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Carbon Nanofiber Electrode for Neurochemical Monitoring

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Abstract

The ability to rapidly detect neurotransmitter release has broad implications in the study of a variety of neurodegenerative diseases. Electrochemical detection methods using carbon nanofiber nanoelectrodes integrated into the Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS) offer many important advantages including biocompatibility, selectivity, sensitivity, and rapid adsorption kinetics. Carbon nanofiber nanoelectrodes exhibit greater selectivity and sensitivity in the electrochemical detection of neurotransmitters compared to macroelectrodes and are able to resolve a ternary mixture of dopamine (DA), serotonin (5-HT), and ascorbic acid as well as to detect individual neurotransmitters in concentrations as low as 50 nM for DA and 100 nM for 5-HT using differential pulse voltammetry. Adsorption kinetics studies and isopropyl alcohol treatments modeled on previous studies on carbon fiber microelectrodes were conducted to investigate the analogous properties on carbon nanofiber electrodes using fast-scan cyclic voltammetry with WINCS and showed analogous results in carbon nanofiber electrodes compared with carbon fiber microelectrodes.

Keywords

Biosensor; Neurotransmitter; Carbon nanofiber; Nanoelectrode

Introduction

Functional electrical stimulation (FES) techniques have long been used in the treatment of neurological disorders [1]. Deep brain stimulation (DBS) and similar techniques have been shown to be effective in the treatment of numerous neurological and neurodegenerative disorders such as Parkinson's disease, schizophrenia, epilepsy, and obsessive-compulsive disorder [1–4]. These techniques are thought to modulate the levels of various neurotransmitters, such as dopamine (DA) and serotonin (5-HT), which play key roles in the regulation of the nervous system [5, 6]. Nonetheless, the mechanisms of FES techniques still remain poorly understood. Advances in the field of neurochemical detection may offer insight into these mechanisms by allowing the real-time monitoring [7] of neurochemical events in the brain as DBS and other techniques are applied. Greater understanding of these mechanisms can lead to improvements in the technologies behind functional electrical stimulation techniques [8]. In this endeavor, electrochemical detection methods are often used [9–11] which offer several advantages; for example, the electrodes are fully miniaturizable and implantable in living systems, and the method offers much faster response times, up to real-time monitoring in vivo [12].

A recently developed technology for electrochemical detection involves using carbon nanofiber-based electrodes. Carbon nanofibers are similar in structure to carbon nanotubes consisting of graphene sheets, but with a bamboo-like interior of interlocking stacked cups. Advantages of carbon nanofiber-based electrodes include high selectivity and sensitivity and fast adsorption kinetics [13]. Carbon nanofiber-based systems have also been shown to be highly biocompatible, suitable for long-term implantation in animals [14, 15]. Furthermore, these nanofibers are easily grown as vertically aligned arrays on a silicon substrate [16, 17]. In the present work, the sensitive and selective detection of neurochemical transmitters by the carbon nanofiber nanoelectrode array is demonstrated.

Methods

Carbon Nanofiber Electrode Fabrication

Arrays of vertically aligned carbon nanofibers were fabricated on a 4-in. silicon wafer as previously described [18]. Briefly, a three-by-three array of $200\text{ }\mu\text{m}\times 200\text{ }\mu\text{m}$ sensing pads were defined using photolithography. The electrodes, contact pads, and contact lines were comprised of 200 nm chromium, and 100-nm-diameter nickel catalyst spots were defined using electron beam lithography. Vertically aligned carbon nanofibers were grown from the nickel catalyst in a plasma-enhanced chemical vapor deposition process with $125\text{-sccm}^3/\text{min}$ acetylene and $400\text{-sccm}^3/\text{min}$ ammonia feedstock. Carbon nanofibers were then encapsulated with SiO_2 using chemical vapor deposition and tetraethylorthosilicate feedstock. The entire device surface was polished using chemical mechanical polishing to expose approximately 50–200 nm of the carbon nanofiber tips. Electrodes were examined using a field emission scanning electron microscope (S-4800; Hitachi, Pleasanton, CA) and an atomic force microscope (Multimode; Digital Instruments, Santa Barbara, CA). Figure 1 shows a three-by-three array of carbon nanofiber-containing pads grown on silicon wafer serving as the electrode.

