Optimization of Coulometric Microdevice for Protein Detection Based on Metallization Principle

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Abstract. Optimization strategies for the protein detection on coulometric microdevice based on metallization principle was presented. The coulometric microdevice detection principle comes from the concept of metallization where the information of analyte in a one reaction chamber was measured by the amount of deposited metal in another chamber. Protein was detected by using the immunoassay (ELISA) method. Several strategies to improve the coulometric microdevice from the previous work were explained. The strategies focus on the reduction of the background/noise by modifying the structure of the device and optimizing the blocking condition to minimize non-specific absorption on the electrode where the ELISA procedure was conducted. These strategies successfully give an improvement on the device sensitivity.

1. Introduction
Optimization of the coulometric-based microdevice for protein detection was presented. In the previous work [1], there were several problems which need to be addressed, particularly the background charge for the detection of alpha-fetoprotein (AFP) was much larger than that for the detection of H₂O₂. A major cause of this was probably the non-specific binding of proteins to the PDMS walls during the incubation process of ELISA. In this study, to reduce the background and improve the sensitivity of the microdevice, the device structure and another experimental parameter for protein detection were optimized. Figure 1 shows the previous device that will be modified and optimized in this study.

There were three approaches presented in this work. First step is optimizing the blocking process using BSA. Next, the structure of the working electrode was modified to minimize the background. Last step is modification of the flow channel for the measurement of the plug due to the possibility of protein losses during the measurement of the plug in the rhombus structure in an auxiliary flow channel. Throughout these steps, the increase of the signal-to-noise ratio are expected to increase.
2. Experimental Section

2.1. Device Fabrication

Fabrication method of the device follows similar method as explained in the previous work [1]. The device was fabricated on a glass substrate with thin-film electrodes formed by the sputter deposition. A PDMS substrate with a flow channel structure was stacked onto it. There are two flow channels (A and B) where oxidation and reduction occur separately. Rectangular platinum and gold electrodes formed in flow channel A were used for oxidizing H$_2$O$_2$ and immobilizing capture antibodies, respectively.

In flow channel B, a platinum working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary electrode were formed. The working electrode in flow channel B was connected with the rectangular platinum electrode in flow channel A. A positive photoresist insulating layer with a pinholes structure was formed on the working and reference electrode areas. The flow channels were connected with a liquid junction formed by pouring agarose (2%) dissolved in a 1.0 M KCl solution at 80°C into the compartment and solidifying it as it cooled down to room temperature. To form the on-chip Ag/AgCl electrode, the silver electrode was immersed in a 0.1 M KCl solution along with a commercial Ag/AgCl
reference electrode and a platinum auxiliary electrode. A constant current of 50 nA was applied to the silver electrode for 15 min with magnetic stirring to grow the AgCl layer.

2.2. General ELISA procedure on this study
Procedure for protein detection through ELISA is shown in Figure 2. Briefly, Complex sandwich structures of capture antibodies, proteins, and detection antibodies were formed on the gold electrode. Glucose solution was introduced to produce the \( \text{H}_2\text{O}_2 \) when reacted with the glucose oxidase enzyme labelled on the detection antibodies. The plug was then delivered from gold patch to the Pt electrode in flow channel A followed with introducing Ag\(^+\) ion solution in flow channel B. Ag was deposited on the microelectrode array in flow channel B. The amount of silver deposited on the platinum electrode was measured by coulometry by introducing 0.1 M KCl solution and applied potential of +0.7 V for 60 s.

![Figure 3. Device used for optimizing blocking with BSA in ELISA.](image)

![Figure 4. Plug volume of glucose substrate was reduced from 3 \( \mu \)L (left) to 1.5 \( \mu \)L (right)](image)

2.3. Optimization of the blocking process for ELISA
Blocking process using BSA was optimized by fluorescence measurement. An Olympus IX73 fluorescence microscope was used for this experiment. A thin PDMS substrate with a through-hole at the center was stacked on a flat PDMS substrate (Figure 3). A droplet of solution was injected into the through-hole. Finally, the compartment was covered with a cover glass and fluorescence measurement was conducted. For the background measurement, a droplet of PBS solution was directly placed on the compartment and fluorescence measurement was conducted. To estimate non-specifically adsorbed proteins on the wall of PDMS flow channels, 5-Aminofluorescein solution was incubated for 30 min.

The solution then flushed out and the compartment was rinsed. PBS was then injected onto the compartment, and the fluorescence measurement was conducted. To optimize the incubation time for BSA, a BSA solution (5.0 mg/mL) was incubated in the compartment for a certain length of time. After rinsing the compartment, a 5-aminofluorescein solution of 10 \( \mu \)M was incubated for 30 min, followed by rinsing the compartment. Finally, PBS was injected into the compartment and the fluorescence measurement was conducted.

2.4. Optimization of the plug volume
In the detection of proteins using the device in previous work [1], the plug of the glucose solution (3 \( \mu \)L) was larger than the minimum volume of the plug to cover the entire area of the gold electrode (1.5 \( \mu \)L).
μL). The enzymatic reaction product in the plug can be accumulated more effectively by reducing the plug volume. In this study, the volume of the glucose plug was reduced to 1.5 μL (Figure 4).

Figure 5. Left figure shows device structure modification for plug measurement (1st modification). Rhombus structure for measuring the plug was removed. Right figure shows modification of the working electrode in flow channel A from rectangular shape into comb-like shape (2nd modification).

Figure 6. Procedure of plug processing on the new design of flow channel A. (A) Introduction of a solution into flow channel A. (B) Splitting of the solution using the air vent. (C) Incubation of the plug on the gold electrode.

