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Label-free Electrochemical Detection of Tetracycline by an Aptamer Nano-biosensor

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Abstract

A novel electrochemical biosensor, comprising a tetracycline aptamer immobilised on nano-porous silicon (PS), was investigated for the rapid detection of tetracyclines. . Electrochemical impedance spectroscopy (EIS) was used to analyse the behavior of the sensor. The specific binding of tetracycline to the aptamer biosensor led to a decrease in impedance. The corresponding impedance spectra (Nyquist plots) were obtained when serial concentrations of tetracycline were added into the system. An equivalent electrical circuit was used to fit the impedance data obtained. The linear range of the sensor was 2.079-62.37nM ..

Keywords: Nano-porous silicon, biosensor, impedance, aptamer, Tetracycline

1. Introduction

Nano-porous silicon (PS) is an interesting material with a diversity of applications, which has recently become available for biosensing (King *et al.*, 2007; Schmedake *et al.*, 2002; Cunin *et al.*, 2002; Drott *et al.*, 1997; Laurell *et al.*, 1996; Orosco *et al.*, 2006; Setzu *et al.*, 2007; Zhang *et al.*, 2009). The advantages of the PS result from several unique features: intrinsically high surface-to-volume ratio, biocompatibility and relatively simple incorporation into biosensors.

Aptamers are DNA or RNA molecules, which have been selected from random pools based on their specificity and affinity to target molecules (Ellington *et al.*, 1992; Torres *et al.*, 2009). In recent years, aptamer biosensors based on fluorescence (Gokulrangan *et al.*, 2005; Liao *et al.*, 2006; Jing *et al.*, 2010), electrochemistry (Bang *et al.*, 2005; Hansen *et al.*, 2006; Xiao *et al.*, 2005), infrared spectroscopy (Kenzo *et al.*, 2009; Liao *et al.*, 2006) and quartz crystal microbalance (QCM) (Hianik *et al.*, 2005) measurements have been reported.

Electrochemical impedance spectroscopy (EIS) is a sensitive and non-destructive technique. It is widely used to characterise the electrical properties of electrode-electrolyte interfaces (Diniz *et al.*, 2003; Yang *et al.*, 2004; Zhang *et al.*, 2010; Zhang *et al.*, 2007).

In the present work, we combined aptamer immobilised onPS with EIS to develop a new inexpensive, label-free and sensitive biosensor. According to the distinct electrical properties of the aptamer and its corresponding target, it is believed that the specific binding of the aptamer to its target elicited a change in the dielectric/surface

properties of the PS.

2. Experimental

2.1. PS Preparation

Nano-porous silicon was obtained from p-type Si (100) with an electrochemical etching process at $220\text{mA}/\text{cm}^2$ for 20 minutes in 48% hydrofluoric acid (HF) solution. The PS chip was then treated with H_2O_2 to oxidise its surface (Zhang *et al.*, 2007). Afterward, the PS chips were scanned with an atomic force microscopy (JEOL JSPM-5200, Shimadzu Corp., Japan).

2.2. Functionalising the PS Surface with NH_2 Groups

The PS chips from above were further immersed in a $\text{NH}_3 \cdot \text{H}_2\text{O} / \text{H}_2\text{O}_2 / \text{H}_2\text{O}$ (1/1/10) mixture at 70°C for 20 min. to make the PS surface highly hydrophilic. Then, the PS chips were silanised in 3-aminopropyltriethoxysilane (10%) for 3 hours, followed by rinsing with water.

2.3. Aptamer immobilisation

The tetracyclines aptamer was made using the SELEX method. Tetracycline was coated on a 96-well ELISA plate. The ssDNA was incubated with the tetracycline coated wells at 37°C for 40 min. Subsequently the unbound ssDNA was washed out.

The bound ssDNA was eluted with phenol-chloroform, and isolated with alcohol.

The above NH₂ modified PS was further dipped into glutaraldehyde (2.5%) solution for 2 hours, and then rinsed with PBS buffer (pH 7.0). Next, 10 μL of aptamers containing solution (5 ng/μL) was applied on the PS overnight at room temperature until the immobilisation was saturated. After thoroughly washing with PBS, the nonspecific sites were blocked with 2 mg/ml bovine serum albumin (BSA) for 30 min.

2.4. Electrochemical analysis

The electrochemical measurements were performed using an Im6e electrochemical workstation (ZAHNER elektrik, Zahner Co Ltd, Germany) using a three-electrode system. The working electrode was the aptamer immobilised PS chip. An Ag/AgCl electrode acted as the reference electrode, and a Pt wire was used for the counter electrode. The electrochemical experiments were carried out in 20 mL PBS buffer (0.2 M, pH 7.0) with serial tetracycline concentrations (2.079—207.9 nM). EIS experiments were carried out by applying a potential perturbation of 5 mV amplitude in the frequency range from 0.01 Hz to 100 kHz. During the measurements, all the spectra were collected at an open circuit potential. Data simulation was performed using commercial software (Zview, Scribner and associates, Charlottesville, VA). The impedance versus tetracycline concentration was plotted. A negative control was obtained using the same method, but in the absence of the aptamer.