Neurochemical Detection by Differential Pulse Voltammetry

DA, 5-HT, and ascorbic acid (AA) were obtained from Sigma (Sigma-Aldrich, St. Louis, MO), and neurochemical mixtures were made using $10\text{ }\mu\text{M}$ DA and 5-HT and 1 mM AA in a solution of 12 mM Tris buffer (Sigma-Aldrich, St. Louis, MO). Differential pulse voltammograms were taken of the neurochemical mixtures using a carbon nanofiber electrode and also a glassy carbon electrode for comparison.

Wireless Instantaneous Neurotransmitter Concentration Sensor System

Wireless Instantaneous Neurotransmitter Concentration Sensor System (WINCS) is a device developed by Mayo Clinic that is capable of providing real-time electrochemical monitoring of analytes using fast-scan cyclic voltammetry (FSCV) and chronoamperometry. WINCS is fabricated with FDA compliant materials and is sterilizable. It is a lithium-ion battery-powered FSCV-capable potentiostat that communicates to a base station on a laptop computer via Bluetooth. WINCS is able to send and record digital signals at up to 100,000 samples per s, thus making it capable of real-time monitoring of neurotransmitters in vivo [19].

Neurochemical Detection Using WINCS

The experiments were conducted in a custom-built flow cell and using a flow injection system that continuously pumps Tris buffer through the cell. Measurements were made by the WINCS, using FSCV parameters including a pyramidal scanning waveform with a baseline voltage of -0.4 V to a peak of 1.0 V against a Ag/AgCl reference electrode. The scan rate was varied between 200 and 1,000 V/s, with measurements being taken at each interval of 100 V/s. At 1-min intervals, 1 mL of a given neurochemical was injected into the cell, and the resulting faradic current was recorded. In order to observe a change in faradic current over the high capacitive current present at such high scan rates, a background scans of only Tris buffer must be subtracted from the scan with the neurochemical present. The flow cell apparatus was flushed out with distilled water and Tris buffer solution between each measurement. To determine the effect of pretreatment with isopropyl alcohol on the signal-to-noise ratio, a separate experiment was conducted by rinsing the electrodes with distilled water and then soaking in reagent-grade isopropyl alcohol for 2 min between each measurement.

Results and Discussion

Detection of Neurochemical Mixtures

An important advantage of carbon nanofiber-based electrodes over the others is the ability to detect and resolve the presence and concentration of individual neurotransmitters in a mixture. Since DA and 5-HT have similar oxidation potentials, the electrochemical measurement system must be specific enough to resolve the differences in these potentials [20]. In the biological system, DA and 5-HT are often found simultaneously and in the presence of interfering agents such as AA. For example, in the human brain, AA can occur at concentrations up to 1,000 times that of DA and has an oxidation potential which is close enough to both DA and 5-HT to cause a significant interference during electrochemical measurements [21, 22]. Compared to the glassy carbon electrode, a carbon nanofiber-based electrode was able to resolve a mixture of DA and 5-HT in the presence of AA using differential pulse voltammetry (Fig. 2a, b).

Carbon nanofiber-based electrodes also offer superior sensitivity and signal-to-noise ratio compared to macroelectrodes due to the difference in scale between the electrode and the target molecules [23]. A large array of multiple carbon nanofibers also allows signal redundancy for statistical reliability. Using differential pulse voltammetry, carbon nanofiber-based electrodes have been able to detect DA down to a concentration of 50 nM (Fig. 2c) and 5-HT down to a concentration of 100 nM (Fig. 2d).

Adsorption of Dopamine on Carbon Nanofiber Electrode

Adsorption kinetics of dopamine is well understood on carbon fiber microelectrodes [24]. Using FSCV, it was shown that the voltammetric scan rate varied linearly with the resulting peak faradic current, demonstrating that DA exhibits adsorption to the surface of the

electrode. In the carbon fiber microelectrode, it is believed that the positive charge on the protonated amine group of dopamine interacts electrically with oxidized functionalities on the carbon surface, which results in the adsorption phenomenon [25].

Compared to carbon fiber microelectrode, the carbon nanofiber-based electrode offers faster detection because the electrode response time varies as a function of the electrode size. This study aims to characterize the adsorption kinetics of DA on carbon nanofibers in a similar manner as carbon fiber microelectrode. Furthermore, AA contains negatively charged carboxyl functionality and, thus, is predicted to exhibit non-adsorbing kinetics due to the electrical interaction between the negative charge and the oxidized groups on the carbon surface. Therefore, the peak faradic current is predicted to vary linearly with the square root of the voltammetric scan rate. In this study, the adsorption kinetics of DA and AA on carbon nanofibers is investigated using FSCV. The peak current for DA is found to vary linearly with the concentration of DA in the range from 50 to 500 μM (Fig. 3a). Moreover, the peak faradic current for DA is found to vary linearly with the voltammetric scan rate (Fig. 3b), suggesting that the DA adsorbs on the carbon surface of the carbon nanofiber electrode, analogous to the previous characterization on the surface of the carbon fiber microelectrode. The redox peak currents for AA vary linearly with the square root of the voltammetric scan rate (Fig. 3d). This demonstrates that the AA exhibits non-adsorbing kinetics on the carbon surface [24].

