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Glucose Biosensors Based on Vertically-Aligned Multi-walled Carbon Nanotubes

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ABSTRACT

Vertically-aligned multiwalled carbon nanotubes (VA-MWCNTs) were grown using plasma enhanced chemical vapor deposition (PECVD) technique. These VA-MWCNTs were then dip coated by Poly methyl methacrylate (PMMA) followed by annealing. Samples were then polished to expose the tips of CNTs. Biological molecules Glucose Oxidase (GOx) were then immobilized on the exposed tips of these nanoelectrode ensembles. Here we present further characterization of these devices, with results on the detection limits and measurement stability. We found that these sensors can be reused for longer than six months when kept in proper storage conditions.

INTRODUCTION

In the efforts to develop better and smaller blood glucose sensors, carbon nanotubes (CNTs) have been used as the electrode materials for these sensors. CNTs possess excellent chemical and physical stabilities to be used in biosensors as reported in literature [1-3]. While preparing CNT based glucose biosensors, the most common and extensively used enzyme, Glucose Oxidase (GOx), is amalgamated with CNTs [4-9]. GOx enzyme was first discovered by Müller [10] in 1928 as the catalyst to for the oxidation of glucose to gluconic acid in the presence of dissolved oxygen [11]. Since CNTs possess fast electro- catalytic speed and electron transfer rate, it is a common assumption that CNTs can capture the electrons from the deeply embedded redox centres in GOx and act as transducers in CNT based glucose biosensors [12]. Wang et al. used CNT composites as the electrode materials for glucose sensors. Simultaneously they filled the mixture of CNTs and GOx in a polyamide tube and the potted the other end with the nafion coating and recorded the current response of the sensor [13]. Since then various techniques such as cross-linking [14], physical adsorption [15], etc. has been used to improve the immobilization of GOx onto surface of different electrodes including CNTs. All these techniques are complicated and involved non-compatible reagents which produces biosensors that do not exhibit good stability and longer life time. Since inception, the issues which has been discussed so far pertains to improving stability, attaining high sensitivity and low limit of detection and response time of CNT based biosensors. However, reports on durability, and reusability of GOx-CNT based biosensors are sparse. In most of the cases the dispersion of CNTs affects the immobilization of enzyme and limits its performance [16]. It is evident that as grown CNTs have closed shell and it does not allow high degree of functionality.

Keeping this in mind Lin et al. employed opened end CNTs and fabricated glucose biosensors based on CNT nanoelectrode nanoensembles (CNNEs) [1]. But again, very less has been discussed about the durability of the biosensors and stability of the enzyme. Overcoming

the limitation faced till date, here we report a durable glucose sensors based on vertically-aligned multiwalled carbon nanotubes (VA-MWCNTs).

EXPERIMENTAL DETAILS

Fabrication of CNT nanoelectrode nanoensembles (CNNEs)

Our samples were prepared by dual RF-plasma-enhanced chemical vapor deposition [17]. In brief, Ni films (10 nm thick) were first deposited on *p*-type Si substrates (1–10 Ω cm) by RF magnetron sputtering. These substrates were then used for the growth of VA-MWCNTs at 450^0C by using pure methane gas. VA-MWCNTs were grown within a circular area (7mm in diameter). The as grown VA-MWCNTs were then dip coated by poly methyl methacrylate (PMMA) followed by annealing at ~100^0C. Samples were then polished to expose the tips of MWCNTs. These CNNEs are ready for immobilization of biological molecule Glucose Oxidase (GOx) for glucose sensing.

Functionalization of VA-MWCNTs with carboxylic group (-COOH)

Figure 1 shows the schematic diagram of functionalization of VA-MWCNTs with Carboxylic (-COOH) group. VA-MWCNTs were pretreated electrochemically with NaOH (1M) at 2.8V for 2 minutes. The modified electrodes were then dried for 30 minutes in air.

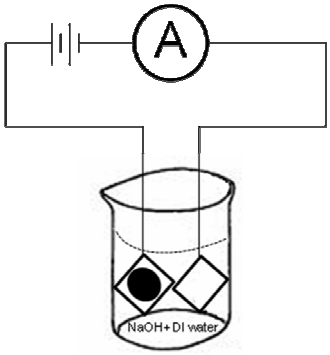

Figure 1. Schematic of functionalization of VA-MWCNTs with Carboxylic groups.

