, Analyst **132** (2007) 1186.

[4] J.W.A. Findlay, W.C. Smith, J.W. Lee, G.D. Nordblom, I. Das, B.S. DeSilva, M.N. Khan , R.R. Bowsher, J. Pharm. Biomed. Anal. **21** (2000) 1249.

[5] E. K. Sackmann, A. L. Fulton, and D. J. Beebe, Nature **507** (2014) 181.

[6] M. Zimmermann, E. Delamarche, M. Wolf, P. Hunziker , Biomed. Microdevices **7** (2005) 99.

[7] R. Usuba., M., Yokokawa, T.N. Ackermann, A. Llobera, K. Fukunaga, S. Murata, N. Ohkohchi, H. Suzuki, ACS Sensors. **1** (2016) 979.