INTEGRATION OF IMMUNOASSAY INTO EXTENDED NANOSPACE FOR ANALYSIS AT SINGLE-MOLECULE LEVEL

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ABSTRACT

We report a novel ELISA (Enzyme-Linked ImmunoSorbent Assay) system utilizing extended nanospace (3µm wide and 300 nm deep) for analysis at single-molecule level. Nanofabrication, nanofluidic system and detector (thermal lens microscope, TLM) were realized. Repeatable immunoassay format was developed by chemisorption of capture antibody at glass wall. The performance was evaluated, and LOD of 20 molecules (60 pL sample volume) were verified. This system will become powerful tool for single cell analysis due to the quite small volume.

KEYWORDS: Extended-Nano, TLM, ELISA, Single Cell

INTRODUCTION

Recently, a new method for understanding gene or protein expression at single cell is increasingly desired in the fields of proteomics, metabolomics research and diagnostics. One of the challenges is loss-less widely applicable molecular recognition method with single-molecule sensitivity in a large amount of co-existing molecules. One of the most selective molecular recognition methods is immunoassay. Especially, ELISA is frequently utilized due to the high sensitivity coming from amplification reaction of dye molecules by the enzyme. Previously, we reported integration of the ELISA system on microchip and verified the fast and sensitive analytical performance combining our thermal lens microscope (TLM) of ultra sensitive detector for non-fluorescent molecules [1]. In microTAS2007, we reported integration of ELISA system into extended nanospace (nanochannel) and investigated the basic principle. However, repeatable measurement in the nanochannel was difficult. In this presentation, we developed a format for repeatable immunoassay in extended nanospace and investigated the performance. Approximately 20 molecules (60 pL sample volume) were detected.

EXPERIMENTAL

Micro- and nano-channels design is fabricated on glass substrate as shown in Figure 1. The sample and reagents was introduced to microchannel by pressure control (100 kPa) and introduced nanochannel by increasing the pressure (400kPa). In the nanochannel, ELISA was realized. For repetitive immunoassay, capture antibody was immobilized by chemisorption and glycine-HCl (PH=1.5) was introduced.
to remove the antigen and immunoassay (changing the antigen concentration) was conducted again [2]. This method is shown in Figure 2. For detection, substrates were introduced and produced dye molecules by enzymatic reaction was detected by TLM. For controlling the sample volume introduced, the flow velocity in the nano-channel was measured by introducing fluorescent solutions and measuring the intensity in two points (300µm distance) in the nanochannel.

**Fig. 1. Chip design and experiment system**

1. Immunoreaction
2. Color reaction
3. TLM detection
4. Detachment of antigen

**RESULTS AND DISCUSSION**

We performed ELISA in the nanochannel repetitively. We detected blank, 10 µg/ml and 100 µg/ml sample in the same channel. The sample volume was 58 pL each time. The signal rose 2 minutes after introducing of substrate and after 5 min-

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222
utes we could measure stable signal. The result of stable signals are illustrated in the
Figure 3. The calibration curve is demonstrated in the Figure 4 and this was the first
time that the concentration dependence of ELISA in the extended-nano space was
indicated. Also from this result and injection volume, we discussed the single-
molecule detection and concluded that the detection limit was 20 molecules from
S/N ratio.

![Fig. 3. Results of stable signal in the nanochannel](image)

![Fig. 4. Calibration curve of ELISA in the nanochannel](image)

CONCLUSIONS

We developed quantitative and high reproducible ELISA. We will realize
the single-molecule detection by reducing nonspecific adsorption and adjusting condition. Then, we will combine this method with single-cell analysis.

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REFERENCES