analytical chemistry

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Polyethylenimine Carbon Nanotube Fiber Electrodes for Enhanced Detection of Neurotransmitters

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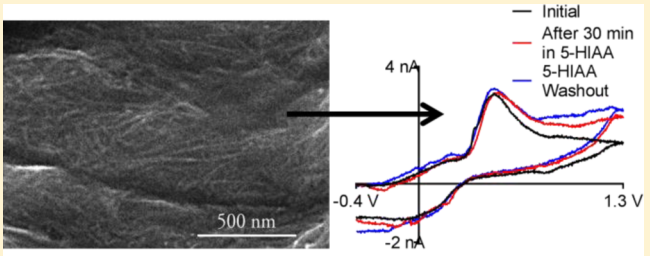
Supporting Information

ABSTRACT: Carbon nanotube (CNT)-based microelectrodes have been investigated as alternatives to carbon-fiber microelectrodes for the detection of neurotransmitters because they are sensitive, exhibit fast electron transfer kinetics, and are more resistant to surface fouling. Wet spinning CNTs into fibers using a coagulating polymer produces a thin, uniform fiber that can be fabricated into an electrode. CNT fibers formed in poly(vinyl alcohol) (PVA) have been used as microelectrodes to detect dopamine, serotonin, and hydrogen peroxide. In this study, we characterize microelectrodes with CNT fibers made in polyethylenimine (PEI), which have much higher conductivity than PVA-CNT fibers. PEI-CNT fibers have lower overpotentials and higher sensitivities than PVA-CNT fiber microelectrodes, with a limit of detection of 5 nM for dopamine. The currents for dopamine were adsorption controlled at PEI-CNT fiber microelectrodes, independent of scan repetition frequency, and stable for over 10 h. PEI-CNT fiber microelectrodes were resistant to surface fouling by serotonin and the metabolite interferant 5-hydroxyindoleacetic acid (5-HIAA). No change in sensitivity was observed for detection of serotonin after 30 flow injection experiments or after 2 h in 5-HIAA for PEI-CNT electrodes. The antifouling properties were maintained in brain slices when serotonin was exogenously applied multiple times or after bathing the slice in 5-HIAA. Thus, PEI-CNT fiber electrodes could be useful for the in vivo monitoring of neurochemicals.



Carbon nanotubes (CNTs) were identified in 1991[1] and have been used extensively to enhance the sensitivity and electron transfer kinetics of electrodes.[2,3] Britto et. al developed the first carbon nanotube paste electrode, which had perfect, Nernstian reversible kinetics for dopamine detection (~30 mV peak separation).[4] The CNT-based electrode displayed faster electron transfer kinetics than typical carbon electrodes because the sp^2 hybridized CNT structure is highly conductive and the ends of CNTs have reactive edge plane sites.[5] CNTs are especially attractive for making smaller electrodes because the high surface-area-to-volume ratio results in a large electroactive surface area for the adsorption of biomolecules. Many different strategies have been developed to modify microelectrode surfaces with CNTs. Dip-coating carbon nanotubes onto carbon-fiber microelectrodes (CFMEs) results in an increase in sensitivity, faster electron transfer kinetics, and a resistance to serotonin fouling, but the CNTs can aggregate on the surface.[6,7] Polymer coatings, such as Nafion or overoxidized polypyrrole, can be used to immobilize CNTs and increase sensitivity for dopamine while repelling anionic interferants such as ascorbic acid.[8−10] The most sensitive CNT-modified CFMEs have aligned CNT forests self-assembled onto the surface, suggesting that CNT alignment is key.[11] However, all of these methods are difficult to fabricate reproducibly, and the electrochemical properties of the carbon fiber core, which vary with different waveforms, can affect the electrochemical properties.[12] Therefore, an electrode material made only from CNTs could avoid these issues.

