Silicon nanowires for high-sensitivity glucose detection

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Silicon nanowires (SiNWs) were investigated as supporting matrices for enzyme immobilization to construct glucose biosensors. Glucose oxidase was adsorbed onto SiNWs after different treatments, either as grown, HF etched, or carboxylic acid (COOH) functionalized. The amperometric biosensor with COOH-functionalized SiNWs performed the best with a detection limit of 0.01 mM glucose (signal-to-noise ratio=3). For real-time detection of glucose, SiNW biosensor showed a linear response in the range of 0.1–15 mM. This work demonstrates the utility of SiNWs as a biosensor component and provides a general method to modify the surface of semiconducting nanomaterials for potential biomedical applications. © 2006 American Institute of Physics.

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One-dimensional nanostructures have been demonstrated to possess unique characteristics for ultrasensitive, miniaturized molecular sensing applications.\textsuperscript{1} The sensing mechanism of semiconductor nanostructures, such as carbon nanotubes,\textsuperscript{2–4} silicon nanowires,\textsuperscript{5,6} SnO\textsubscript{2} nanowires,\textsuperscript{7} and In\textsubscript{2}O\textsubscript{3} nanowires,\textsuperscript{8} is generally believed to be related to change of surface charge of nanostructures in the presence or the absence of analyte species. The high surface-to-volume ratio of nanostructures renders them extremely sensitive to surface species so that small volume detection becomes possible. Silicon nanowires (SiNWs) are of special interest because their surface can be easily modified to act as both electron-transfer mediators and immobilizing matrices for biological or chemical molecules.\textsuperscript{9,10}

Amperometric enzyme-based biosensors combine enzyme specificity with the sensitivity and convenience of electroanalytical techniques in a compact form to facilitate analysis. On the other hand, the benefits of immobilized enzymes for biosensor applications include enhanced stability, repeated use, facile separation from reaction mixtures, and prevention of enzyme contamination in products. The commonly used methods for enzyme immobilization include covalent bonding,\textsuperscript{11,12} physical adsorption,\textsuperscript{13} and physical entrapment.\textsuperscript{14–17} Recent works have demonstrated that nanomaterials could be a promising mediator for enzyme immobilization, leading to increased selectivity and sensitivity of amperometric biosensors.\textsuperscript{9,18–20}

In this study, we report the use of SiNWs as both electron-transfer mediator and immobilization matrix to construct an amperometric biosensor. The capacity of glucose oxidase (GOx) to catalyze glucose oxidation to gluconic acid was used as a model system for the design of glucose sensors. The principle of glucose determination by enzymatic method is based on measuring enzymatically produced hydrogen peroxide during oxidation of glucose in the presence of molecular oxygen.

To further enhance sensitivity and lower detection limit, we used and compared three different surface-treated SiNWs for the interfacial component of glucose biosensors and investigated the electrocatalytic performance of the resulting biosensors. We demonstrate that SiNWs have good biocompatibility with the immobilized enzyme molecules, and SiNW-based biosensors have the potential of highly sensitive, real-time, and label-free detection of glucose in aqueous solution. Our results further show that surface treatment of SiNWs significantly affects the capacity of enzyme immobilization and the electron-transfer conductivity of SiNWs.
The Pt electrode was coated with 15 mg/mL glucose oxidase (GOx, EC 1.1.3.4, Type VII-S, Aspergillus niger) in 6 mg/mL glucose oxidase/de-ionized distilled water, and then mixed with 40 mg/mL with a SCE and platinum wire used as reference and counter electrodes. An electrochemical workstation was evaluated in a three-electrode cell controlled by CHI 802. The characteristics of the SiNW-modified glucose biosensor were measured in 0.1 M phosphate-buffered saline (PBS, pH = 7.0) at a scan rate of 5 mV/s. The Pt electrode was stored at 4 °C before use. The performance and detection sensitivity of the resulting biosensor. After functionalization with carboxyl groups, the morphology of COOH-SiNWs remained similar to that of HF-SiNWs.

Figure 2 shows the linear sweep voltammograms of SiNW-based glucose biosensors at a scan rate of 5 mV/s. A sigmoid response to glucose was obtained (curves b–d in Fig. 2). The current response began to increase at +0.2 V, and reached a plateau at 0.4 V, indicating that GOx trapped in the SiNW matrix could respond to glucose sensitively. To enhance the current signal, SiNWs were treated with HF to remove the outer oxide layer of SiNWs, which would inhibit electron transfer between the enzyme and the base electrode. The surfaces of HF-etched, oxide-free SiNWs are passivated by hydrogen and exhibit moderately high reactivity. It can be seen from Fig. 2 that the amperometric response (curve c) of the biosensor based on HF-etched SiNWs is greater than that of based on as-grown SiNWs (curve b) at 2 mM glucose solution, possibly due to the increased conductivity of SiNWs after oxide removal. HF-etched SiNWs offer both a suitable biocompatible microenvironment for the entrapment of glucose oxidase and a mediator for electron transfer between the enzyme and electrode. We have also linked the enzymes covalently with SiNWs modified with carboxyl groups. The response of the COOH-SiNW biosensor (curve d in Fig. 2) exhibits the highest current values among the three different types of biosensors.