3. Results and discussion

3.1. Characterisation of the PS

We have previously carried out and reported many PS surface modification and studies on their properties (Zhang *et al.*, 2007, 2009, 2010). When the surface is modified with NH_2 groups, both DNA and protein can be immobilised and detected. If the surface is treated with BSA, no DNA can be detected on the BSA modified surface.

AFM was performed in Multimode (tapping mode) using a NanoScope IIIa controller on a dry sample. No significant difference was observed with AFM between aptamer-modified and non-aptamer modified surface. Stable PS chips were fabricated successfully using an electrochemical etching process. The porous structure of the surface can be seen clearly from the AFM images (Fig. 1).

3.2. Impedance Spectroscopy Characterisation of Aptamer-immobilised PS

We have measured the faradic impedance of the PS in 0.2 M PBS solution. The corresponding Nyquist plots of the aptamer-immobilised PS chip and the free PS chip are shown in Fig. 2. The immobilisation of the aptamer led to a decrease in impedance. The decrease in impedance is thought to be due to a change in dielectric constant after

aptamers were immobilised on the PS surface.

3.3. The Tetracycline Determination with EIS

The PS chips were exposed to different tetracycline concentrations from 2.079 to 207.9 nM. The corresponding impedance spectra (Nyquist plots) are shown in Fig. 3. A typical faradic impedance result could be observed at the aptamer-immobilised PS chips (Fig. 3A), while the negative control did not show any obvious change (Fig. 3B).

The sensing principle used is based on changes in dielectric properties, charge distribution and conductivity change, when the tetracycline binds to the surface of the PS chips. The impedance spectra consist of a semicircle corresponding to an electron transfer limiting process. The diameter of the semicircle representing the electron-transfer resistance (R_{ct}), can be used to describe the interface properties of the electrode. Indeed, the decrease of its value after reacting with different tetracycline concentrations provides evidence for successful binding with the analyte. The modified Randle's equivalent circuit (Fig. 4) was chosen to fit the obtained impedance data. The proposed circuit includes the following three elements: R_L , the ohmic resistance of the electrolyte solution, C_d , the capacitance of PS electrode, that is associated with the interface capacitance of the semiconductor, the insulation capacitance and the double layer capacitance, and R_{ct} , the electron transfer resistance of the PS electrode. A negligible change in R_L compared with R_{ct} values was observed

from the fitted values. Therefore, the change of R_{ct} values was chosen as a way of following the interaction between the fully functionalised PS and tetracycline. Fig. 5 shows the fitted values of R_{ct} versus the corresponding concentrations of tetracycline; the linear range of the sensor was between 2.079 and 62.37 nM. In all cases, a significant decrease of the R_{ct} value was observed, due to the specific adsorption of tetracycline on the surface of aptamer immobilised PS.

Fig. 5a shows that when the Tetracycline concentration was lower than 100 nM, the R_{ct} decreased rapidly. When the concentration was higher than 100 nM, the R_{ct} remained at a constant level. That is the maximum binding concentration was 100 nM. Since the experiments were carried out in 20 mL PBS buffer, the maximum amount of Tetracycline bound to aptamer immobilised PS chip was 2 nmol. Therefore, the actual amount of Tetracycline bound was the detected concentration multiplied by 0.02 (20 mL divided by 1000 mL).

4. Conclusion

In summary, a new impedance nanobiosensor for the rapid detection of tetracycline was investigated. The nano-porous silicon used offers many advantages, such as large surface for aptamer immobilisation, a semiconductor environment for label-free impedance determination, easy fabrication, stability, and low cost. This study illustrates that the PS may provide a novel electrode material for practical electroanalytical applications. Impedance affinity biosensors can be constructed by

immobilising recognition elements, such as aptamers on the surface of PS, and measuring changes in the dielectric/surface properties when the analyte binds. A linear relationship between the impedance and the concentration of tetracycline was obtained across the whole concentration range of 2.079—62.37nM investigated, and the minimum detectable amount of analyte was about 2 nM. Therefore, this biosensor has the potential to be developed into a fast, simple and sensitive detection technique.

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Figure captions

Fig. 1 AFM images of the PS structure, where (A) is the 2D structure, and (B) is the 3D structure.

Fig. 2 Electrochemical impedance spectra: (a) is from the free PS chip; (b) is from the aptamer immobilised PS chip.

Fig. 3 Electrochemical impedance spectra of the aptamer immobilised PS chips (A) and the negative control PS (B), reacted with different tetracycline concentrations of: (a) 0, (b) 2.079, (c) 10.40, (d) 20.79, (e) 41.58, (f) 62.37, (g) 104.0, (h) 207.9 nM.

Fig. 4 Equivalent circuit employed for all data fits.

Fig. 5 Fitted values of R_{ct} versus the corresponding concentrations of tetracycline from 2.079—207.9 nM. Where (a) is from the aptamer immobilised PS and (b) is from the negative control PS. The inset is the linear range of the sensor. The error bars correspond to 1 SD and triplicate experiments were performed.

Figures

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5