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Fluorescent biosensor for alkaline phosphatase based on fluorescein derivatives modified silicon nanowires

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ABSTRACT

A fluorescent sensor for alkaline phosphatase (ALP) was realized by covalently immobilizing fluorescein molecules onto the surface of silicon nanowires (SiNWs) and phosphorylation of the immobilized fluorescein group. Based on the fluorescence enhancement, the new type of sensor exhibited good selectivity and sensitivity to ALP, and displayed a linear relationship between the fluorescence intensity and the ALP concentration in the range of 0.0175–0.3 U/mL. A single nanowire-based sensor was successfully employed to monitor the ALP activity and examine the inhibition effect of levamisole on the ALP activity. The fluorescence images of the single nanowire-based sensor showed a high spatial resolution. The present sensor paves a way to directly assay the activity of intracellular ALP by inserting a single nanowire-based sensor into a cell.

1. Introduction

Alkaline phosphatase (ALP) is a phosphomonoester hydrolase enzyme that catalyzes the hydrolysis and transphosphorylation of a wide variety of phosphate compounds to the corresponding alcohols (or phenols) and phosphate ions [1,2]. ALP consists of a group of isoenzymes that are widely distributed in mammalian tissues, especially concentrate in liver, bone and kidney. In clinical practice, ALP is one of the most regularly assayed enzymes because an abnormal level of serum ALP is usually associated with several diseases such as liver dysfunction, bone disease, breast and prostatic cancer, diabetes and so on [3,4]. Despite the substantial studies on ALP, the detailed mechanisms of ALP activity regulation during pathological process are still unclear. For the purpose of biological and clinical research, exploring a rational approach which allows real-time tracking of the ALP activities at a single-cell level would be useful to understand the mechanisms of diseases. Different methods such as fluorescent [5–11], colorimetric [12], chromatographic [13], chemiluminescent [14], bioluminescent [15,16], electrochemical [17,18] and surface enhanced resonance Raman scattering (SERS) [19] etc. have been developed to monitor the activity of ALP. Among these methods for ALP assay, the fluorescence-based assay shows great advantages over others due to its high sensitivity and spatial resolution when combined with microscopy [5]. Therefore, the development of appropriate fluorescent probes that can be finely localized at specific position in a cell is highly desirable to investigate the dynamic ALP activity at a single-cell level and reveal the biological roles of ALP.

Based on their huge surface area, the nanostructures including zero-, one-, two and three-dimensional configurations are considered to be a good candidate to be able to enhance the sensors' sensitivity and selectivity [20–25]. In recent years, several fluorescent sensors for ALP based on semiconductor quantum dots, silver nanoclusters or nanogold particles have been reported [26–28]. Although these zero-dimensional nanoparticles have been employed to construct fluorescent sensors for ALP, it is anticipated that one-dimensional nanowire is better suited for direct detection of intracellular materials [29–31]. Modifying the target-responsive fluorophores onto the large surface of a single nanowire, a single nanowire-based sensor could be developed. By utilizing a micro-operation system to precisely insert the single modified nanowire into the desired location of a cell, the intracellular ALP could be detected with a high spatial resolution [32]. Among one-dimensional nanomaterials, silicon nanowires (SiNWs) are particularly favorable to be used as matrix to fabricate sensors due to their stability, easy assembly as an array for the device, biocompatibility and potential application in a multiplex environment [33–39]. Based on the virtues of SiNWs and fluorescence-based assay in ALP detection, a SiNWs-based ALP sensor (MSiNWs) was constructed by immobilizing fluorescein molecules onto the SiNWs

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and phosphorylation of the fluorescein group. This new type of sensor exhibited high sensitivity and selectivity to ALP. A single modified silicon nanowire (MSiNW) was employed to monitor the ALP activity, and the fluorescence images of the single MSiNW showed a high spatial resolution. We believe that the present sensor has a potential application in direct assay of intracellular ALP by employing the micro operation.

2. Experimental

2.1. Reagents and materials

3-Buten-1-amine and mesitylene were purchased from Alfa Aesar. Alkaline Phosphatase (ALP) was purchased from Tokyo Chemical Industry. Other reagents were purchased from Beijing Chemical Regent Co. All reagents and chemicals were AR grade and used directly without further purifications unless otherwise noted. Water used for measurement was purified by Millipore filtration system.

2.2. Instruments and spectroscopic measurements

TEM images were recorded by the JEOL-2100 at an acceleration voltage of 200 kV. SEM images were recorded by a Hitachi S-4300FEG. Fluorescence spectra were obtained with F-4600 spectrophotometers. Fluorescence images were taken with Olympus BX51TRF microscope (λ_{ex} = 450–480 nm).

2.3. Preparation of SiNWs

SiNWs were prepared by a typical chemical vapor deposition (CVD) method [40]. An alumina boat containing SiO powder (Aldrich, 99%) was placed at the center of a horizontal alumina tube mounted inside a high temperature tube furnace. The system was evacuated to 10^{-3} Pa by mechanical pump and molecular pump. Ar (95%) and H_2 (5%) as carrier gas was introduced at the flow rate of 49 sccm, then the system pressure was rised to 800 Pa and maintained for ten minutes. And then the furnace was heated to 1350 °C, while the system pressure was maintained at 10^4 Pa. The system was maintained for 6 h and then cooled to room temperature naturally. The slightly yellow cottonlike product was collected. The structures and morphologies of the as-prepared SiNWs were characterized using transmission electron microscopy. It was found that the as-prepared SiNWs have a crystalline Si core of 8–10 nm in diameter and a silicon oxide sheath of 2–3 nm in thickness, as shown in Fig. 1(a).