2.5. Optimization of the device structure for the plug volume measurement
Structure of the device from Figure 1 for processing of the solution plug was modified to minimize the loss of analyte proteins during the measurement of the plug volume. In the modified structure, the auxiliary flow channel with the rhombus structure was removed. Figure 5 shows the modified structure of flow channel A to measure the volume of the plug (left figure; 1st modification). The plug volume was measured between two air vents. Figure 6 shows the procedure for processing the plug. First, the solution was introduced into flow channel A from the right inlet. A hydrophobic negative resist pattern help stop the solution when it reached the left air vent. The right air vent was used to cut the solution by applying air pressure. The separated plug was flushed out of flow channel A. A 1.5 μL of a solution plug was measured between these two air vents. Working electrode structure in flow channel A was modified from the rectangle to comb-like shape (Figure 5; right figure; 2nd modification). The width of each electrode strip was 30 μm. Total number of electrode strips was 40 and the electrode span the same distance (edge-to-edge) as the previous rectangular electrode.
3. Results and discussion

3.1. Optimization of the BSA blocking
Optimization of the BSA was conducted through a simple fluorescence measurement. In this strategy, the amount of the adsorbed fluorescein particles on the PDMS wall reflecting the amount of the adsorbed GOD-antibody complexes which causing the non-negligible background. From Figure 7, incubation time of 60 min of BSA was enough to obtain a small fluorescence intensity, in comparison to the background condition. Our previous selection of 30 min BSA incubation in previous work was not enough for the blocking process [1]. From this result, BSA incubation for 60 min was selected as a new parameter for the ELISA experiment in this study. A good blocking condition during the ELISA procedure can minimize the interference that comes from non-specific bonding/deposition of the biomolecules to the surface of gold.

3.2. Optimization of the plug volume
ELISA procedure from Section 2.2 with BSA blocking of 60 min was selected. AFP concentration of 10 ng/mL and the background measurement were tested to see the performance of the device. Figure 7 shows the result. By adjusting the plug volume to the size of the gold electrode, H2O2 produced from the ELISA experiment was accumulated more effectively. As a result, the output charge from silver deposition was increased. The ratio of output charge and the background was improved from 3.8 to 4.9.

3.3. Optimization of the plug measurement method
ELISA procedure described in Section 2.2 was conducted to check the effect. Blocking by BSA was conducted for 60 min and AFP concentration of 10 ng/mL was selected to see the performance of the device. Figure 8 shows the result. By removing the rhombus structure, the loss of the analyte could be reduced. The increase of the response charge indicates that more deposited silver was obtained. The ratio of output charge and the background was improved from 4.9 to 8.6.

![Figure 7](image1.png)  
*Figure 7. Change in fluorescence intensity on different blocking condition.*

![Figure 8](image2.png)  
*Figure 8. Change in coulometric responses after optimization of the plug volume. Data A was the output charge collected using glucose plug of 3.0 µL volume. Data B was the output charge collected using glucose plug of 1.5 µL. Background charges in both A and B conditions were shown.*
Figure 9. Change in coulometric response after structure modification of the device for plug measurement (data C). Data B was the previous result obtained when the plug volume was optimized. Data B was shown to compare the change of device performance.

Figure 10. Change in coulometric response after modification of the working electrode structure in flow channel A (data D). Data C was the previous result obtained when the structure for measuring the plug was optimized. Data C was shown to compare the change of device performance.

3.4. Optimization of the working electrode in flow channel A
Reduction of the area of the working electrode will reduce the background as similarly shown in the other work [3]. ELISA procedure explained in Section 2.2 was conducted to check the effect. BSA blocking was conducted for 60 min and AFP concentration of 10 ng/mL was selected. Figure 9 shows the reduction of the background when the shape of the electrode was change from rectangular shape (1st modification) to an array of strips (2nd modification). The background was reduced as the area of the working electrode area (or the sensing area) for the decomposition of H$_2$O$_2$ solution was reduced. This H$_2$O$_2$ came from the non-specific binding of the protein. However, the charge for the detection of AFP was also reduced in this case. The ratio of output charge and the background was improved from 8.6 to 18.2.

Considering the improvement strategies used in this work, the calculated detection limit could be increased 18.2 times from 1.4 ng/mL in previous work [1]. Detection limit of 1.4 ng/mL was selected because the improvement sensitivity by repeating the incubation of glucose plug every 30 min was not conducted here. Using this strategy, the detection limit of current work become 0.76 ng/mL. Most of the current published works on microfabricated chip for protein detection are based on amperometry and the publication using coulometry was still limited. Continuation of this work on coulometry may help further exploration of the technique in future.

| Method          | Analyte                  | Detection limit | Reference |
|-----------------|--------------------------|-----------------|-----------|
| Cyclic voltammetry | mouse IgG                | 10 ng/mL        | 4         |
| Amperometry     | α-fetoprotein            | 2 ng/mL         | 5         |
| Amperometry     | carcinoembryonic antigen | 0.25 ng/mL      | 6         |
|                 | α-fetoprotein            | 0.13 ng/mL      |           |
| Coulometry      | α-fetoprotein            | 1.4 ng/mL       | Previous work [1] |
| Coulometry      | α-fetoprotein            | 0.76 ng/mL      | This work |

Table 1. Comparison with other works on microfabricated protein chips.
4. Conclusion
Improvement strategies of the coulometric-based microdevice for protein detection were presented. Strategies for reducing the background signal and increasing device sensitivity through structure modification were conducted. Detection limit of the current work was 0.76 ng/mL for AFP detection. This strategy could be useful for improving the microdevice biosensor in general.

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