Fibers made from CNTs would be an ideal microelectrode material because they could be directly fabricated into electrodes in a manner similar to carbon fibers, rather than coating an existing electrode with CNTs. CNT fibers grown via chemical vapor deposition and twisted into yarns have faster electron transfer kinetics than CFMEs and have been used to measure stimulated dopamine release in brain slices.[13] The sensitivity at one type of CNT yarn is independent of scan frequency, giving them enhanced ability to make fast measurements.[14] The Poulin group developed a method of making carbon nanotube fibers through polymer wet spinning.[15] They separated carbon nanotube bundles in an aqueous surfactant solution to overcome van der Waals forces of attraction and aggregation. The suspended nanotubes were then pushed into a streaming solution of poly(vinyl alcohol) (PVA) which displaced the surfactant and formed nanotube ribbons which subsequently collapsed in air into fibers.[15] Wang's group examined these PVA-CNT fibers as electrodes for the detection of NADH, peroxide, and dopamine using hydrodynamic voltammetry and amperometry; however, the

Received: January 23, 2014
Accepted: August 13, 2014
Published: August 13, 2014

   dx.doi.org/10.1021/ac5003273 | *Anal. Chem.* 2014, 86, 8568−8575

concentrations tested were much higher than physiological levels.[16] PVA-CNT fiber electrodes have been used to codetect complex mixtures of dopamine and ascorbic acid,[8] electrocatalytically oxidize NADH,[17,18] detect glucose in enzymatic sensors,[17] and reduce electrode fouling from large concentrations of dopamine.[19]

Other polymer-CNT fibers have been developed but not been tested as microelectrode materials. Polymer-CNT fibers wet-spun with polyethylenimine (PEI), for example, are 100 times more conductive than PVA-CNT fibers because of the physisorption of the amine to the CNT wall.[20] The amine intercalates into bundles of SWCNTs and initiates a charge transfer. In this study, we compare the electrochemical properties of PEI-CNT fiber microelectrodes to PVA-CNT fiber microelectrodes and carbon-fiber microelectrodes. PEI-CNT fiber microelectrodes have lower limits of detection and better electron transfer kinetics than PVA-CNT fibers. Dopamine detection is adsorption controlled, and the signal is stable for 10 h. The PEI-CNT fibers are resistant to fouling by serotonin and 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolism product, which will make them useful for the detection of neurotransmitters in vivo.

METHODS AND MATERIALS

Chemicals and Materials. Dopamine was purchased from Sigma (St. Louis, MO, U.S.A.). A 10 mM stock solution was prepared in 0.1 M perchloric acid and diluted to 1.0 μM daily with phosphate-buffered saline (PBS) (131.5 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4 with the pH adjusted to 7.4) (all from Fisher Scientific, Fair Lawn, New Jersey, U.S.A.). All aqueous solutions were made with deionized water (EMD Millipore, Billerica, MA, U.S.A.). Diethylenetriamine hardener was used as received from Fisher Scientific.

Instrumentation. Fast-scan cyclic voltammetry (FSCV) was performed using a ChemClamp potentiostat (Dagan, Minneapolis, MN, U.S.A.). Data were collected and analyzed with Tarheel CV software (gift of Mark Wightman, UNC, Chapel Hill, NC, U.S.A.) using custom data acquisition hardware previously described.[21] A triangle waveform was applied to the electrode from a holding potential of −0.4 to 1.3 V and back at a scan rate of 400 V/sec and a frequency of 10 Hz, unless otherwise noted. A silver−silver chloride wire was used as the reference electrode. Samples were tested in a flow injection analysis system consisting of a six-port, stainless steel HPLC loop injector mounted on a two-position air actuator (VICI Valco Instruments, Co., Houston, TX, U.S.A.). Buffer and samples were pumped through the flow cell at 2 mL/min using a syringe pump (Harvard Apparatus, Holliston, MA, U.S.A.).

Scanning Electron Microscopy. Scanning electron microscope (SEM) images were collected on a FEI Quanta 650 microscope with a secondary electron detector using an accelerating voltage of 5 kV and a working distance of 5.6 mm.

