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Porous silicon photoluminescence biosensor for rapid and sensitive detection of toxins

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ABSTRACT

A rapid and low cost photoluminescence (PL) immunosensor for the determination of low concentrations of Ochratoxin A(OTA) and Aflatoxine B1 (AfB1) has been developed. This biosensor was based on porous silicon (PSi) fabricated by metal-assisted chemical etching (MACE) and modified by antibodies against OTA/AfB1 (anti-OTA/anti-AfB1). Biofunctionalization method of the PSi surface by anti-OTA/ anti-AfB1 was developed. The changes of the PL intensity after interaction of the immobilized anti-OTA/anti-AfB1with OTA/AfB1 antigens were used as biosensor signal, allowing sensitive and selective detection of OTA/AfB1 antigens in BSA solution. The sensitivity of the reported optical biosensor towards OTA/AfB1 antigens is in the range from 10^{-3} to 10^{2} ng/ml.

Keywords: porous silicon, biosensor, Ochratoxin A, Aflatoxine B1, biofunctionalization.

1. INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by some fungal species. Among all mycotoxins, ochratoxin A (OTA) and aflatoxin B1 (AfB1) have received much attention due to severe health effects in animals and humans [1,2]. Porous silicon (PSi) has become one of the most popular materials for the biosensor technology in the last years. In the present study, we have developed a sensitive, cost-effective, and comparatively fast method for OTA and AfB1 detection. PSi samples fabricated by metal-assisted chemical etching (MACE) were used to develop photoluminescence (PL) biosensor for OTA/AfB1 detection. PSi samples were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), reflectance and PL spectroscopy. The main structural and chemical parameters of PSi were obtained. We explored the ability of PSi samples, as a new material to enhance the performance of optical biosensors for OTA and AfB1 detection. This biosensing system using PSi and PL signal analysis was proposed in our knowledge for the first time for OTA/AfB1 detection. The PL biosensor exhibited a wide detection range from 0.094 to 100 ng/mL (linear range from 0.001 to 100 ng/mL) of toxins. Immobilization of the biosensitive layer onto PSi surface was found to decrease the intensity of PL. A phenomenon of changes in PL of PSi samples upon the interaction with toxins was proposed to be used as a platform for the optical biosensor development.

2. EXPERIMENTAL

Preparation and characterization of PSi. The PSi samples were fabricated from (111) oriented and highly doped p-type Si (B-doped, ρ= 0.005 Ω cm) utilizing metal-assisted chemical etching (MACE) according to our previous report. [3] Fabricated PSi samples were evaluated by a high resolution field emission scanning electron microscope (SEM) SU-70 (Hitachi, Japan) with an accelerating voltage 30 keV. Structural properties of PSi were characterized using Grazing Incidence X-ray Diffraction (GIXRD; BrukerD5000).

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Biofunctionalization of the PSi surface and biosensor testing. The cleaned PSi samples were immersed into a 4% solution of APTES ((3-Aminopropyl)triethoxysilane) in toluene vapors for 1 h at 70º C. Than samples were removed from the solution and rinsed with toluene and dried at 70º C for 30 min. The APTES modified PSi samples were washed in PBS (Phosphate-buffered saline) and allowed to react with 10% glutaraldehyde in PBS (pH 7,4) for 20 min at room temperature. This was followed by thoroughly rinsing the PSi with DI water to avoid non-specific adsorption of the protein A. The glutaraldehyde-activated surface was then reacted with 5 μg/ml of capture protein A in PBS buffer at room temperature for 30 min to get a protein layer. Then 5 μg/ml antibodies against OTA/AfB1 were deposited on PSi samples for covalent capturing by protein A to get an antibody layer. Finally, 5 μg/ml of BSA (Bovine serum albumin) was added to block the remaining surface (fig. 1).