2.4. Synthesis of MSiNWs

100.5 mg of the SiNWs prepared by CVD method were immersed in 4% hydrofluoric acid (HF) solution for 2 min to remove the oxide layers and bring Si—H bonds onto the surface of the SiNWs. The H-terminated SiNWs were quickly rinsed with excess amounts of deionized water and dried under vacuum at 50 °C for the following chemical modification.

The modification route of the sensor was shown in Scheme 1. Mono fluorescein carbaldehyde (CHO-fluorescein) was synthesized according to the literature [41]. 50 mg of the dried H-terminated SiNWs were suspended in 10 mL anhydrous mesitylene (distilled) under nitrogen in a two-neck round bottomed flask. Then 0.3 mL (3.28 mmol) of 3-Buten-1-amine was added by syringe. The mixture was heated to 180 °C slowly, and stirred for 3 h. After cooling down to room temperature, the product was collected with micro filter and rinsed successively with acetone, ethanol, and dichloromethane. The product was defined as NH_2-SiNWs.

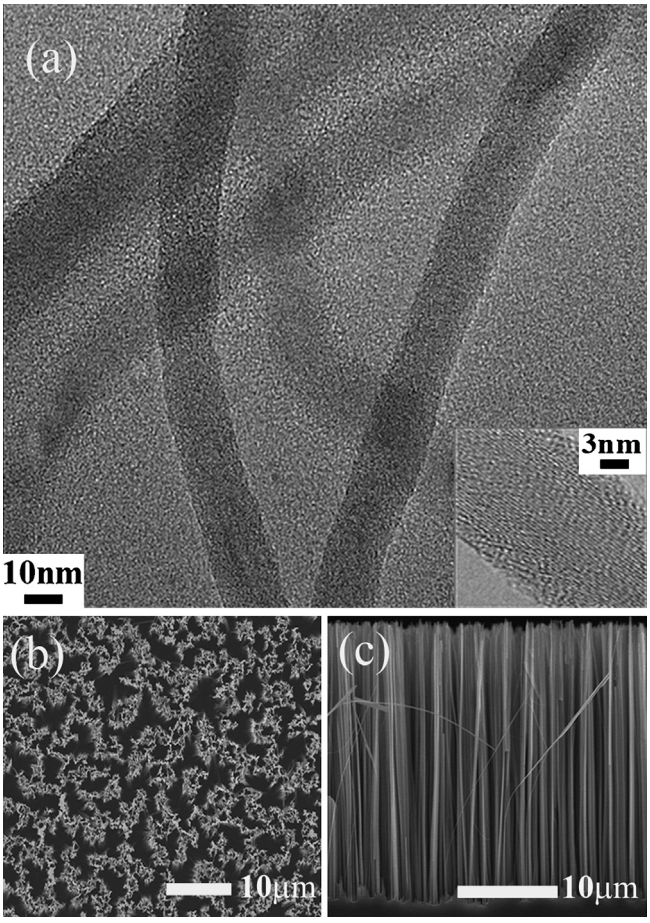

Fig. 1. (a) The TEM and HRTEM image (insert) of SiNWs by CVD method. (b and c) SEM images of the top view (b) and the side view (c) of the SiNW arrays by CE method.

40 mg of NH_2-SiNWs were suspended in 5 mL ethanol solution containing 4.5 mmol CHO-fluorescein. After stirring for 1.5 h at room temperature, the product was collected by filtration and repeatedly washed with ethanol. Unreacted organic materials were removed completely by monitoring the fluorescence of the washing liquid. Then, the products were dispersed in 8 mL ethanol solution of 0.03 mmol mL^{-1} sodium triacetoxyborohydride. After stirring for 3.5 h at 50 °C, the product were collected by micro filter and repeatedly washed with ethanol and then dried under vacuum for 10 h. The product was defined as FLU-SiNWs.

The fluorescein group of FLU-SiNWs were phosphorylated by phosphorus oxychloride [42]. FLU-SiNWs (22.5 mg) and CH_2Cl_2 (5 mL) were added into a flame-dried round flask. The suspension was cooled to 0 °C and pyridine (1.2 mmol), phosphorus oxychloride (0.5 mmol) were orderly added in. The reaction mixture was stirred for 1 h under N_2 at 0 °C. The reaction was then quenched by dropwise addition of 1:1 acetone: water (0.5 mL). The product was filtered and washed successively with deionized water, acetone and dichloromethane. After drying under vacuum, the modified SiNWs fluorescent sensor (MSiNWs) was obtained.

2.5. Preparation of SiNW arrays

The High-quality SiNW arrays were fabricated by the chemical etching (CE) method [43]. A clean n-type silicon wafer with [100] orientation was immersed into 5% hydrofluoric acid (HF) solution to bring Si-H bonds on the surface of the wafer. Afterwards, the wafer was soaked in 5 mM silver nitrate solution to deposit Ag particles on