CNT Fiber Fabrication. Poly(vinyl alcohol) (PVA) carbon nanotube (CNT) fibers were prepared as previously described.[15] HiPCo CNTs (0.4%, high pressure carbon monoxide, Unidym, Sunnyvale, CA) were suspended in 1.2% aqueous solution of sodium dodecylbenzenesulfonic acid (SDBS, Sigma) by sonicating for 60 min in a tissue sonicator. The CNT suspension was pumped through a 30 G syringe needle (flow rate 0.5 mL/min) into a 4% aqueous solution of poly(vinyl alcohol) (PVA) (Aqua Solutions, Deer Park, TX, MW = 124 000−186 000). The PVA solution was revolved using a custom built rotating stage. CNT ribbons were subsequently purified and rinsed in water and then methanol, which washed away the excess polymer. Ribbons collapsed into fibers upon being allowed to dry in air and then were placed in the oven for 1 h at 180 °C.

Polyethylenimine (PEI) CNT fibers were formed as previously described.[20] HiPCo CNTs (0.4%) were suspended in water with SDBS (1.2%) and were pumped into a rotating solution of 40% PEI (branched, MW = 50 000−100 000, MP Biomedicals, LLC, Santa Ana, CA) in methanol. The CNT ribbons were subsequently purified in methanol. CNT fibers were dried in air and then 180 °C for 1 h.

Electrode Construction. Carbon nanotube fiber microelectrodes were made with epoxy insulation.[22] Each channel in a Teflon mold (channels 30−40 μm wide and deep)[22] was filled with Armstrong Resin C7 and 0.8% Armstrong Activator A2 (Ellsworth Adhesives, Germantown, WI, U.S.A.). A single carbon nanotube fiber or carbon fiber was manually inserted into each channel, and the epoxy was allowed to cure for 3 h at 165 °C before the electrode was removed from the mold. Silver epoxy (H20E, equal portions of Parts A and B, Epoxy Technology, Billerica, MA, U.S.A.) was applied with to one end of the epoxied carbon fiber and connected to a gold pin (0.035 in. × 0.249 in., Digikey, Thief River Falls, MN, U.S.A.) to connect to the potentiostat. The silver epoxy was cured for 1 h at 150 °C. CNT fibers were cut at the surface at an angle of 90° to form "disk-like" electrodes. Cylindrical carbon-fiber microelectrodes were made by cutting at 100 μm length to give equivalent surface areas.

Glass insulated cylindrical carbon-fiber microelectrodes (for serotonin fouling experiments) and PEI-CNT fiber microelectrodes (for brain slice experiments) were made by aspirating a single carbon fiber/CNT fiber into a glass capillary (1.2 mm by 0.68 mm, A-M Systems, Inc., Carlsborg, WA, U.S.A.). The capillary was pulled to form two electrodes on a vertical pipet puller (Narishige, model PE-21, Tokyo, Japan), and the fiber cut to length. Glass electrodes were epoxied with Epon 828 resin and phenylenediamine hardener (Miller-Stephenson, Morton Grove, IL, U.S.A.) and heated for 24 h at 150 °C. Glass-insulated PEI-CNT fiber microelectrodes were polished at 45° for brain slice studies.

All CNT fiber microelectrodes were equilibrated by scanning with the applied waveform for 1 h before testing with the exception of electrodes tested using the serotonin waveform (0.2 to 1.0 to −0.1 to 0.2 at 1000 V/sec)[23] or for stability experiments (upper limit = 1.0 V) that were equilibrated with their aforementioned waveforms for 10 min. The limit of detection (LOD) was calculated using a S/N ratio of 3 from 1 μM measurements for serotonin and 100 nM measurements for dopamine. Surface areas were estimated by either integrating the current and multiplying by time or using the background current at 0.25 V. These currents were divided by specific capacitance times scan rate to produce surface areas. Both methods gave similar areas. The specific capacitance value used was 24 $\mu F/cm^2$,[24] a standard capacitance for glassy carbon. Although this specific capacitance might vary by fiber material (the range for carbon is typically 20−40 $\mu F/cm^2$),[24] it allows a rough estimation of surface area.

