Electrochemical Nanoneedle Biosensor Based on Multiwall Carbon Nanotube

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We report the fabrication and analytical functions of a biosensor based on a nanoneedle consisting of a multiwall carbon nanotube attached to the end of an etched tungsten tip. The devised electrode is the smallest needle-type biosensor reported to date. The nanoneedles prepared in this work are 30 nm in diameter and 2–3 μm in length. Dopamine and glutamate, which are physiologically important neurotransmitters, were successfully detected using these nanoneedles. Bare nanoneedles detected dopamine in the range from 100 to 1000 μM by differential pulse voltammetry, and enzyme-modified nanoneedles were able to respond to glutamate in the 100–500 μM range by potentiostatic amperometry.

Ever since carbon nanotubes (CNTs) were first discovered,¹ they have attracted much interest that was directed toward exploiting unique geometrical, mechanical, electronic, and chemical properties.²–³ Moreover, many potential and practical applications of CNTs have been reported in the fields of chemical sensing,¹⁰ biosensors,⁶⁻⁷ cell counters,⁶ energy storage,⁹ field emission materials,¹⁰ catalyst supports,¹¹ high-sensitivity nano-balance,¹² scanning probe microscopy (SPM)¹³,¹⁴ and many others.

The first attempt to use an individual CNT as an electrochemical probe was reported by Crooks et al.,¹⁵ who described the fundamental electrochemical behavior of CNT electrodes. It has also been reported that individual multiwalled carbon nanotubes have been mounted on tungsten tips,¹⁶⁻¹⁷ but no more work has been published on chemical sensors or enzyme electrodes. The present work is about the smallest nanoneedle-type biosensor ever produced and its potential for detecting biologically important analytes. An enzyme-modified glucose sensor based on a single-wall carbon nanotube was previously reported by Azamian et al.;¹⁸ however, this sensor was immobilized on a fixed substrate and, thus, could not be moved to a desired location.

We report here on an interesting application, namely, a needle-type nanobiosensor. In the present study, nanoneedles were fabricated on the basis of one multiwall carbon nanotube (MWCNT) on an etched tungsten tip and used as electrochemical biosensors to determine dopamine (DA)¹⁹,²⁰ and glutamate²¹,²² levels. DA is a neurotransmitter that is released at synaptic clefts in many

[References and Footnotes]

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EXPERIMENTAL SECTION

The MWCNTs soot required for the fabrication of the nanoneedles was synthesized by arc discharge, as previously described. A tungsten tip was electrochemically etched to a sharp, long tip geometry, to which a MWCNT was easily attached (see Supporting Information). This attachment process was carried out in a field emission scanning electron microscope (JEOL, JSM-6700F) equipped with two piezoelectric nanomanipulators. The nanoneedle tungsten portion was sealed with a UV-hardening polymer (Norland optical adhesive 68) to insulate it from the solution under study and to provide mechanical support. As a result, only the MWCNT was exposed to solutions.

Dopamine (3-hydroxytyramine hydrochloride, Aldrich) solutions were prepared at 100, 200, 400, 600 and 1000 μM concentrations in HEPES buffer solution (pH 7.4). The quantitative analysis of dopamine was conducted by differential pulse voltammetry using a potentiostat (CH 750A, CH Instruments). The reference electrode and the counter electrode were a Ag/AgCl electrode and a Pt wire, respectively.

Glutamate is an electrochemically inactive species, unlike dopamine, nanoneedles require enzyme modification for glutamate detection. To immobilize glutamate oxidase (GLOx) on the nanoneedles, electrodes were immersed in a solution containing 2.5 mg of GLOx, 0.0025% of glutaraldehyde, and 10 mM m-phenylenediamine (m-PD). m-PD was electropolymerized onto the electrode, and GLOx was trapped inside poly-m-PD during voltage cycling from 0 to +0.8 V at a scan rate of 2 mV/s. Using enzyme-modified nanoneedles, glutamate was detected in HEPES buffer solution (pH 7.4) in the range from 100 to 500 μM by amperometry.

RESULTS AND DISCUSSION

The nanoneedles in this work were fabricated by attaching a MWCNT to a tungsten tip using a nanomanipulator in a high-vacuum chamber. The MWCNTs employed for this purpose were no thicker than 50 nm in diameter and 2–3 μm in length. In the vacuum chamber of the field emission scanning electron microscope (FE-SEM), the end of the tungsten tip is touched to an individual MWCNT out of the bundle of carbon nanotubes (Figure 1A). After locating the MWCNT, it is pulled out of the bundle, a sufficient voltage is applied, then a weak site of the carbon nanotube is cut, and the length is 2–3 μm, as displayed in Figure 1B. The close-up view of the MWCNT stuck on the tip is shown in Figure 1C. Potassium hydroxide (KOH) concentration in the etching solution, the immersion depth of the tungsten tip, and the applied bias voltage should all be carefully controlled to produce a tungsten tip with a high tip aspect ratio. This tip aspect ratio depends more on the immersion depth than on the KOH concentration or bias voltage (see Supporting Information, Figure 2). Although etching processes can sharpen the tips effectively, tungsten tip sizes are still larger than 100 nm in diameter (inset of Figure 1A). A single MWCNT of ~30 nm in diameter (Figure 1C) was attached to the tungsten tip to obtain a probe with an even higher aspect ratio.

