ABSTRACT

We developed a new rapid and easy-to-use immunoassay platform to simultaneously evaluate multiple biomarkers in a single sample. As proof of principle, we demonstrated a multiplex assay measuring three biomarkers: $\alpha$-fetoprotein (AFP), C-reactive protein (CRP), and prostate-specific antigen (PSA). The assay was completed within 12 min, and the limit of detections (LODs) were several tenths of pg/ml for the three biomarkers.

KEYWORDS: Immunoassay, Multiplex, Easy-to-use, Photopolymer, Polystyrene beads

INTRODUCTION

The immunodetection of multiple biomarkers in a single sample is very important for many applications including clinical diagnosis, drug discovery, and so on. However, the current methods, such as planer arrays and beads-based suspension arrays, require a long reaction time, relatively large sample volume, and troublesome procedures [1, 2]. From viewpoint of practical use, it requires a rapid, small sample volume, easy-to-use and inexpensive method. Our platform developed here satisfies these requirements, and furthermore, offer rapid, easy-to-use, and high sensitive immunoassay platform for multiplex assay.

EXPERIMENTAL

Figure 1 shows a schematic illustration of fabrication method of triplex immunoassay device. A mixture of anti-AFP, anti-CRP, and anti-PSA antibodies which are immobilized on polystyrene beads (1\(\mu\)m-diameter) and photopolymer (poly(ethylene glycol)-based polymer) containing a photo initiator was introduced into a straight microchannel. UV light was irradiated through a photomask on the microchannel. The photopolymer was photopolymerized only in the exposed parts, and the exposed parts became hydrogel pillars including many biomarkers immobilized beads (Fig.1) [3]. The devices we used are made in cyclic olefin copolymer (COC) substrates (70 mm $\times$ 30 mm). The microchannels were fabricated by a conventional injection molding technique. The width and depth of the microchannel were 1000 $\mu$m and 50 $\mu$m, respectively. Since the microchannel with hydrogel pillars is not crowded as shown in Fig.2, the introduction and removal of the sample (a mixed solution of three biomarker antigens), the reagent (a mixed
solution of appropriate fluorescently labelled secondary antibodies: Dylight 647-labelled anti-rabbit IgG, Alexa 488-labelled anti-mouse IgG, Alexa 555-labelled anti-mouse IgG, and the for washing were easily achieved by capillary force and mild suction. The assay procedure in detail was described elsewhere [3]. The sample and reagent quantities required for assay were only 0.25 µl, respectively. The immune complexes in hydrogel pillars were detected by using a fluorescence microscope with wavelength of 488 nm, 555 nm, and 633 nm, respectively.

Figure 1. Fabrication of the triplex immunoassay device and the schematic illustration of hydrogel pillars including three biomarkers inside the microchannel.

Figure 2. (a) Photograph of immunoassay device. The COC were used as the device substrates (70cm × 30cm). The length, width, and depth of the microchannels were 10 mm, 1000 µm and 50 µm, respectively. (b) Expanded view of the microchannel. The diameter and height of hydrogel pillars inside the microchannel were 200µm and 50 µm, respectively.

RESULTS AND DISCUSSION

Figure 3 shows the calibration curves of AFP, CRP, and PSA. The total assay time was only 12 min. The limits of detection (LODs) were 10, 20, and 10 pg/ml for
AFP, CRP, and PSA, respectively. In spite of short assay time, these values were superior to that of conventional ELISA (enzyme-linked immunosorbent assay) using microtiter plates. Furthermore, we assayed serum samples by using our platform, and obtained successful results.

CONCLUSIONS
We successfully developed a new multiplex immunoassay platform which provides rapid, easy-to-use, small sample volume, inexpensive, and high sensitivity. We believe that this platform has a great potential for a wide range of practical application fields especially practical use such as point-of-care testing.

ACKNOWLEDGEMENTS
The authors would like to acknowledge Kansai Paint Co., Ltd. for kindly providing us with photo-crosslinkable resins and a photoinitiator, and also acknowledge Tomohiko Ebata and Hidekatsu Tazawa of Institute of Microchemical Technology, Co., Ltd. For helpful discussion. This work was supported by NEDO (07A07009a).

REFERENCES

Figure 3. Calibration curves of (a) AFP, (b) CRP, and (c) PSA. Total assay time is 12 min.