Brain Slice Experiments. All animal experiments were approved by the Animal Care and Use Committee of the University of Virginia. Male Sprague−Dawley rats (250−350 g, Charles River, Wilmington, MA) were housed in a vivarium and

dx.doi.org/10.1021/ac5003273 | Anal. Chem. 2014, 86, 8568−8575

given food and water ad libitum. Brain slice experiments were performed as previously described.[10] Rats were anesthetized with isoflurane (1 mL/100 g rat weight), beheaded, and the brain removed. A vibratome (LeicaVT1000S, Bannockburn, IL) was used to collect 400 μm slices of the caudate-putamen, which recovered in oxygenated aCSF (95% oxygen, 5% CO_2) for an hour before the experiment. During the experiment, slices were perfused with aCSF maintained at 35−37 °C at a rate of 2 mL/min. The PEI-CNT electrode was inserted 75 μm into the tissue.

For serotonin fouling experiments, 25 μM serotonin was pressure ejected into brain slices from a pulled glass pipet placed 20−30 μm from the working electrode using a Parker Hannifin picospritzer (Picospritzer III, Cleveland, OH). The ejection parameters were 20 psi for 50−100 ms. High concentrations of serotonin are needed in order to detect serotonin at the electrode due to diffusion and rapid uptake. To test for fouling, the same amount of serotonin was exogenously applied every 2 min for 10 min (total of 5 ejections). Then, the effect of 5-HIAA fouling was tested in the same slice. The slice was perfused with 10 μM 5-HIAA in oxygenated aCSF for 30 min, and serotonin was exogenously applied at the end of the 30 min. The 5-HIAA was then washed out with normal aCSF, and serotonin was exogenously applied again. The peak oxidative currents for serotonin before, after 30 min of 5-HIAA perfusion, and after washout were compared. Statistics were performed in GraphPad prism and considered significant at the 95% confidence level.

RESULTS

Synthesis and Characterization of PEI-CNT Fibers. Polyethylenimine (PEI) CNT fibers were constructed by a wet spinning procedure, in a manner similar to that of PVA-CNT fibers.[20] SWCNTs were suspended in water using a charged surfactant, SDBS, and sonication. PEI, similar to other amines, physisorbs to the sidewall of single-wall carbon nanotubes and can facilitate electron transfer by intercalating between adjacent CNT bundles.[20] The conductivity of the fiber is expected to be increased by 2 orders of magnitude when replacing PVA with PEI in polymer-CNT fibers because of the electron donation from the amine group of PEI to SWNT sidewalls.[18]

Scanning electron microscope images show PEI-CNT fibers have diameters of 15 to 25 μm. The diameter is dependent on the flow rate of the syringe pump and the rotation speed of the stage and can be controlled by varying these two parameters. Figure 1A shows the side of a fiber. The surface of the fiber is primarily composed of SWCNTs with distinct regions of PEI that were not fully removed during the rinse. Fewer regions of polymer impurities are observed on the outside of the CNT fiber walls for PEI-CNT fibers than for PVA-CNT fibers.[15] Figure 1B shows an end of a CNT fiber. The CNTs appear to be in thick bundles on the surface and are coated in PEI polymer.

Comparison of PVA-CNT and PEI-CNT Fiber Electrodes. As an initial comparison, the electrochemical detection of 1 μM dopamine was compared at PVA-CNT and PEI-CNT fiber disk microelectrodes. Our lab and the Ewing lab have previously used PVA-CNT fiber microelectrodes to detect dopamine using fast-scan cyclic voltammetry,[19,22] and others have characterized their performance with other analytes and electrochemical techniques.[8,16−18] The potential was scanned from −0.4 to 1.3 V at a scan rate of 400 V/s and a repetition frequency of 10 Hz. The example cyclic voltammograms (CVs),

