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Detection of small toxin molecules by Si photovoltaic integrated circuit

Chan Woo Park*, Jong-Heon Yang, Chil Seong Ah, Chang-Geun Ahn, Yo Han Choi, Kwang Hyo Chung, Wan-Joong Kim, Gun Yong Sung

Biosensor Research Team, Electronics and Telecommunications Research Institute (ETRI)
218 Gajeongno, Yuseong-gu, Daejeon 305-700, Republic of Korea

Abstract

We propose a new type of photosensitive biosensor with a CMOS compatible Si photovoltaic integrated circuit, for the high-sensitive detection of small mycotoxin molecules requiring competitive assay approach. In this work, a photodiode is connected to the gate of a field effect transistor (FET) so that the open circuit voltage (V_{OC}) of the illuminated photodiode is transferred into the drain/source current of the FET. The sensing scheme employs competitive binding of toxin molecules (within the sample solution) and toxin-BSA conjugates (immobilized on the photodiode surface) with Au-nanoparticle-labeled antibodies, followed by silver enhancement to generate opaque structures on the photodiode surface. As the amount of toxin increases, less opaque becomes the photodiode surface resulting in a higher V_{OC}. By monitoring the channel current of the FET whose gate is driven by the V_{OC}, quantitative detection of Aflatoxin B1 has been achieved in the range of 0~15 ppb.

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Keywords: Photosensitive biosensor; CMOS compatible; Photodiode; Competitive assay; Mycotoxin

1. Introduction

The photosensitive biosensor employing metallic nanoparticle labels has been considered a promising candidate for overcoming some drawbacks of conventional fluorescence-based methods [1-3]. In previous works of photodiode biosensors [1-3], the coverage of metallic nanoparticles has been measured from the amount of photocurrent, which provided a high enough sensitivity to detect pM to nM range of proteins

* Corresponding author. Tel.: +82-42-860-5249; fax: +82-42-860-6594.
E-mail address: chanwoo@etri.re.kr

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[1] or DNA [2,3] by sandwich assays. However, in the photocurrent-based approach, the overall level of photocurrent becomes too low near the full coverage of metal particles (where most of the incident light is blocked), which causes the degradation of signal resolution [1].

In the present work, we propose a new type of photosensitive biosensor with a CMOS compatible Si photodiode integrated circuit, for the high-sensitive detection of small mycotoxin molecules. In this work, a photodiode is connected to the gate of a field effect transistor (FET) so that the open circuit voltage (V_{OC}) of the illuminated photodiode is transferred into the drain/source current (I_{DS}) of the FET. The sensing scheme employs competitive binding of toxin molecules (within the sample solution) and toxin-BSA conjugates (immobilized on the photodiode surface) with Au-nanoparticle-labeled antibodies, followed by silver enhancement to generate opaque structures on the photodiode surface. As the amount of toxin increases, less opaque becomes the photodiode surface resulting in a higher V_{OC}. By monitoring the channel current of the FET whose gate is driven by the V_{OC}, quantitative detection of Aflatoxin B1 has been achieved in the range of 0~15 ppb.

2. Experimental

2.1. Fabrication of Si photosensitive biosensors

Photosensitive biosensors were fabricated from 8 inch silicon-on-insulator (SOI) wafers with a 100-nm-thick top silicon layer and a 200-nm-thick buried oxide layer. First, the initial top Si layer was thinned down to 40 nm by dry oxidation and wet etching cycles, and then the active area for p-MOSFETs was defined by a local oxidation of silicon (LOCOS) process. For forming photodiodes on the bottom p-type Si substrate, the oxide covering the photodiode region was removed by wet etching in a HF solution. Then, the exposed Si surface was sequentially implanted with 2.0e13 cm^{-2} and 5.0e15 cm^{-2} of phosphorus ions at 300 keV and 80keV, respectively, followed by drive-in anneal at 1000 °C for 3 h. For forming p-MOSFETs on the top Si layer, the active area was implanted with 2.5e13 cm^{-2} of phosphorus ions at 25 keV, and then the gate oxide of 80Å was grown. After patterning the poly-Si gate, the drain/source region was implanted with 2.0e15 cm^{-2} of boron ions at 40 keV, and rapid thermal annealed at 950 °C for 30s. Finally, metal electrodes were formed as a stack of Au/Cr/Al (50 nm/5 nm/50 nm) by electron-beam evaporation and lift-off technique, followed by annealing at 400 °C for 30 min.

2.2. Immobilization of toxin-BSA conjugates on the Si photodiode surface

The Si photodiode surface was activated with hydroxyl (-OH) groups by low power oxygen plasma treatment (50 W, 5 min), and then functionalized with 3-aminopropyltriethoxysilane (APTES) within a home-made Teflon reactor at 120 °C for 10 min. The amine-functionalized Si surface was immersed in a 25 wt% glutaraldehyde aqueous solution with 160 mM sodium cyanoborohydride ($NaBH_3CN$) for 4 h, followed by rinsing with de-ionized water. During the reaction with glutaraldehyde, the amine-modified Si surface was functionalized with aldehyde groups. The immobilization of toxin-BSA conjugates was achieved by exposing the aldehyde-functionalized Si surface to 100 μg/ml of Aflatoxin B1-BSA conjugates in 10 mM phosphate buffer solution (pH 8.4) with 4 mM $NaBH_3CN$ for 12 h. Finally, the unreacted surface aldehyde was blocked with 1 % casein in 10 mM phosphate buffer solution for 30 min.

