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A capacitive biosensor based on an interdigitated electrode with nanoislands

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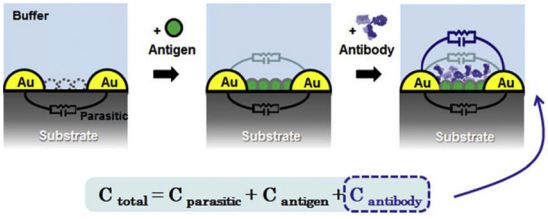
HIGHLIGHTS

- An interdigitated electrode (IDE) with nanoislands was developed for capacitive biosensor.
- The sensitivity of capacitive immunoassay could be enhanced by the IDE with nanoislands.
- A parylene film was coated on the IDE to improve the immobilization efficiency of receptors.
- The parylene film coated electrode was applied to detect hepatitis surface antigen (hHBsAg).

GRAPHICAL ABSTRACT



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ABSTRACT

A capacitive biosensor based on an interdigitated electrode (IDE) with nanoislands was developed for label-free detection of antigen-antibody interactions. To enable sensitive capacitive detection of protein adsorption, the nanoislands were fabricated between finger electrodes of the IDE. The effect of the nanoislands on the sensitive capacitive measurement was estimated using horseradish peroxidase (HRP) as a model protein. Additionally, a parylene-A film was coated on the IDE with nanoislands to improve the efficiency of protein immobilization. By using HRP and hepatitis B virus surface antigen (HBsAg) as model analytes, the effect of the parylene-A film on the capacitive detection of protein adsorption was demonstrated.

1. Introduction

Capacitive biosensors based on interdigitated electrodes (IDE) have been of interest for non-labeled detection of interactions between biomolecules because of their high sensitivity and their relative simplicity [1–8]. Recently, capacitive biosensors based on IDE have been reported for the detection of C-reactive protein (CRP) [9], IL-6 [10], CEA [11], Johne's disease specific antigen [12], Hev b1 [13], and Nampt [14], as summarized in Table 1. The capacitive detection of IDE is based on the following equation of capacitance: $C = \varepsilon_0 \times \varepsilon_r \times A \times d^{-1}$, where ε_0 and ε_r represent vacuum permittivity and the relative permittivity of a dielectric material between electrodes, respectively, and where d and A represent

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Table 1
Comparison of sensing parameters of capacitive biosensors based on IDEs.

Authors	Target analyte	Linear range (ng mL^{-1})	Limit of detection
Kallempudi and Gurbuz 2011 [9]	CRP	1 ~ 250	1 ng mL^{-1}
Qureshi et al., 2010 [10]	IL-6	0.025 ~ 25	25 pg mL^{-1}
Altintas et al., 2014 [11]	CEA, hEGR	0.02 ~ 1	20 pg mL^{-1}
Li et al., 2014 [12]	Anti-bovin IgG	–	1 ng mL^{-1}
Sontimuang et al., 2011 [13]	Hev b1	10 ~ 900	10 ng mL^{-1}
Park et al., 2012 [14]	Nampt	1 ~ 50	1 ng mL^{-1}
(This work)	HBsAg	0.1 ~ 1000	<100 pg mL^{-1}

electrode distance and area, respectively. As the relative permittivity of a protein ($\varepsilon_r = 20$) is significantly different from that of water ($\varepsilon_r = 80$), a change in capacitance can occur due to the adsorption of proteins on the electrodes. The equation above shows that the change in capacitance is enhanced if the distance between the electrodes is shortened. Therefore, IDE have been developed to minimize the distance between the finger electrodes for sensitive capacitive detection. In previous studies [9–14], most IDE have been fabricated with UV-photolithography with a linewidth limit of 1–5 μm [15]. The IDE-type electrodes with a < 1 μm distance between the finger electrodes are known to require costly and time-consuming processes such as E-beam lithography [16], focused ion-beam fabrication [17], and a mass-producible submicron-gap IDE via step photolithography [18]. In addition to the IDE, nanoislands have also been applied to the capacitive detection of proteins by fabrication with field effect transistor (FET) devices and Pt-carbon nanotube electrodes [19–22]. Such nanoislands have been fabricated with various methods such as thermal annealing, intense pulsed light, nanoporous alumina blocks, and e-beam evaporation [19–21,23]. In this work, IDE with nanoislands were fabricated between the finger electrodes of an IDE in order to shorten the distance between the finger electrodes of the IDE, as shown in Fig. 1(a). From the simulation results, analysis of the nanoislands determined that they concentrated the electric fields on IDE far higher than the conventional IDE, which had widely distributed electric fields into the electrolytes. The improvement in sensitivity of the IDE with nanoislands in detecting the changes in capacitance and impedance was demonstrated using model analytes in comparison with the conventional IDE.