Immobilization of GOx on CNNEs

For all the experiments described hereafter, deionized water was used as the solvent. For the immobilization of GOx, we use standard water soluble coupling agent EDC (1-ethyl-3-3-dimethylaminopropyl carbodiimide) and Sulfo-NHS (N-hydroxy-sulfo-succinimide) as reported by Lin et al. [1]. First, CNNEs were immersed in 20ml freshly prepared EDC solution (10 mg/ml). 300 mg of sulfo-NHS was then added to the solution. Chemical reaction was allowed to occur at room temperature for 3 hours. Finally the CNNEs were washed and immersed in 20 ml of GOx solution (0.1M) prepared in phosphate buffer solution (pH 7.2). GOx immobilization was allowed to occur at room temperature for 3 hours. The functionalized CNNEs (glucose biosensors) were stored at 4^0C when not in use. The overall processes described so far are schematically summarized in figure 2.

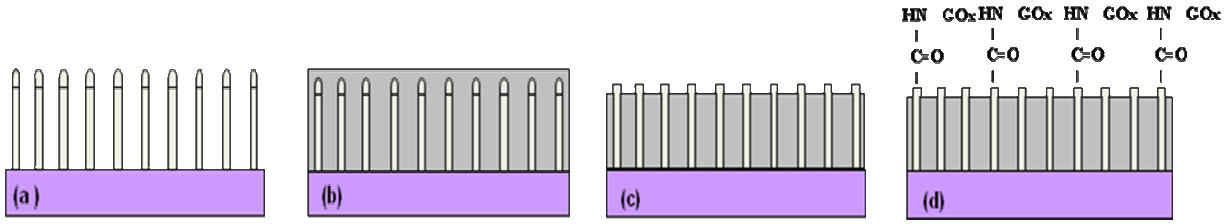

Figure 2. Schematic diagram of the fabrication steps of CNNEs. (a) As grown VA-MWCNTs (b) VA-MWCNTs dipped coated with PMMA (c) The polished PMMA coated VA-MWCNTs with exposed tips and (d) CNNEs functionalized with GOx.

Assembly of glucose biosensor and measurements

The fabricated glucose biosensor was incorporated into a three-electrode electrochemical cell as the working electrode as shown in figure 3. Phosphate buffer solution (PBS, pH 7.2) was used as the medium. A platinum wire was used as the counter electrode together with Ag/AgCl as a reference electrode. Amperometric response of the sensors was obtained using a computer controlled potentiostat. This electrochemical set up was allowed to equilibrate for at least 500 s before adding glucose solution to the system for sensing.

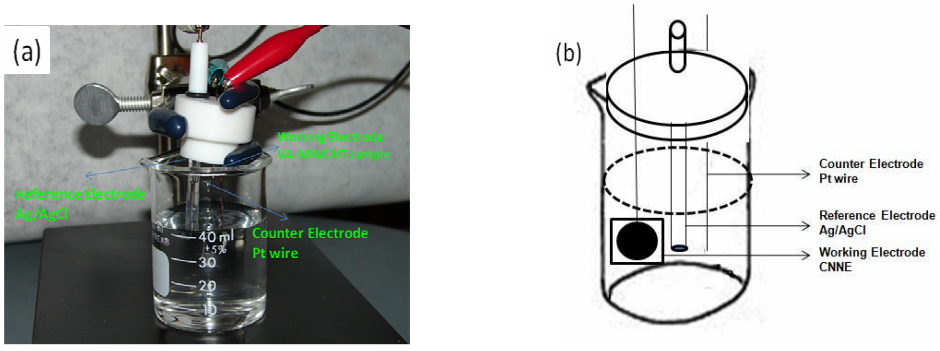

Figure 3. (a) Picture and (b) Schematic diagram of the three-electrode electrochemical cell.

For each sensing measurement, the background response of GOx-VA-MWCNTs was first recorded at an applied potential of 0.2V. When the current became stable, then glucose solution (in PBS) was added to the cell. When the biosensor was not in use it was stored at 4^0C in PBS.