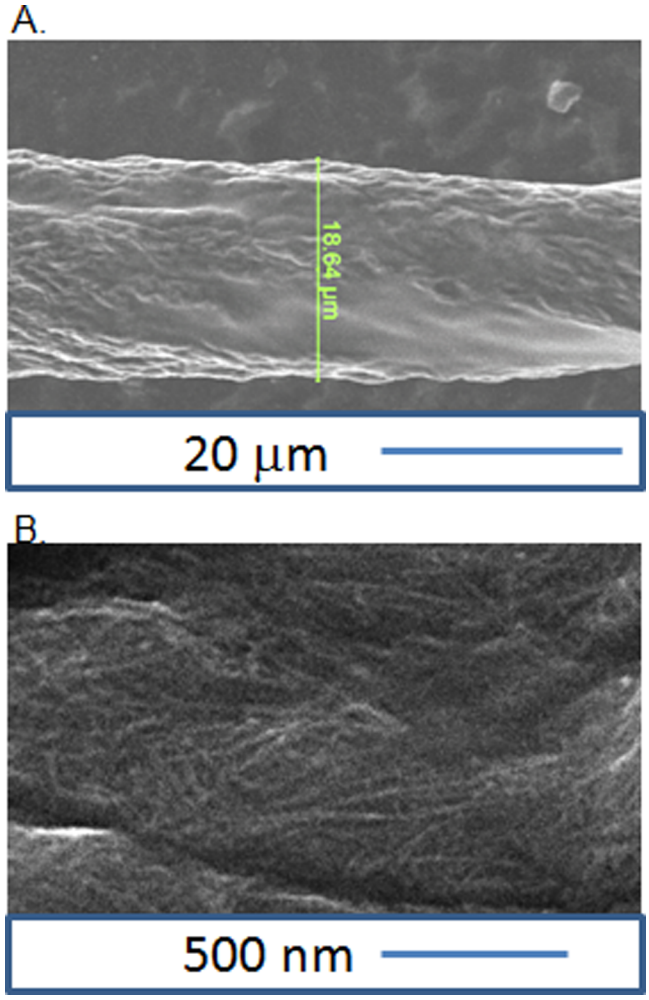


Figure 1. SEM Image of PEI-CNT Fiber. (A) SEM Image of a CNT fiber with darker regions containing more conductive CNTs. (B) Zoomed in SEM image of a CNT fiber end. Thin bundles of CNTs are seen coated in polymer.

Figure 2A, show that the PEI-CNT fiber microelectrode has a higher oxidation current for dopamine as well as a smaller potential separation between the peaks (ΔE_p) than the PVA-CNT microelectrode. The background CVs in Figure 2B show a larger background current at the PEI-CNT fiber microelectrode compared to the PVA-CNT fiber microelectrode, even though the microelectrodes had a similar diameter (about 15 μm) and should have a similar surface area. The larger capacitive current indicates that the PEI-CNT fiber microelectrodes have a larger electroactive surface area or greater surface roughness than PVA-CNT microelectrodes. On average, PEI-CNT fiber microelectrodes have a 6-fold greater oxidation current for 1 μM dopamine compared to PVA-CNT fiber microelectrodes (Figure 2C, n = 6 each, $p < 0.0001$, t-test). Figure 2D shows a significant difference in ΔE_p between the two CNT fibers with the peak separation of PEI-CNT microelectrodes about 300 mV less than PVA-CNT microelectrodes (n = 6 each, $p < 0.0001$, t-test). This suggests that the electron transfer kinetics may be faster at PEI-CNT fiber microelectrodes, and PVA may slow the kinetics. The increased ΔEp could also be caused by differing double-layer capacitances, uncompensated resistance, or ohmic drop. However, because both the electrolyte and the size of the electrodes are similar, ohmic drop is an unlikely cause. Because

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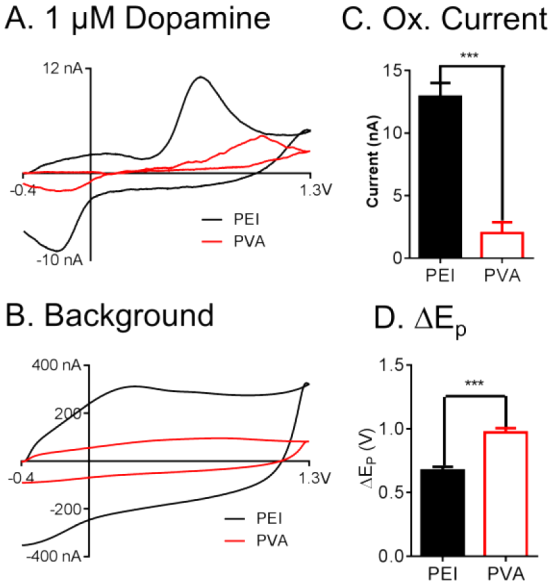

