Preparation of Immunosensors Using a Microfluidic Device with an Interdigitated Array Electrode Modified with Antibodies

Yu HIRANO,a,* Tomoyuki YASUKAWA,b Yoshihiro SAWAYASHIKI,c Hitoshi SHIKU,c Fumio MizUTANI,b and Tomokazu MATSUEc,*

aResearch Institute of Genome-based Biofactory, National Institute of Advanced Industrial, Science and Technology (AIST) (2-17-2 Tsukisamu-Higashi, Toyohira, Sapporo 062-8517, Japan)
bGraduate School of Material Science, University of Hyogo (3-2-1 Kouto, Kamigori, Ako, Hyogo 678-1297, Japan)
cGraduate School of Environmental Migrations, Tohoku University (6-6-11 Aramaki Aoba, Sendai 980-8579, Japan)

Received August 28, 2009 ; Accepted December 18, 2009

We prepared a microfluidic device with an interdigitated array electrode modified with antibodies to develop immunosensors. A comb-type array (W1) was used for forming immunocomplexes and another (W2) for detecting mediators generated by the enzyme reaction. We used electropolymerization and avidin-biotin complexes for an addressable immobilization of antibodies on W1. Since the microfluidics significantly accelerated the formation of the immunocomplexes, a period as short as 10 min was sufficient to detect the responses. The current observed at bare array (W2) increased with increasing analyte (mouse IgG) concentration in the range of 1.0–100 ng/mL. Therefore, present procedure is suitable for rapid immunosensing in a simple device.

Key Words : Microfluidic Device, Immuno-sensing, Addressable Immobilization

1 Introduction

Enzyme-linked immunosorbant assays (ELISAs) are widely used as one of the most important analytical methods to measure biomaterials in biological and clinical fields. Electrochemical detection systems have received considerable attention for improving the sensing performance.1-3 In electrochemical immunosensing, the amount of a labeled enzyme captured on an electrode via the formation of immunocomplexes is estimated by detecting the mediators generated by the enzyme reaction.4-7 The electrochemical measurement system incorporated into a microfluidic channel allows rapid, simple, and sensitive immunosensing.8,12 Increasing the ratio of surface area to volume results in a rapid reaction between the antibody immobilized on the solid surface and the analyte in the solution. In addition, the fluidic stream flushes unreacted immunomolecules from the signal detection area. Therefore, the combination of electrochemical detection with a microfluidic system is a powerful analytical tool for heterogeneous immunosays.

In the present study, we used a microfluidic device with an interdigitated array (IDA) electrode, consisting of two comb-type arrays facing each other (W1 and W2), to develop immunosensors. W1 was used for the formation of immunocomplexes and W2 for the detection of mediators (ferrocenemethanol, FeOH) generated by the enzyme reaction of horse radish peroxidase (HRP), which is commonly applied as a labeled enzyme for immunosensing. We used electropolymerization of the biotinylated poly-succinimidyl 3-(pyrrol-1-yl) propionate (poly-SPP) film to immobilize antibodies, allowing an addressable immobilization of the protein by combining cross-linking chemistry.10 The addressable immobilization of the antibodies on the poly-SPP film was estimated by scanning electrochemical microscopy (SECM), which is widely used for imaging the catalytic activity of biomolecules over a localized area.17,19 The amount of labeled enzyme captured on the polymer on W1 via an immunoreaction was determined at W2 by reducing the electroactive products. The current response observed at W2 increased with increasing analyte concentration in the range of 1.0–100 ng/mL. The present procedure is therefore suitable for rapid and sensitive immunosensing in a simple device.

2 Experimental

We fabricated an Au IDA electrode on a glass substrate by conventional photolithographic methods. The IDA electrode was composed of two pairs of a microband array with a set of 30 elements. The width of each element and the gap between the elements of arrays were 5 μm, respectively. Poly-SPP films were obtained by oxidative electropolymerization of 10 mM SPP monomer (Daichi Pure Chemicals, Tokyo, Japan) with a potential cycling method (potential range −0.3– +1.2 V vs. Ag/AgCl, scan rate 20 mV/s) in an acetonitrile solution containing 0.1 M tetra-n-butylammonium perchlorate (TBAP, Nakalai Tesque, Kyoto, Japan). The thickness of the film fabricated with 6 cycles was 220 nm. Fluidic channels were fabricated as reported previously.20 Briefly, a layer of positive photoresist was patterned on the IDA. We then used a silicone sealant to cover the device surface. After curing, the resist inside the channels was eluted by immersing the device in acetone for approximately 1 h. The width and height of the channel were 100 and 20 μm, respectively. We prepared
the auxiliary and reference electrodes at about 3 and 4 mm downstream, respectively, from the IDA.

We successively filled the channel with a 10 mM 5-biotinamidine-pentylamine (Pierce, IL)/DMSO solution for 2 h and 1.25 μg/mL avidin conjugated anti-mouse IgG antibody solution for 30 min. After washing with phosphate buffered saline (PBS, pH 7.4) containing 0.05% Tween20 (PBST), the substrates were immersed in blocking reagent (Block Ace, Dainippon Pharmaceutical, Osaka, Japan) for 2 h. Then, mouse IgG (Bethyl Laboratories, TX) and 2.4 μg/mL HRP-labeled anti-mouse IgG were successively injected into the channel for 10 min to create sandwich structures on the substrate. A syringe pump was used for precise control of flow rate for the solutions.

For electrochemical measurements, we injected PBS containing 0.5 mM FeOH and 0.5 mM hydrogen peroxide into the channel. The potential of W2 was set at 0.05 V to detect the oxidized form of FeOH (Fe⁺OH) generated by the enzyme reaction of HRP captured on W1.