A tungsten tip was used as a structural support for the carbon nanotube because it is stronger than other metals; however, it can cause a higher background current or induce undesirable reactions in electrochemical systems if exposed to the system. Thus, the tungsten was insulated selectively with a UV-hardening polymer (Norland optical adhesive 68). It is a suitable material for making a thin and insulating film on the tungsten tip by shining a UV light. First, a slide glass was placed at the focused position in a microscope, and the UV-hardening polymer was dropped on the slide glass. The tungsten portion of the nanoneedle was then dipped into the epoxy carefully using a micromanipulator, lest the carbon nanotube part be contacted with the epoxy. Finally, the nanoneedle was lifted and exposed to UV light for the epoxy to be hardened. This process was repeated 3–4 times for complete insulation. Figure 2 shows the microscopic images of the insulation process. The carbon nanotube is at the right in the white circle.

The observed redox behavior of ferricyanide (Fe(CN)$_6^{3-}$) at the nanoneedle electrodes confirmed that such nanoneedles can function as reliable electrochemical probes. The cyclic voltammogram of Fe(CN)$_6^{3-}$ at a MWCNT-based nanoneedle is shown in Figure 3A. Figure 3B is the differential pulse voltammogram under the same conditions as Figure 3A. The nanoneedle electrode showed quasireversible behavior ($E_{pc} = 0.213$ V, $E_{pa} = 0.380$ V, $\Delta E = 0.167$ V). This quasireversibility supposedly stems from the intrinsic electronic properties of the MWCNT, which requires further study.

DA is a well-known catecholamine that can be electrochemically oxidized to the o-quinone form. No modification of electrodes
is required for its oxidation, which involves a two-electron transfer ($E^o = 0.155 \text{ V vs Ag/AgCl}$). 29

Figure 4A shows the cyclic voltammogram of DA, which was obtained using a bare nanoneedle electrode in HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer solution (pH 7.4). The observed redox behavior is in good accordance with the literature. Figure 4B displays a differential pulse voltammogram of DA at various concentrations of dopamine ($100-1000 \mu M$), in which peak currents are dependent on DA concentration. It was confirmed that the electrochemical signals were not being generated by the tungsten component but, rather, by the MWCNT from the repetitive measurements employing a tungsten tip only. The differential pulse voltammogram of DA shown in Figure 4 of the Supporting Information was recorded using a tungsten tip which was completely insulated except for the very end of the tungsten tip. 29

Figure 1. SEM images of MWCNT nanoneedle electrode. (A) Contact of a single MWCNT with tungsten tip from MWCNT bundle. Inset is a tungsten tip after etching. (B) Attachment of a single MWCNT to tungsten tip after a sufficient voltage is applied. (C) MWCNT image magnified from (B).

Figure 2. Microscope image during the insulation process.

Figure 3. Voltammetric behavior of MWCNT nanoneedle electrode for electrochemical application. (A) Cyclic voltammogram of $\text{Fe(CN)}_6^{3-}$ at a nanoneedle electrode. (B) Differential pulse voltammogram $\text{Fe(CN)}_6^{3-}$ at the nanoneedle electrode.

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No DA oxidation peak was observed; only a minute background current was detected, which was clearly distinguishable from that of the MWCNT-based nanoneedle in Figure 4B. The calibration curve of peak current vs concentration is shown in the inset of Figure 4B, which indicates that peak current is proportional to the DA concentration in a given range.

On the other hand, the oxidation of glutamate by glutamate oxidase (GLOx) produces hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and \(\alpha\)-ketoglutarate. The concentration of glutamate can be determined from the electrochemical oxidation current of \(\text{H}_2\text{O}_2\) on the electrode surface. The reactions taking place at the electrode are as follows:

\[
\text{L-glutamate} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GLOx}} \alpha\text{-ketoglutarate} + \text{H}_2\text{O}_2 + \text{NH}_3
\]

\[
\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2e^-
\]

Figure 5 shows the responses of the MWCNT-based nanoneedle biosensor to glutamate when 0.4 V was applied vs Ag/AgCl. The nanoneedle biosensor linearly responded to glutamate in the range from 100 to 500 \(\mu\text{M}\), which is low enough to detect glutamine secretion inside a synaptic junction.

**CONCLUSIONS**

The nanoneedles were fabricated by attaching a MWCNT to a sharp-etched tungsten tip. The nanoelectrodes produced can serve analytical functions as reliable electrochemical nanosensors detecting electroactive species. Voltammetric analysis of DA was successfully demonstrated, and it suggests that many other redox-active species, such as ascorbic acid, acetylcholine, and cytochrome c\textsuperscript{30} can be detected in volumes of <1 \(\mu\text{L}\) using these nanoneedles without any chemical modification. Moreover, chemical or biological modification of these nanoneedles could lead to the development of a variety of nanobiosensors. The nanoscale enzyme electrode in this study is an example of a biologically modified nanoneedle. Such modified nanoneedles make it possible to determine the concentration of electrochemically inactive biomaterials, as shown for sensing glutamate in this report. The nanosensors based on the nanoneedles show that there may be a number of powerful applications for molecular biology and chemical analysis.

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**SUPPORTING INFORMATION AVAILABLE**

Experimental details, manufacture of CNT nanoneedles, selective insulation of the tungsten tip at nanoneedle, and confirmation of sensitivity of an carbon nanotube as a biosensor. This material is available free of charge via the Internet at http://pubs.acs.org.

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