Figure 2. Comparison of PEI-CNT and PVA-CNT fiber microelectrodes. All electrodes were scanned from −0.4 to 1.3 V and back at 400 V/s at 10 Hz. (A) Example cyclic voltammograms of 1 μM dopamine for PEI-CNT (black) and PVA-CNT (red) fiber electrodes of about 15 μm in diameter. (B) Example background charging current for the same electrodes. (C) Average peak oxidative currents (nA) for 1 μM dopamine are significantly different (n = 6 each, $p < 0.0001$, t-test, error bars SEM). (D) The ΔE_p values of the electrodes are significantly different (n = 6 each, $p < 0.0001$, t-test, error bars SEM).

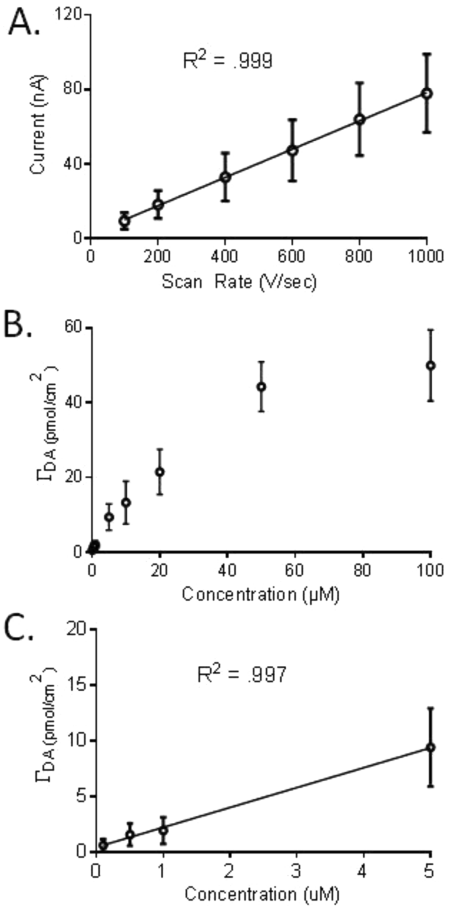

Figure 3. Adsorption Studies (A) Effect of scan rate. A linear relationship was observed between scan rate and peak oxidative current for 1 μM dopamine denoting adsorption control (n = 3, R^2 = 0.999). (B) Concentration Study. Dopamine concentrations were varied from 100 nM to 100 μM (n = 4). (C) Dopamine surface coverage is linear with concentration up to 5 μM (R^2 = 0.997). All error bars are SEM.

the PEI-CNT fibers are reported to be 100-fold more conductive than PVA-CNT fibers, faster charge transfer is expected at PEI-CNT fibers.[20]

In this study, we used untreated CNT fibers, but post treatments have been developed for similar fibers. Acid treatment, oxidizing with polyoxymetalate agents, or heating to high temperatures above 1000 K have been used to remove PVA from PVA-CNT fibers, as well as to oxidize the surface and increase conductivity of the fiber.[8,17,18] However, these procedures are often tedious and lack reproducibility.[18] At high temperatures, the carbon nanotube fibers become more conductive as nongraphitic carbon is either removed or graphitized, but heat treatment must be carried out in inert-atmosphere or under vacuum to avoid excessive oxidation and combustion of the carbon in the fiber.[25] Although heat treatment may further improve PEI-CNT microelectrodes, the FSCV data show that as-fabricated PEI-CNT fibers are suitable for dopamine detection, and heat treatment is not necessary.[20] Thus, untreated PEI-CNT fiber microelectrodes are simple to use and have enhanced performance for dopamine detection compared to untreated PVA-CNT fibers.