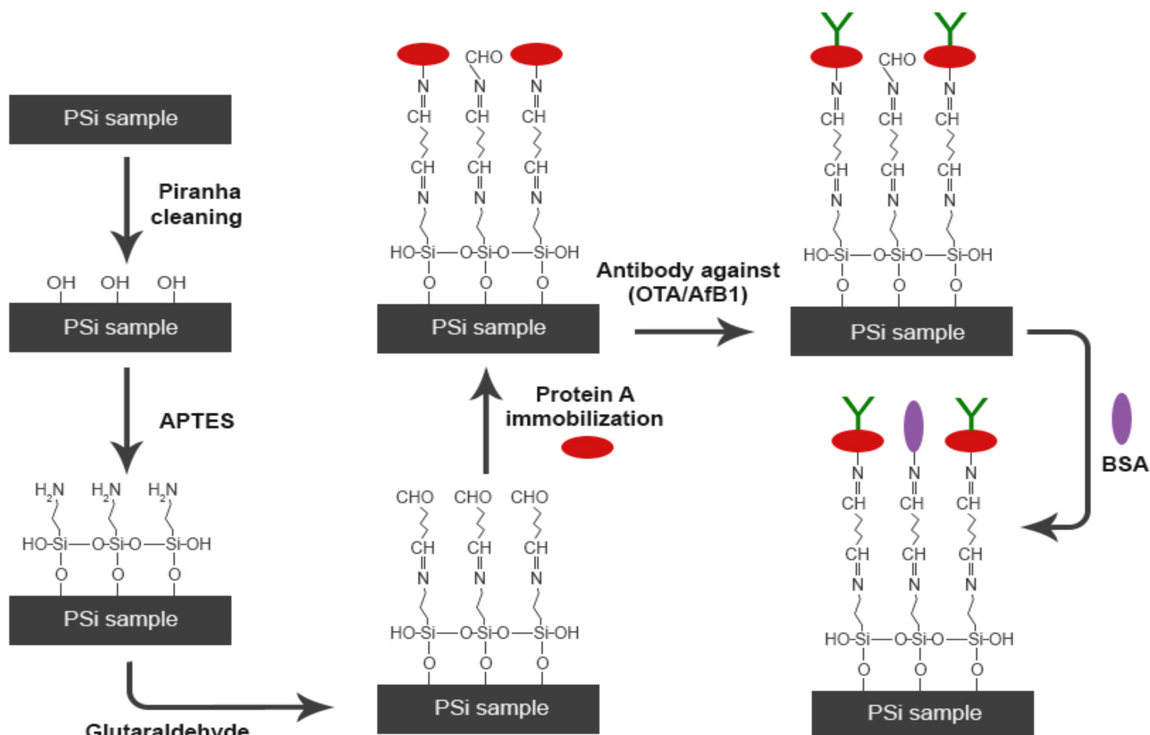

Figure 1. Functionalization of PSi samples surface and immobilization of antibody against of OTA/AfB1.

3. RESULTS AND DISCUSSIONS

Morphology and optical properties of PSi samples. Before evaluating the biosensor performance, it is important to characterize the structural properties of the PSi layer. The morphology of PSi in this work was monitored by scanning electron microscopy (SEM). Figure 2a shows the plain-view SEM image of macroporous silicon (macro-PSi) obtained by MACE. The average pore size is approximately 2−3 μm. High-resolution SEM showed that the surface of an individual macropore consists of mesoporous silicon (meso-PSi) with an average pore size ranged 10−50 nm (figure 2b). We have already observed and explained the formation of this complex porous structure by the model where etching occurs in different directions because of high concentration of holes in silicon [4].

