GLASS FIBER SHEET ON A CHIP: FOR RAPID, LOW-COST, AND CONTAMINATION-FREE QUANTITATIVE IMMUNOASSAY

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ABSTRACT

This paper describes a quantitative immunoassay chip harnessing EOF (electroosmotic flow) in glass fiber sheet. The chip consists of a glass fiber sheet-based channel covered with PMMA (polymethylmethacrylate) frames and antibody-immobilized microbeads at the observation spot (Fig. 1). Applying EOF, another labeled-antibody and rinsing buffer sequentially migrate through the antigen-antibody complex on the beads and stain the beads depending on the sample (antigen) concentration. We demonstrated a quantitative assay of insulin and succeeded in determining the concentration in 20 min. The developed chip would provide a rapid, low cost and contamination-free assay system.

KEYWORDS

Glass fiber sheet, Immunoassay, Electroosmotic flow

INTRODUCTION

Immunoassay has been utilized for food inspection, clinical diagnostics and detection of biological/chemical hazards. For quantitative immunoassay, ELISA (Enzyme-linked immunosorbent assay) is one of the most popular methods owing to its high sensitivity, but it is time-consuming and the commercial system/kits are expensive. Quantitative heterogeneous immunoassay requires controlled B/F (bound/free) separation which usually needs several manual procedures (e.g. adding sample and/or ligand, counting time, and washing).

Various microfluidic platforms have been developed for the immunoassays since the miniaturization of the assay system enables to reduce the sample consumption, accelerate the detection rate, and enhance the sensitivity. There have been numbers of reports that proved the applicability of the microfluidic devices to the broad analytes such as proteins, chemical substances, etc [1-5]. Recently, in the field of clinical treatment, quantitative POC (point-of-care) immunoassay testing is strongly desired. They are essential in emergency department, moreover doctors' office, schools, workplaces and homes which have no central laboratory. Upon these requests, disposable and power-free microfluidic devices have been studied for POC testing [6-10]. These devices, however, demand multiple adding and washing procedures to the operators.

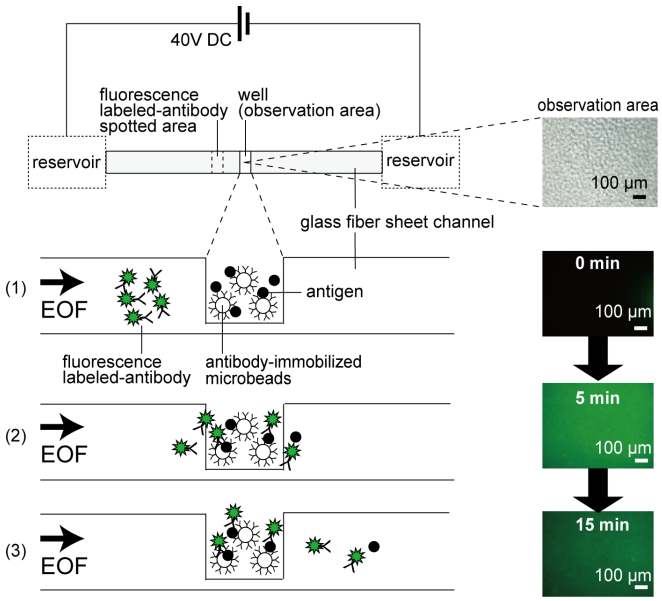

Fig. 1 Conceptual diagram of B/F (bound/free) separation and quantification of the antigen in the glass fiber sheet chip by EOF (1-3) and fluorescent microscopic images of the well (sample: 50 ng/mL insulin, see also Fig. 3). The increased intensity (at 5 min) reduced after the B/F separation (at 15 min).

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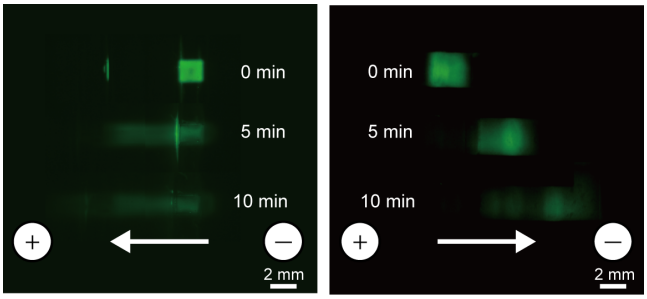

Fig. 2 Series of microscopic images of a fluorescent-labeled antibody migration. Left: In agarose gel, electrophoresis phenomenon is dominant. Right: In the glass fiber sheet, EOF became dominant and the antibody was flowed toward the cathode.

Here we present an alternative quantitative immunoassay chip to solve the abovementioned problems. We chose glass fiber sheet as the key material that allows the generation of EOF. Due to the combination with EOF, the glass fiber sheet-based chip enables to save the multiple applying/washing manipulations and to avoid the contamination by enclosing the sample-liquid within the chip. The developed chip would provide a rapid, low cost and contamination-free assay system.