Effects of Isopropyl Alcohol Pretreatment on Sensitivity

Isopropyl alcohol has previously been shown to significantly improve the signal-to-noise ratio on carbon fiber microelectrode when used to pretreat the surface before conducting measurements using FSCV [24–27]. To determine the effect of pretreatment with isopropyl alcohol on the signal-to-noise ratio, a separate experiment was conducted. The electrodes were rinsed with distilled water between each measurement and then soaked in reagent-grade isopropyl alcohol for 2 min and rinsed again with distilled water before buffer was introduced into the flow cell apparatus. Pretreatment with isopropyl alcohol seems to increase the signal from 100 μM DA by approximately twofold compared with the untreated version (Fig. 4a). Moreover, a similar level of signal intensity is achieved using a much lower concentration of DA when combined with isopropyl alcohol pretreatment (Fig. 4b).

Conclusion

The detection and monitoring of neurotransmitters have implications in numerous neurological and neurodegenerative disorders such as Parkinson's disease, schizophrenia, epilepsy, and obsessive–compulsive disorder. The multiplexed carbon nanofiber array electrode has been successfully integrated with the WINCS to provide optimal temporal and spatial resolution in neurochemical event detection. Moreover, the ability of carbon nanofiber-based electrodes to simultaneously detect and resolve individual neurotransmitters in a mixture containing interfering agents as well as the increased sensitivity to dilute concentrations of analytes is of significant importance in biological applications. Carbon nanofiber-based electrodes offer fast response times in the detection of electroactive species with well-characterized adsorption kinetics and isopropyl alcohol responsiveness in agreement with well-established results on carbon fiber microelectrode. Therefore, carbon nanofiber-based electrodes show great promise for the selective detection of neurochemical transmitters.

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Fig. 1. SEM image of the carbon nanofiber nanoelectrode array. **a** Three-by-three array of carbon nanofiber electrode pads ($\times 60$ magnification). **b** Close-up of individual sensor pad ($\times 400$ magnification). **c** Vertically aligned individual carbon nanofiber growth shown on a silicon substrate ($\times 5,000$ magnification). **d** Close-up of individual carbon nanofibers ($\times 18,000$ magnification)

Fig. 2.

Comparison of simultaneous detections of neurotransmitters in a ternary mixture. **a** Baseline-corrected differential pulse voltammetry plot of a mixture of 1 mM AA, 10 μ M DA, and 10 μ M 5-HT measured using carbon nanofiber electrode. **b** Baseline-corrected differential pulse voltammetry plot of a mixture of 1 mM AA, 10 μ M DA, and 10 μ M 5-HT measured using glassy carbon electrode. **c** Calibration curve for DA. **d** Calibration curve for 5-HT

Fig. 3.

Adsorption kinetics of dopamine. **a** Linear dependence of signal on a concentration of DA: Plot of DA concentration vs. voltammetric peak faradic current. The linear regression is shown with equation $i_p = 0.275(\text{DA}) + 22.4$ and a correlation coefficient of 0.9924. **b** Plot of voltammetric scan rate vs. peak faradic current in a solution of 500 μM DA in Tris buffer. The linear regression is shown with equation $i_p = 0.211R + 74.3$ and a correlation coefficient of 0.9927. **c** Plot of voltammetric scan rate vs. peak faradic current in a solution of 2,000 μM AA in Tris buffer. **d** The data from **b** plotted using the square root of the voltammetric scan rate vs. peak faradic current instead. The linear regression is shown with equation $i_p = 1.35\sqrt{v} - 10.9$ and a correlation coefficient of 0.9896.

Fig. 4.

Comparison of isopropyl alcohol (IPA) pretreatment with control. Measured peak faradic current is plotted against the voltammetric scan rate for two experiments. **a** The experiment conducted with IPA pretreatment showed approximately twice the signal as the untreated version when detecting 100 μ M of DA. **b** Two experiments are showing similar levels of signal; however, the experiment using IPA pretreatment also had significantly lower concentration of DA