In order to study structural properties of PSi XRD have been used. XRD spectrum allows defining basic parameters of porous silicon such as: size of PSi crystalline, porosities, and morphology. A strong peak (fig. 2c) at $2\theta = 69{,}3^{\circ}$ was assigned to the (400) plane of crystalline Si. A small peak at around $2\theta = 68{,}9^{\circ}$ indicates the presence of PSi layer. The peak position is shifted due to the crystal lattice expansion of PSi [5]. D. Bellet et al. have shown interrelation between

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the deformation of PSi crystal lattice ($\Delta a/a$) and porosity of PSi [4]. We have calculated the deformation $\Delta a/a$ and its value was approximately $14 \cdot 10^{-3}$ corresponding to the porosity of 90 %.

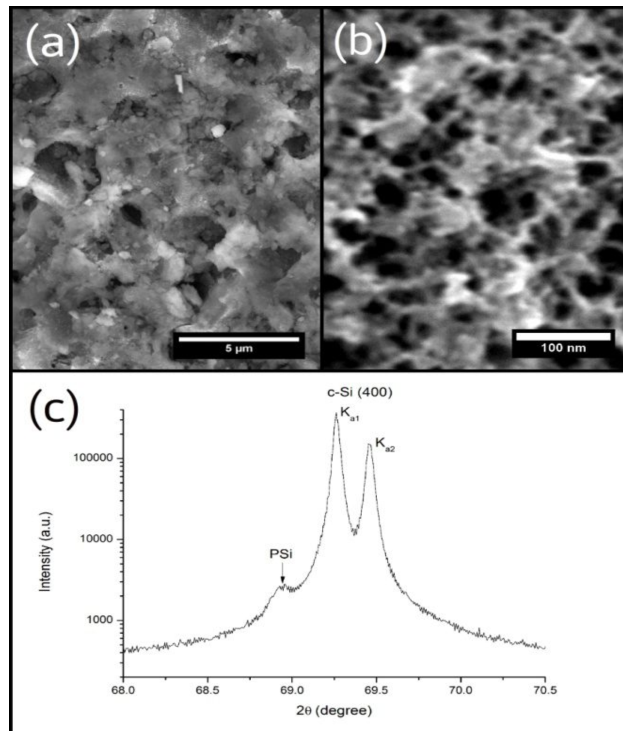

Figure 2. (a,b) SEM images; (c) XRD peak of PSi (100) sample

Testing of PSi photoluminescence biosensor for OTA/AfB1 detection. A schematic of a cell for the kinetic measurement based on photoluminescence is shown in figure 3. This instrument contains a planar sensor in which the antibodies (OTA/AfB1) are immobilized to the surface, while the antigen (OTA/AfB1) is flowed over the surface. To generate photoluminescence, the laser beam (405 nm) is directed onto the PSi surface. It passes through quartz glass and a solution and interacts with PSi surface. Then the emitted light enters an optical fiber and a spectrometer and, finally, processed on a computer using standard software. Changing the PL spectrum occurred due to a charge distribution in the near-surface layer of PSi after the antibodies (OTA/AfB1) and antigens (OTA/AfB1) interaction.