![Fig. 1. SEM images of (a) as-grown SiNWs and (b) HF-treated SiNWs.](image)

SiNWs used in this study were prepared by oxide-assisted growth by simple thermal evaporation of silicon monoxide powder as the single source. The as-grown SiNWs nominally have a crystalline Si core of 15–20 nm in diameter and a Si oxide sheath of 3–5 nm in thickness. The as-grown SiNWs were dispersed in 40 mg/mL, 6 mg/mL glucose oxidase (GOx, EC 1.1.3.4, Type VII-S, Aspergillus niger, Aldrich-Sigma) completely. The Pt electrode was coated with 15 μl of the resulting mixture, and the enzyme immobilization reaction was allowed to occur at 4 °C for 24 h. The SiNW-modified enzyme electrode was stored at 4 °C before use. The performance and characteristics of the SiNW-modified glucose biosensor were evaluated in a three-electrode cell controlled by CHI 802 electrochemical workstation (CH Instruments, USA). The SiNW-modified electrode was used as the working electrode, with a SCE and platinum wire used as reference and counter electrodes, respectively. β-D-(+)-glucose was obtained from Aldrich-Sigma (USA). The supporting electrolyte was an aqueous solution buffered with phosphate.

Figure 1 shows the scanning electron microscopy (SEM) images of as-grown SiNWs and HF-etched SiNWs. The morphology of as-grown SiNWs [Fig. 1(a)] changed after HF treatment [Fig. 1(b)]. The as-grown SiNWs typically had a crystalline silicon core measuring 20–30 nm in diameter and sheathed with a 3–5 nm thick oxide layer. The etched SiNWs were slightly reduced in diameter, and coated with some clusters of Si nanoparticles. The nanoparticles were formed due to breakup of SiNWs or Si nanochains upon HF etching. The nanoparticles cluster would increase the surface-to-volume ratio and contribute to the improved detection sensitivity of the resulting biosensor. After functionalizing with carboxyl groups, the morphology of COOH-SiNWs remained similar to that of HF-SiNWs.

![Fig. 2. Linear sweep voltammograms of biosensors in pH 7.0 phosphate buffer solution without (a) and with [(b), (c), (d)] of 2 mM glucose at a scan rate of 5 mV/s. (b) SiNWs/GOx; (c) HF/SiNWs/GOx, and (d) COOH/SiNWs/GOx.](image)

![Fig. 3. Typical current-time response curve for successive addition of glucose. [(a) and (b)] 0.1 mM; [(b) and (c)] 0.2 mM; [(c) and (d)] 0.5 mM at HF/SiNWs/GOx electrode in 0.1M PBS (pH=7.0). The operating potential is 0.6 V vs SCE.](image)
Glucose oxidase was adsorbed onto SiNWs of differently functionalized SiNW, COOH/SiNWs/Gox, and COOH/HD/SiNWs/Gox, respectively. The applied potential is 0.6 V vs SCE. Inset shows the calibration curves in the range of 0–2 mM.

The amperometric response of the HF-etched SiNW-based biosensor following each successive addition of glucose is presented in Fig. 3. The biosensor showed excellent bioelectronic oxidation activity for glucose at a static applied potential (+0.6 V). The reaction occurring at the biosensor takes 10–15 s to reach a dynamic equilibrium upon each addition of the sample solution. Significantly, the reaction time is sufficiently fast for practical sensing applications.

A series of experiments was performed to determine the pH dependence of the biosensor response (more data are shown in Fig. S3 in the supporting material). The optimum response current was observed at pH 6.0, which is in good agreement with the previous data for soluble GOx. It shows that the SiNW matrix used to immobilize the enzyme did not affect the optimal pH value for the electrocatalytic reaction of GOx with glucose, indicating that this structure provided a good biocompatible microenvironment around the enzyme.

The corresponding calibration plots for SiNW-based glucose biosensors with different surface treatments are shown in Fig. 4. A fast response was observed after the addition of 0.1 mM glucose. The biosensor based on the as-grown, HF-etched, or COOH-functionalized SiNWs all showed a linear response to glucose concentration within the range of 0.1–15 mM (2.70 g L−1). Therefore, the dynamic range covers both physiological and pathological conditions (normal values: 0.70–11.0 g L−1). The inset in Fig. 4 shows the current-time recordings for a series of successive additions of 0.1 mM glucose. Detection limit of less than 0.01 mM for glucose (1.80 mg L−1 at signal-to-noise ratio = 3) was observed for COOH-SiNW-based biosensor. The present sensitivity results are considerably better than those previously reported, which showed the best detection limit of 0.02 mM (Ref. 28) and a linear range from 0.48 to 12 mM. It is clear that the sensitivity of the SiNW-modified electrodes increased with the treatment of HF and carboxyl group, and the greatest response was obtained from COOH-functionalized SiNW. The sensitivity of COOH-SiNW-based biosensor was more than three times greater than that of HF-SiNW-based biosensor indicating that SiNWs modified with carboxylic acid may provide high enzyme loading capacity as well as high conductance and transducing ability.

In summary, SiNWs were used as supporting matrices for enzyme immobilization to construct glucose biosensors. Glucose oxidase was adsorbed onto SiNWs of differently treated surfaces, either as grown, HF etched, or carboxylic acid (COOH) functionalized. Among the three surfaces, COOH-functionalized SiNW biosensor performed the best with a detection limit of 0.01 mM [signal-to-noise ratio (S/N) = 3]. For real-time detection of glucose on SiNW biosensor, a linear response was obtained in the range of 0.1–15 mM. The optimal pH value for enzyme immobilization on SiNW matrix is 6.0, indicating good bio compatible microenvironment around the enzyme. SiNW-based biosensors showed fast response and high sensitivity to glucose, demonstrating the potential of SiNWs as a biosensor component. The present study also suggests the possibility of using semiconductor nanowires to construct biosensors for sensitive, label-free, real-time detection of a wide range of chemical and biological species.

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