EXPERIMENT

Fabrication of glass fiber sheet chip

We made immunoassay chip with glass fiber sheet and PMMA. A piece of glass fiber sheet (thickness 370 μm) was cut to the channel shape (width 2 mm) and the PMMA frames were micromachined for the cover of the fiber sheet. At the middle of the channel, the observation area (well, $2\times1\times0.5$ mm^3) was designed for setting the antibody-immobilized microbeads. The other spot area was for the injection of the fluorescence-labeled antibody. All three parts, PMMA channel frame, glass fiber sheet, and bottom PMMA frame, were thermally compressed and bonded at 140 °C for 5 min. Both ends of the fiber sheet were connected to the reservoirs, and a DC voltage for the EOF was applied via the reservoirs (Fig. 1).

Quantitative immunoassay of insulin

The insulin sample (2.5 μL) was mixed with the standard amount of microbeads, which immobilized anti-insulin antibody, for 5 min, and the mixed beads were set to the well (observation area). Then the reservoirs and channels are filled with TBE (Tris-borate-EDTA) buffer, and finally 1 μL of fluorescence-labeled anti-insulin antibody was spotted. After 40 V DC was applied for fifteen minutes, the fluorescence intensity of the beads was detected by fluorescence microscopy, which would correspond to the insulin intensity. The assay took 20 min in total.

RESULTS AND DISCUSSION

Migration direction of target molecules with EOF

Firstly, the migration direction of a fluorescence-labeled antibody on the chip was confirmed under a DC voltage. The fluorescence-labeled antibody used in this experiment has a weak negative net-charge at a neutral pH range. The migration on the developed chip was compared with that on an agarose gel system.

In the glass fiber sheet, the fluorescence-labeled antibody was migrated against the net charge (from anode to cathode, see Fig. 2). This result suggests that the EOF is predominantly strong in glass fiber sheet and the target molecules will be constantly carried towards the cathode, regardless of their net charge.

In contrast, the fluorescence-labeled antibody was migrated from cathode to anode in agarose gel since the applied voltage dominantly promotes the negative net charge of the antibody towards the anode. This means that the migration direction/rate of the target molecules depends on the charge of the molecules.

B/F separation with EOF

Glass fiber sheet is bonded-fiber fabric. Inside the glass fiber sheet, there are glass fiber networks and a series of porous structures, on which surface a considerable amount of ionizable silanol groups present. By the application of an electric field, electrical double layers are generated on the fiber surfaces and induce EOF. Since the generated EOF dominates the direction of the antibody migration, the developed chip allows the sequential step of the B/F separation, that is, the labeled-antibody and the rinsing buffer sequentially migrate through the antigen-antibody complex on the beads and stain the beads depending on the antigen (insulin) concentration. As shown in the fluorescence images in Fig. 1, we observed the increase in the fluorescence intensity with the arrival of the labeled antibody at the well, and then the decrease in intensity due to the appropriate washing step by the rinsing buffer (B/F separation).

Calibration curve of insulin

Finally, we conducted the quantification of insulin concentration as a model sample with the developed chip to demonstrate B/F separation by the EOF generated in the glass fiber sheet. As shown in Fig. 3, we successfully obtained the calibration curve of the insulin concentration, which is applicable to determine the unknown sample down to 12.5 ng/mL.

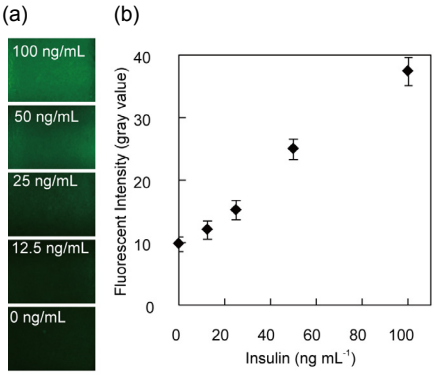

Fig. 3 Determination of the insulin (antigen) concentration by the developed chip. (a) Fluorescent microscopic images of the observation spot after B/F separation. The fluorescent intensity increased with the insulin concentration. (b) Calibration curve of the insulin concentration. The error bars reflect the standard deviation (n = 3). The concentration will be determined as low as 12.5 ng/mL.

CONCLUSION

In this study, we aimed at a POC device which needs no washing manipulations in the middle of heterogeneous immunoassay. The EOF generated in the glass fiber sheet enabled to flow the labeled antibody and the rinsing buffer sequentially, allowing a sufficient B/F separation. Also the system design confined the fluid within the chip, showing the potential for contamination-free assay. These characteristics of the chip, i. e. quantitative, rapid, low-cost and contamination-free, will give a great chance to assay various hazardous substance/viruses in food security and clinical diagnostics.

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