LOC(랩온어칩) 응용을 위한 마이크로패턴에 형성된 전기화학식 면역 바이오센서의 신호검출 방법

윤현철, 양해식, 고종수, 김윤태
한국전자통신연구원 바이오소자팀

A Signaling method from micropatterned immunoelectrodes for LOC(Lab-on-a-chip)

Hyun C. Yoon, Haesik Yang, Jong Soo Ko, Youn Tae Kim
BioMEMS Team, Electronics and Telecommunications Research Institute (ETRI),
161 Gajeong-dong, Yuseong-gu, Daejeon 305-350, Korea. hcyoon@etri.re.kr

Introduction

With the advent of LOC(Lab-on-a-chip) and μ-TAS(total analysis system) technology, there has been an increasing demand for efficient methodologies for DNA, protein, and metabolites analyses (biosensing). This presentation describes a strategy of signal registration from micropatterned immunosensors that converts antigen-antibody binding reaction into electrochemical signals. Of the transduction techniques in use such as electrochemical, optical, piezoelectric, etc., amperometric signaling was chosen for the relatively direct application of the microelectronics, which is advantageous for sensor miniaturization and automation. The cyclic voltammetric method, tracking biocatalyzed precipitation of insoluble products onto the sensing surface and subsequent decrement in the electrode area, was used for signal registration. As a model immunosensing with the micro-fabricated device, we have investigated the functionalization of ferritin (antigen) to the PAMAM dendrimer-assisted sensing surface and its biospecific interaction with anti-ferritin antiserum.

Experimental

The strategies for immunosensor surface construction and signal registration as well as a typical sensor response are shown in Figures 1 and 2. Detailed experimental steps are described in the literatures referenced.[1, 2]

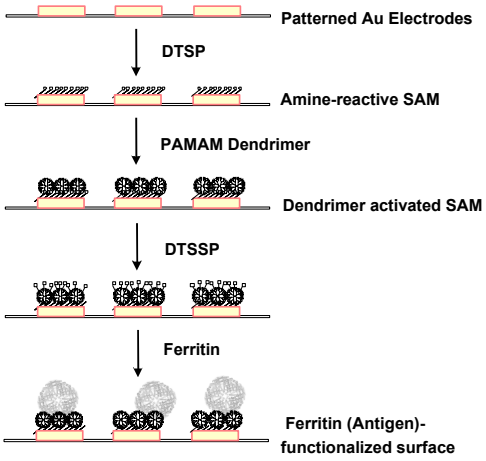

Figure 1. Schematic representation of the immunosensor surface construction

Results & Discussion

As shown in Figure 1, micropatterned gold electrodes were functionalized by ferritin antigen with dendrimer-assisted SAM as the base template. PAMAM(G4) dendrimer-assisted surface was used, because the modified surface exhibits friendly environment for biofunctionalization while maintaining the permeable structure for electrochemical signal registration. For efficient signaling from the immunosensor, antigen-modified surface should exhibit high permeability for the signal tracer (Fc-MeOH in this work). If the surface permeability were significantly hindered from the antigen immobilization, as usually the case with mixed alkanethiol SAM-modified surfaces, the sensor detection range would narrow down significantly. With the PAMAM(G4) dendrimer-assisted surface, however, the surface still exhibit high permeability, supporting its advantage as the base template for affinity surface construction. In addition, the dendrimer-assisted surface led to higher association level of protein than the polyamine (PLL) layer or various SAMs including DADOO-assisted SAM, resulting in above 80% surface coverage by protein molecules. This is likely to originate from the structural feature of the dendrimer monolayer such as the surface exposure of derivatizable functional groups and a corrugated surface.

The experimental protocol adopted in this study was an extended version of our recent publication.[1] However, we made two important progresses in this work. The one is that the immunosensing was conducted with a microfabricated electrode array, confirming the applicability of the developed sensing method to microscale biosensors. And the other is that the developed immunosensor was used for analysis with a real serum sample. The target analyte, ferritin, is regarded as a general biomarker for cancers. And a recent study suggested the relation of ferritin level to hepatocellular carcinoma.[3]

With the ferritin-functionalized microfabricated immunosensor, we performed immunoassays for anti-ferritin antiserum. We have developed a strategy of signal generation for immunosensors that transduces biospecific affinity reactions into electrochemical signals. The cyclic voltammetric method,

tracking the precipitation of insoluble products onto the sensing surface and subsequent decrement in the electrode area, is chosen for signal registration (Figure 2). Precipitation of insolubilities is induced by the catalytic reaction of enzymes, which were labeled to the biospecifically-attached secondary antibody molecules.