2.3. Competitive immunoassay and enhancement of metallic nanoparticles

Immunogold conjugates were prepared by the conjugation of gold nanoparticles (AuNPs) (10 nm in diameter) with monoclonal antibodies of Aflatoxin B1. Aflatoxin B1 molecules were dissolved in 70 %

methanol, and then diluted with 1xPBS solution at various concentrations of 0, 5, 10, and 15 ppb. The Aflatoxin B1-BSA conjugates-immobilized photodiode surface was incubated within the Aflatoxin B1 and immunogold conjugate solution for 1 h at 20 °C, followed by rinsing in 1xPBS several times. For further enhancement of captured AuNps, the photodiode surface was immersed in a 1:1 mixture of silver enhancer solutions A and B (Sigma-Aldrich) for 12 min, followed by rinsing in de-ionized water.

3. Results and discussion

Figure 1(a) shows the top image of a sensor circuit fabricated by a CMOS compatible process, where the FET gate length and the photodiode area are 0.5 μm and 300 μm x 300 μm, respectively. With the n-type side of the photodiode being connected to the gate of a p-channel enhancement type FET, when the photodiode is illuminated, a negative gate voltage is applied and the channel current increases. The coverage of silver-enhanced Au nanoparticles blocking the incident light decreases as the toxin concentration increases, as a result of the competitive binding reaction within the solution.

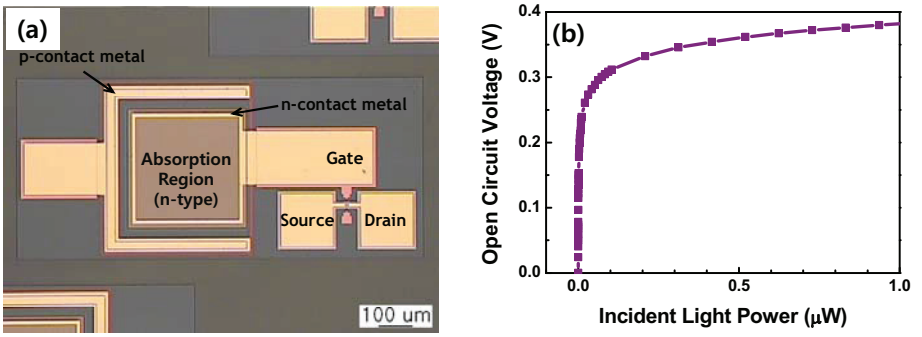

Fig. 1. (a) Optical microscope image of a sensor circuit fabricated on a SOI substrate (FET gate length: 0.5μm, photodiode area: 300μm x 300μm). (b) Dependence of the open circuit voltage of a 300μm x 300μm Si photodiode on the incident light power.

In previous works of photodiode biosensors [1-3], the amount of light blocked by silver-enhanced Au particles is measured from the change of photocurrent. In that case, the sensing resolution becomes low near the full coverage of enhanced particles, as the overall level of photocurrent is too low [1]. However, as shown in Fig. 1(b), the V_{OC} of the Si photodiode increases abruptly in the vicinity of the dark point. Such behavior implies that the measurement of V_{OC} is more suitable for the detection of small molecules requiring a competitive assay, as the sensor can provide a higher resolution in the low concentration range.

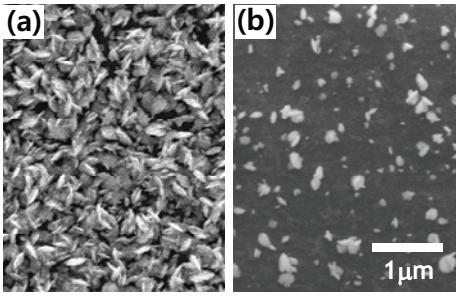

Fig. 2. SEM images of the photodiode surface reacted with (a) 5 ppb and (b) 15ppb of Aflatoxin B1, and then silver-enhanced for 12 minutes.

Figure 2(a) and (b) show scanning electron microscope (SEM) images of the photodiode surface reacted with 5 and 15 ppb of Aflatoxin B1, respectively, and then silver-enhanced for 12 min. The coverage of opaque metal structures is higher at the lower toxin concentration, due to the higher number density of captured Au nanoparticles. Accordingly, the channel current of the photodiode-driven FET also shows a clear dependence on the toxin concentration. As shown in Fig. 3, as the toxin concentration varies from 0 to 15 ppb, the channel current increases almost linearly by about 6 times providing a sufficiently large output change for the interval of 5 ppb. Such results demonstrate that quantitative analysis of the toxin concentration is possible by the sensor circuit.

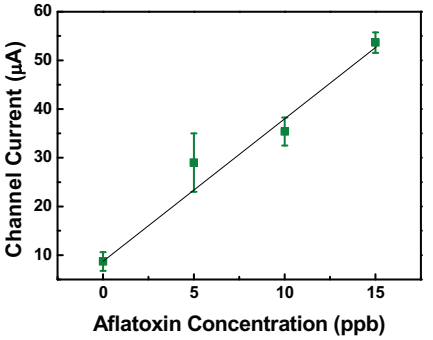

Fig. 3. Dependence of the drain/source current of a photodiode-driven FET on the concentration of Aflatoxin B1 (V_{DS}=500mV).

4. Conclusion

We developed a new type of photosensitive biosensor with a CMOS compatible Si photodiode integrated circuit, which enables the high-sensitive detection of small mycotoxin molecules requiring a competitive assay. By employing AuNPs as labels to generate opaque metal networks on a photodiode surface and monitoring the V_{OC} instead of the photocurrent, we could achieve a high signal resolution at low concentrations even with the competitive assay, which is owing to the abrupt change of V_{OC} with the light intensity under nearly dark conditions. Utilizing optical input and electrical output signals, the new V_{OC}-based photosensitive biosensor provides a useful tool for high-sensitive and low-cost detection of mycotoxins, which can contribute to the development of point-of-use applications for food safety.

Acknowledgements

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