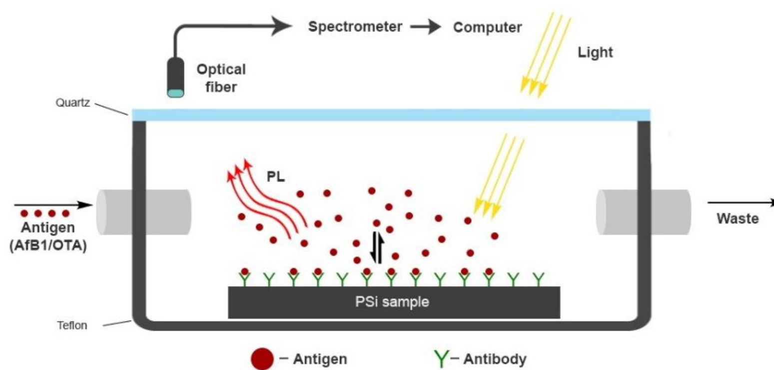

Figure 3. Scheme of a cell for kinetic measurement based on photoluminescence

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Biofunctionalized PSi samples were reacted with antigens of OTA/AfB1 in PBS solutions with concentrations of 10^{-3}–10^{2} ng/ml (fig.4). A decrease of the PL intensity of PSi samples was observed after the interaction with antigens of OTA/AfB1 sample due to the formation of immune complex between antigens and antibodies. It was found that the PL spectra did not change after the interaction of PSi samples with OTA/AfB1 solutions of $>10^{2}$ ng/ml for structures with analyte-sensitive layers (antibodies against OTA/AfB1 on PSi surface), formed from highest applied concentrations of antigens of OTA/AfB1. The hyperbolic function of the PL signal vs concentration of analyte is related to the 'saturation' of the bio-recognition layer by analyte at their higher concentrations [7].

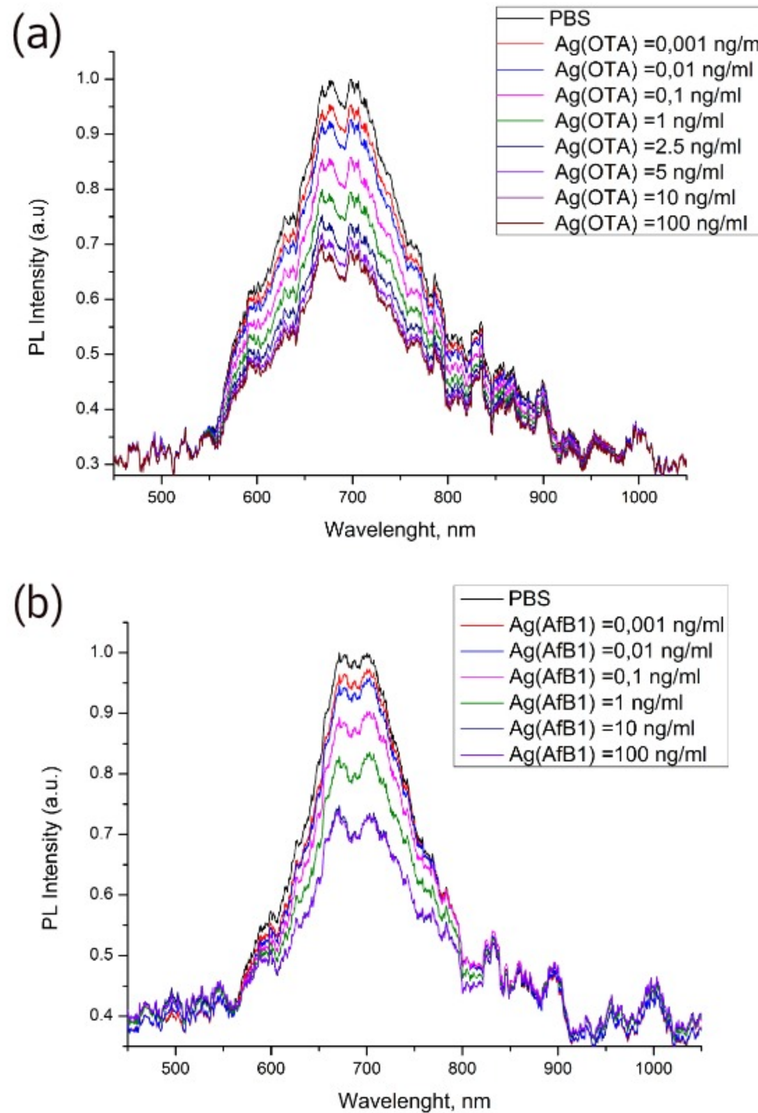

Figure 4. Pl spectra of PSi under different concentration of antigen OTA (a) and AfB1(b).