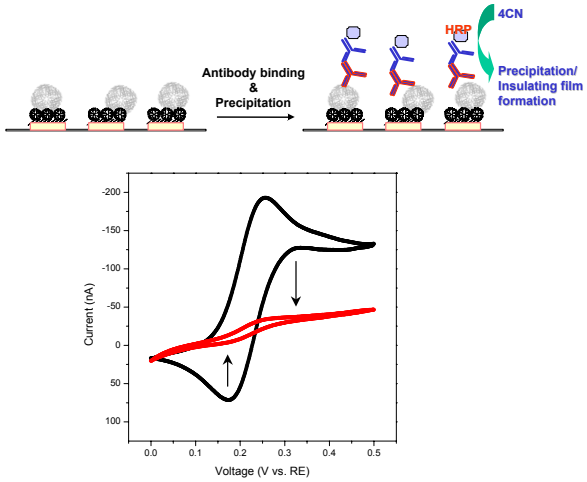

Figure 2. Schematic representation of the proposed immunosensing principle and typical cyclic voltammetric traces for sensor signaling.

Fabrication of the micropatterned chips was performed with conventional semiconductor fabrication techniques. Essentially, base substrates for the affinity sensing monolayers were thin film gold surfaces, prepared by e-beam evaporation of 200nm Au onto Ti(40nm)-primed Si[100] wafers. Two types of electrode geometry were designed, including rectangular (100μm×500μm) and circular (r. 50μm) immunoelectrodes, and fabricated. The fabricated immunosensor chip surface is as shown in Figure 3. Also, the color change at the exposed electrode surface upon signaling reaction was discernible with naked eyes, and the photographs representing the result are shown in Figure 3.

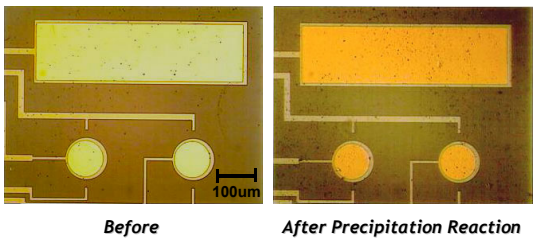

Figure 3. Photographs of an immunosensor surface upon signaling reaction.

For the evaluation of analytical performance of fabricated immunosensor, calibration tests were conducted. As shown in Figure 4, sensor signals were registered at different target protein concentrations with anti-ferritin antiserum for each type of immunoelectrodes. Electrochemical signals

were collected from the background-subtracted cyclic voltammograms. It should be noted that the circular-type (r.50μm) electrodes exhibited characteristics of microelectrodes such as sigmoidal cyclic voltammograms, while the rectangular-type (100μm×500μm) electrodes maintained features of bulk electrodes. (Data not shown) Thus, in case of sufficient signal magnitude, circular-type immuno-micro electrode should be advantageous for sensor operation to bulk-type one, because of the small current change with unexpected potential drift. However, both types of immunosensors exhibited satisfactory calibration curves of similar dynamic detection range for target protein (Figure 4), but of different signal levels in proportion to the electrode surface area.

The presented signaling method could be applicable for microfabricated affinity sensors and LOCs(Lab-on-a-chip).

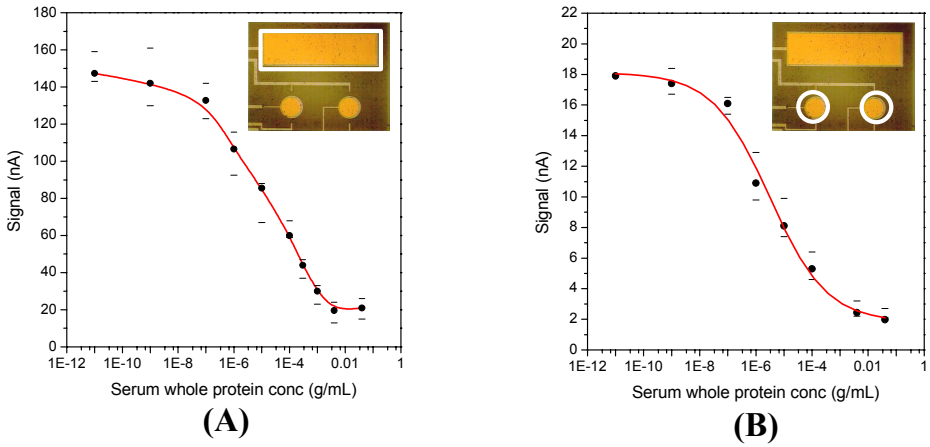

Figure 4. Calibration curves for the anti-ferritin antibody affinity electrodes. (A) Rectangular (100μm×500μm) and (B) circular (r.50μm) type immunoelectrodes.

References

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3. Park KS, Kim H, Kim NG, Cho SY, Choi KH, Seong JK, Paik YK. *Hepatology*, 35 (2002) 1459-1466.