Characterization of Dopamine Detection at PEI-CNT Fiber Microelectrodes. To characterize the properties of PEI-CNT fiber microelectrodes, the scan rate was varied from 100 to 1000 V/s. Figure 3A shows that current for 1 μM dopamine increases linearly with respect to scan rate. This indicates that dopamine oxidation at PEI-CNT microelectrodes is an adsorption controlled process, similar to dopamine oxidation at CFMEs, and is likely to be dependent upon oxide groups at the surface of the microelectrode.[26]

PEI-CNT fiber electrodes were used to detect different concentrations from 100 nM to 100 μM dopamine (Figure 3B). The estimated surface area is 3.8×10^{-5} cm^2 for PEI-CNT fibers, which is about 10-fold greater than the geometrical surface area calculated for a disk of 10 μm radius (3×10^{-6} cm^2). The larger area than just a disk was expected, as the end may fray upon cutting, and the surface is nanostructured. Normalizing each electrode to the electrochemically estimated surface area, a plot of surface coverage versus concentration reveals that the surface coverage plateaus about 40 pmol/cm^2, which is about the same order of magnitude as previous studies of carbon fiber microelectrodes.[26] At higher concentrations, there are contributions from both diffusion and adsorption, so the plot will not completely plateau. The surface coverage is linear up to 5 μM dopamine (Figure 3C).

The LOD for PEI-CNT microelectrodes was estimated from the 100 nM dopamine CVs and was 4.7 ± 0.2 nM. PEI and PVA CNT fiber microelectrodes can be easily compared because they are formed from the same carbon source, HiPCO carbon nanotubes, and the different coagulating polymer solution has no effect on the size and diameter of each individual CNT fiber.[15] PVA-CNT fiber microelectrodes have a limit of detection of 53 ± 5 nM (n = 6), which is an order of magnitude higher than PEI-CNT fibers. The LOD for epoxy-insulated, cylindrical CFMEs was found to be 24 nM in our previous work.[22] The surface area for the CFMEs in that study was 4.3×10^{-5} cm^2, similar to surface area of 3.8×10^{-5} cm^2 for

dx.doi.org/10.1021/ac5003273 | Anal. Chem. 2014, 86, 8568−8575

PEI-CNT fibers because cylindrical carbon fiber microelectrodes were used. Thus, the LODs for PEI-CNT fibers are lower than CFMEs even though the areas are similar.

Microelectrodes are typically used in vivo for hours at a time to measure neurotransmission in behavioral or pharmacological experiments.[27−29] Therefore, electrodes must have a stable electrochemical response for several hours. The stability of PEI-CNT fiber microelectrodes was investigated by continuously applying the potential waveform to the microelectrode for an extended period of time and injecting a bolus of dopamine every 2 h. Over a 10 h period, there was no significant change in peak oxidative current at PEI-CNT fiber microelectrodes with a potential waveform of −0.4 to 1.0 V at a scan rate of 400 V/s, as seen in Figure 4A. Both the sensitivity and the stability of PEI-CNT microelectrodes indicate that these microelectrodes are suitable for in vivo experimentation.

To optimize dopamine detection, the effect of increasing the switching potential was tested. Large positive potentials can cause oxidation of the surface carbons and can modify the electrochemical properties of carbon electrodes.[30,31] At CFMEs, overoxidation of the electrode surface occurs past 1.3 V, where carbon is functionalized with electron-rich oxide groups, resulting in an increase in the sensitivity toward dopamine.[30] Higher switching potentials can also break carbon−carbon bonds, which alters surface roughness and increases adsorption sites for dopamine.[30,31] Figure 4B shows that the measured oxidation current at PEI-CNT microelectrodes increases with increased switching potentials, and potentials of 1.2 V and below result in lower currents. Although larger peak currents are observed at switching potentials of 1.4 and 1.5 V, the signal-to-noise ratio decreased at potentials above 1.3 V. At higher switching potentials, water oxidation likely causes an unstable background, and background subtraction errors result in a greater increase in noise than in signal. Thus, the overoxidation behavior of PEI-CNT fibers is similar to carbon fibers, and the optimal switching potential for improved sensitivity is 1.3 V. A 1.3 V switching potential was chosen for most experiments because it provided some surface activation and was away from the potential for water oxidation.