We also characterized the labeled HRP captured on the poly-SPP film with SECM. We employed a 5.7 μm diameter carbon microdisk electrode as the SECM probe. The z-position of the microelectrode tip was set 4 μm above the substrate. The electrode tip was scanned over the sandwich structures on the film at a scan rate of 9.8 μm/s and a voltage of 0.05 V.

### 3 Results and Discussion

We characterized HRP-labeled antibodies captured on the poly-SPP film with SECM. Figure 1 shows the optical microscopic image of the modified IDA and the SECM image based on the reduction current of Fe⁺OH. The electrodes were treated with (Fig. 1b) and without (Fig. 1c) 10 μg/mL mouse IgG. The white bands in the image indicate a large reduction current and coincide with W1 modified with poly-SPP films (Fig. 1a and 1b). However, current responses obtained from the unmodified bands and glass areas were significantly lower than those from the modified bands, suggesting that the addressable immobilization of proteins was achieved and that SECM could detect the HRP immobilized in the localized area. We did not observe any response in the SECM image obtained from the IDA without the mouse IgG treatment (Fig. 1c), indicating that HRP-labeled antibodies were not captured on the films.

Fe⁺OH generated by the electrochemical oxidation of FeOH at W1 was reduced by W2 to optimize the flow rate of the 0.5 mM FeOH injection. The oxidation current of FeOH and the reduction current of Fe⁺OH increased with increasing flow rate and saturated at more than 5 μL/min. The collection efficiency, defined by the ratio between the reduction and oxidation currents, decreased with increasing flow rate and plateaued at about 60% at flow rate greater than 1.0 μL/min. Therefore, we selected 5 μL/min as the flow rate to characterize HRP immobilized on the poly-SPP film in the microfluidic devices.

The HRP conjugated with avidin was immobilized on the biotinylated poly-SPP film on W1. We injected PBS solution containing 0.5 mM FeOH and various concentrations of hydrogen peroxide into the channel at 5 μL/min. Figure 2 shows amperograms for Fe⁺OH generated from the HRP immobilized on the poly-SPP film. Reduction current at W2 immediately increased and reached a steady state 10 s after the injection of the mixture of 0.5 mM FeOH and hydrogen peroxide. After injecting PBS, the responses returned to the original level, suggesting that Fe⁺OH was constantly supplied by the enzyme reaction on W1 and transferred to W2 by the constant flow. The steady-state current also increased with increasing concentration of hydrogen peroxide and saturated just over 0.5 mM. Therefore, we used 0.5 mM hydrogen peroxide for immnosensing using the microfluidic systems.

To demonstrate immnosensing, we introduced PBS solutions containing various concentrations of mouse IgG into the channel. Figure 3 shows a typical calibration curve for a quantitative sandwich-type assay using mouse IgG as a model, and illustrates that reduction current increases with increasing concentration of mouse IgG in the sample solution. The maximum sensitivity

![Fig. 1](image1.png)  (a) Au IDA electrode modified with poly-SPP films. Typical SECM images of sandwich structure constructed on the poly-SPP films for (b) 10 mg/mL and (c) 0 mg/mL mouse IgG in citric buffer solution (pH 4.5) containing 0.5 mM FeOH and 0.5 mM hydrogen peroxide.

![Fig. 2](image2.png)  Reduction current responses of Fe⁺OH generated by the enzyme reaction of the HRP label immobilized on the poly-SPP film after injecting 0.5 mM FeOH and various concentrations of hydrogen peroxide. Concentrations of hydrogen peroxide injected: (a) 0, (b) 0.01, (c) 0.02, (d) 0.05, (e) 0.10, (f) 0.20, (g) 0.50, and (h) 1.0 mM.
was in the range of 1.0 – 100 ng/mL. The relative standard deviation for three measurements of 10 ng/mL mouse IgG was 4.8%. In the present conditions, 10 min flows of antigen and labeled antibodies were sufficient to produce significant signal differences. The microfluidic system is faster and simpler than conventional ELISA using microtiter plates; the assay format reported here required less than 40 min (from the trapping of analyte), compared with 2 h or more for a conventional microplate assay. Only 50 μL of sample and labeled antibody solution were required. Moreover, the present system possesses a tremendous advantage of simple and easy sensing because the unreacted immunomolecules automatically flowed out of the IDA via the fluidic stream. Although FeOH/Fe^3+OH responses obtained from the electrode modified with poly-SPP were less than 10% as compared to those obtained from an unmodified electrode in the fluidic condition, an unmodified electrode could be used to detect Fe^3+OH generated by the enzyme reaction of HRP immobilized on the poly-SPP film because of the low permeability of FeOH/Fe^3+OH through the poly-SPP film. Immuno-reactions between the HRP labeled antibody and the antibody (or antigen) on the poly-SPP film could be easily incorporated into this system to develop rapid immunosensing.

4 Conclusion

This paper proposes a new immunosensing method based on an IDA electrode incorporated into microfluidic channels. One member of the set of the IDA was used for the immobilization of HRP and the other for the detection of the redox species generated by the enzyme reaction of HRP. We applied electropolymerization for the addressable immobilization of the antibodies on one member of the set of the IDA. The immunocomplexes created on the poly-SPP films on the bands were confirmed by SECM measurements. By using the microfluidic systems, we obtained a good correlation between current intensity and mouse IgG concentration in the range of 1.0 – 100 ng/mL. The model system described above demonstrated that the microfluidic device allows a full immunoassay to be completed in 40 min. Furthermore, the fluidic system allowed automatic separation of unreacted molecules from the IDA area.

Acknowledgment

This work was supported by a Grant-in-Aid for the Creation of Innovations through Business-Academic-Public Sector Cooperation (No. 13504). It was partly supported by a Grant-in-Aid for Scientific Research (No. 15201030) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References