RESULTS AND DISCUSSION

As described, in figure 1, electrochemical treatment was done in order to form carboxylic group (-COOH) on the exposed tips of VA-MWCNTs. The presence of the carboxylic acid group was confirmed by comparing the Fourier-Transformed Infrared (FTIR) spectra of the as grown VA-MWCNTs and the electrochemically treated VA-MWCNTs. As shown in figure 4a and figure 4b. The appearance of the carboxyl group (C=O) absorption peak at ~1746cm^{-1} was detected after electrochemical treatment as reported in literature [18].

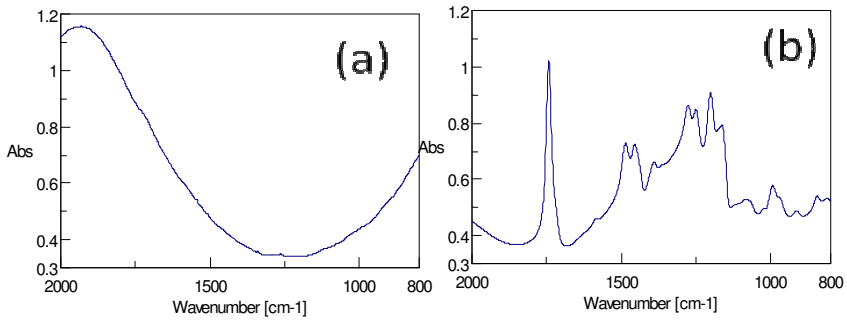

Figure 4. FTIR spectra of (a) the as grown and (b) electrochemically treated VA-MWCNTs.

The recognition of biocatalytic activity of VA-MWCNTs-GOx was electrochemically analyzed. Figure 5a shows the cyclic voltammogram (CV) profiles of the CNNEs in PBS buffer solution (pH 7.2) without immobilization of GOx. We can clearly see the absence of any redox peaks and leaking currents in the CV curve. This means, our CNNEs are fully electrochemically sealed except the exposed nanotubes tips. In the next step, CV of GOx functionalize CNNEs was performed and the result is shown as figure 5b. A pair of symmetrical redox peaks can now be detected. These redox peaks correspond to the direct electron transfer reaction of GOx [19].

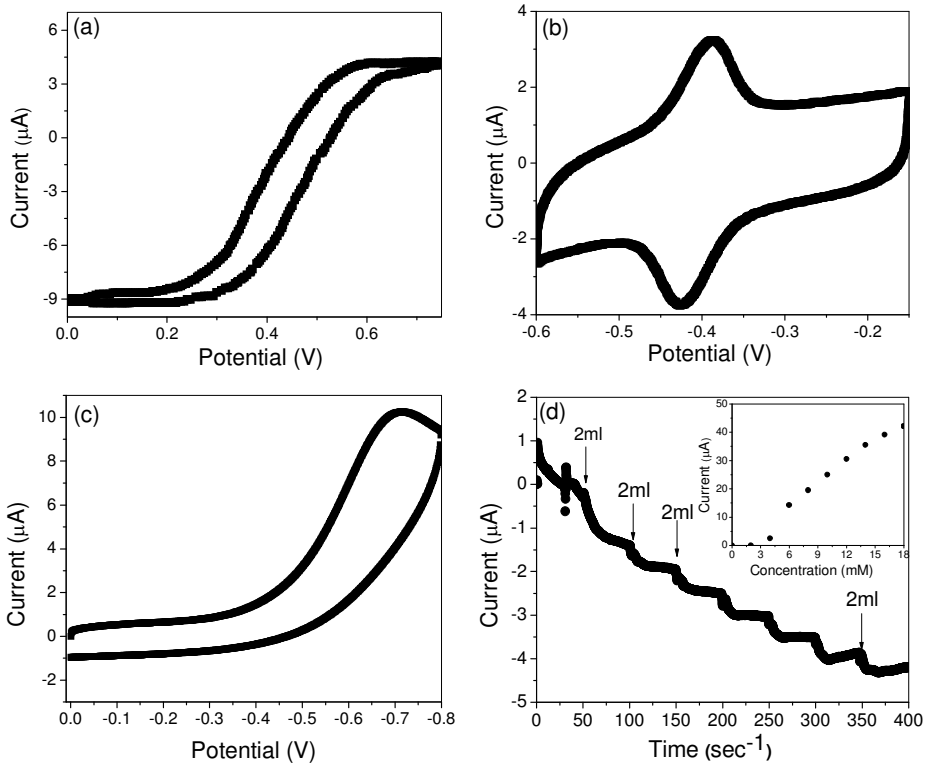

Figure 5. Cyclic Voltammogram (CV) of (a) CNNE without GOx, (b) CNNE with GOx, and (c) CNNE with GOx and glucose. (d) The amperometric response of CNNE biosensor with successive addition of 20mM glucose. Inset shows the calibration curve.