Results of sensitivity tests to Ag (OTA/AfB1) were analyzed for biofunctionalized PSi samples (fig. 5). The sensitivity was calculated according to the formula [8]:

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$$S = \frac{I_0 - I}{I_0}, \qquad (1)$$

where I_0 and I are PL intensities of biofunctionalized PSi samples at peak positions registered before (I_0) and after (I) interaction with antigens of OTA/AfB1 in PBS solution. We suppose that the interaction of AFB1 occurred according to the first order kinetics, which is the most probable in the case when dissolved materials are adsorbing on the surface of interphase and/or interacting with specific molecules (in this particular case such molecules are anti-OTA/AFB1) immobilized on this surface.

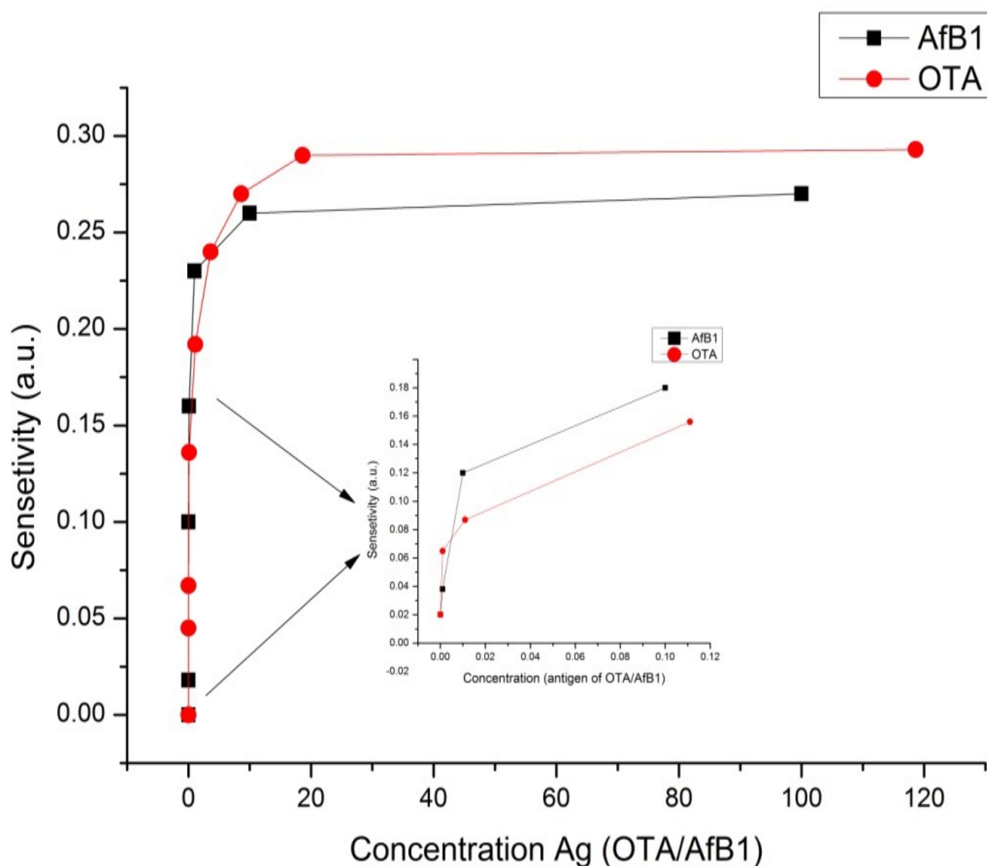

Figure 5. PL response of PSi/protein-A/anti-OTA and PSi/protein-A/anti-AFB1 based structures towards different OTA/AFB1 concentrations.

CONCLUSION

In this paper, we have reported on the development of photoluminescence biosensor based on PSi for optical detection of Ochratoxin A (OTA) and Aflatoxin B1 (AfB1). The sensing experimental design proposed allowed to detect (OTA/AfB1) 0.01ng/ml that it is comparable to high-sensitive traditional methods for the determination of OTA/AFB1 are based on chromatography and ELISA-Kits.

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