The creation of such an IDE with nanoislands aimed to improve the sensitivity in the measurement of impedance and capacitance during immunoassays. For highly sensitive immunoassays, the receptor proteins (antibodies or antigens) should be immobilized on the sensor surface with a high surface density. Recently, parylene films with functional groups such as amines and formyl groups were reportedly used for the covalent immobilization of proteins and peptides for highly sensitive immunoassays. Among such parylene films with functional groups, parylene-A is a polymer of *p*-xylene with primary amine groups, which can be used for the covalent binding of proteins or peptides with linker molecules such as glutaraldehyde or succinimide derivatives [24]. In particular, parylene-A was used for the immobilization of small proteins and peptides and achieved a far higher efficiency than using physical adsorption and self-assembled monolayers [25]. In this work, parylene-A was applied to the IDE with nanoislands to enhance the sensitivity of immunoassays. For application to the IDE with nanoislands, the parylene-A film should be coated as thin as possible (<50 nm) to achieve the sensitive capacitive detection by on-line thickness monitoring [26–29].

Here, the fabrication of the IDE with nanoislands is presented, and the morphological analysis of nanoislands was carried out by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The effect of nanoislands on the sensitive capacitive measurement was characterized by immunoassays with horseradish peroxidase (HRP) as a model protein. Then, a parylene-A film was coated on the IDE with nanoislands to enhance the sensitivity of capacitive detection, and immunoassays with the parylene-A coated electrode were conducted using HRP and hepatitis B virus surface antigen (HBsAg) as model analytes.

2. Materials and methods

2.1. Materials

Horseradish peroxidase (HRP), an anti-HRP antibody, ferricyanide, and other chemicals of analytical grade were purchased from Sigma–Aldrich Korea (Seoul, South Korea). Hepatitis B virus surface antigen (HBsAg) and anti-HBsAg antibodies were purchased from AbCam Co (Cambridge, UK).

2.2. Fabrication of an IDE with nanoislands

The IDE was fabricated on a glass substrate using a UV-photolithographic process, shown in Fig. 1(b). For the fabrication of the IDE, a SU-8 layer of 1 μm thickness was spin-coated on a 4-inch glass wafer. After soft-baking at 110 °C for 2 min, the UV-photolithography process was carried out using a mask for the IDE. The IDE was designed to have 100 pairs of finger electrodes with a width of 5 μm, and the space between electrodes was set to be 5 μm. After developing the IDE pattern, a Ti layer was sputtered as an adhesive layer with a thickness of 5 nm, and then a gold layer was sputtered with a thickness of 100 nm. The IDE pattern was obtained after a lift-off process. Nanoislands were prepared on the IDE by thermal evaporation and heat treatment. First, the gold layer was thermally evaporated with a thickness of 1 nm, and then nanoislands were produced by heat treatment under argon gas (150 sccm) at a temperature of 500 °C for 1 h. Additionally, the surface density of nanoislands was controlled by thermal annealing of the gold layers with different thicknesses of 2, 5, 8 nm which resulted in the surface density of 1.1×10^9, 6.6×10^8 and 7.8×10^7 nanoislands cm^{-2}, respectively.

2.3. Thermal deposition of the parylene-A film

As previously reported, parylene-A (Femto Science Co., South Korea) was thermally deposited by the following polymerization steps: (1) evaporation of parylene dimers at a temperature of 160 °C, (2) pyrolysis for the production of highly reactive *p*-xylene radicals at a temperature of 650 °C, and (3) deposition of the parylene-A film to the substrate at room temperature [26–29]. The whole coating procedure was reproducibly carried out using a microprocessor-controlled parylene coater from Femto Science Co. (South Korea). To control the thickness of the parylene-A film, the QCM response was measured from the beginning of the evaporation step, and the thermal deposition was finished when the QCM frequency shift reached a value corresponding to the targeted thickness of the parylene-A film [29,30].