Figure 5c shows the CV of GOx functionalized CNNEs with the addition of a drop (2 ml) of 20mM β-D-glucose. As shown, the shape of the CV signal has changed and the anodic current has increased. This indicates that the GOx attached to the MWCNTs is catalyzing the redox reaction described as follows. GOx is a dimer with two molecules of flavin adenine dinucleotide (FAD). It is well known that GOx enzyme binds to β-D-glucose and aids in breaking it into its metabolites. In the GOx catalyzed redox reaction, FAD works as the initial electron acceptor and is reduced to $FADH_2$. After that $FADH_2$ is oxidized by molecular oxygen (O_2). O_2 is then reduced to hydrogen peroxide (H_2O_2). Overall redox reaction catalyzed by GOx is given by:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconolactone} + H_2O_2$$

This indicates that the GOx functionalized CNNEs can be used to detect glucose.

For this purpose the amperometric response of the GOx functionalized CNNEs was recorded for each successive addition of 2ml of 20mM glucose solution as shown in figure 5d. The inset shows the calibration curve of this amperometric response as plotted in current versus accumulated glucose concentration. As shown, for the range of concentrations tested here, the sensor response is linear in nature. This allows a linear regression to calculate the detection limit. The limit of detection was estimated to be 14.4μM at a signal to noise ratio of 3. The slope of the calibration curve gives the glucose sensor a sensitivity of 410nA/mM/cm^2 by considering the sensor active area of 0.385cm^2. This sensitivity is comparable with most other glucose sensors [19].

As shown in figure 5d, well defined current responses were observed for our GOx functionalized CNNE biosensors. The reaction occurring at the biosensor is very fast in reaching equilibrium upon each addition of the glucose solution, generating a steady state current signal within 50 seconds. The linear response of the glucose biosensor to glucose molecules is up to about ~20mM of glucose. This is higher than needed for the practical use in the detection of high blood glucose level. Furthermore we tested our glucose biosensors for reusability. First we studied amperometric response of our sensors for 24 hours after addition of 0.0054 mM of glucose. Current decreases sharply at the point of addition of glucose but recover to its initial value. This suggests that the GOx-VA-MWCNTs electrode can be continuously operated for 24 hours.

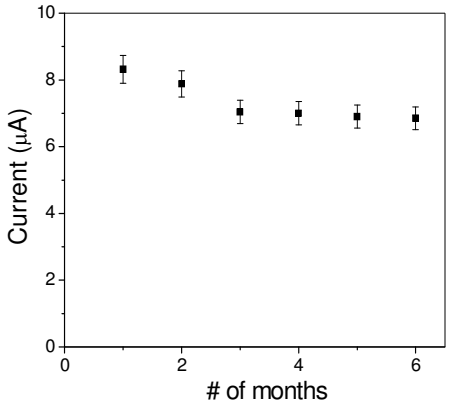

Figure 6. Stability curve of the biosensors.

We further monitored the functionality of our sensors for nearly six months. After each month, current measurements were conducted at regular interval of 5-6 days by using 0.0035mM glucose in PBS. Figure 6 shows stability of current response for six months. It can be seen that current response remains pretty stable. This means, the immobilized GOx on VA-MWCNTs is durable and can be used for more than six months if kept in proper condition.

CONCLUSIONS

In conclusion we have developed a durable glucose biosensor using arrays of VA-MWCNTs. The biocatalytic activity of the enzyme GOx is retained. Preliminary results show that minimum limit of detection is 14.4 μM with the sensitivity of 410nA/mM/cm^2. Furthermore results show that our glucose biosensor can be reused for six months when stored in proper conditions.

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