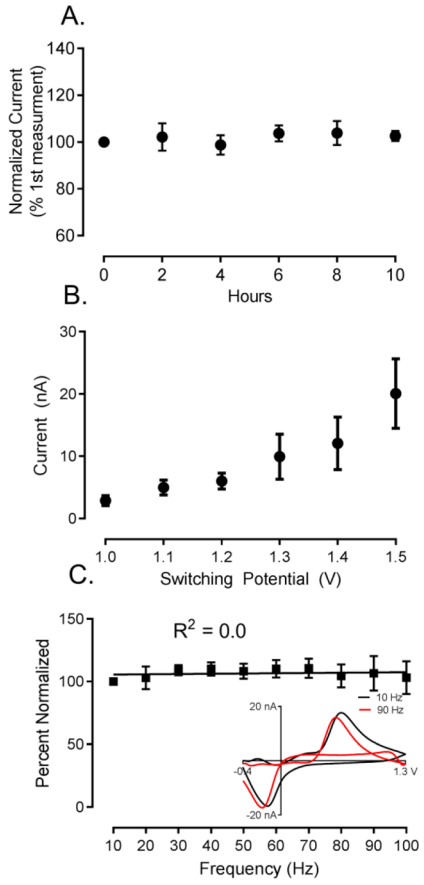

Figure 4. Stability, switching potential, and frequency studies. (A) The stability experiment was performed by testing the response of a PEI-CNT to 1 μM dopamine every 2 h for 10 h. There was no change in sensitivity over 10 h. The electrodes were scanned from −0.4 to 1.0 V at 400 V/sec at 10 Hz. Error bars are SEM (n = 3). (B) The switching potential was varied from 1.0 to 1.5 V, and the response to 1 μM dopamine was measured. Each waveform was applied for 10 min before dopamine was measured. Overoxidation occurs at higher switching potentials, which increases sensitivity toward dopamine. Error bars are SEM (n = 6). (C) The peak oxidative current does not change upon increasing the wave application frequency from 10 to 100 Hz, R^2 = 0.0. Inset: cyclic voltammograms of 1 μM dopamine for a PEI-CNT fiber electrode at 10 and 90 Hz.

The effect of scan repetition frequency was also tested. At PEI-CNT fiber microelectrodes, the oxidation current for dopamine is independent of scan repetition frequency from 10 to 100 Hz (Figure 4C). In contrast, CFMEs lose about 80% of their signal when the repetition frequency is 90 Hz compared to 10 Hz.[26] CNT yarn microelectrodes also have the same frequency-independent current, which was attributed to different rates of dopamine and dopamine o-quinone desorption at the CNT yarn microelectrodes.[14] Future studies could examine adsorption and desorption properties for PEI-CNT fibers and compare them to CNT yarns, but these experiments suggest PEI-CNT fiber microelectrodes could be useful for detection of dopamine with fast repetition rates.

Characterization of Serotonin Detection at PEI-CNT Fiber Microelectrodes. Serotonin is an important electroactive indolamine neurotransmitter in the brain that is important for neurological disorders such as anxiety and depression.[32] The serotonin oxidation peak at CFMEs is typically around 0.6 V with FSCV, and the reduction peak is around 0 V. With the waveform scanning from −0.4 to 1.3 V and back with a scan rate of 400 V/s, PEI-CNT fiber electrodes were more sensitive for serotonin than CFMEs, with a limit of detection of 15 ± 2 nM compared to 24 ± 2 nM (p < 0.05, t-test, n = 3).

With the standard FSCV waveform, oxidative products of serotonin can passivate the CFME surface and block serotonin adsorption sites, resulting in serotonin fouling of the CFME surface.[23] Alternative waveforms exist to reduce serotonin fouling; however, these waveforms do not detect a reduction potential for dopamine and, thus, cannot codetect both dopamine and serotonin.[6] To determine if PEI-CNT microelectrodes are fouled by serotonin in a manner similar to that of CFMEs, 25 consecutive flow injection experiments of serotonin were run using the same waveform we used for dopamine analysis, −0.4 to 1.3 V and back at 400 V/s. In each experiment, the electrode was exposed to a flowing 5 s bolus of 1 μM serotonin, followed by 10 s of flowing buffer, and then a subsequent serotonin injection was performed. Figure 5A compares oxidative currents for serotonin, normalized to the first injection, at CFMEs and PEI-CNT microelectrodes. Over the 25 serotonin injections, the oxidation current of serotonin decreased by 50% at CFMEs, indicating passivation of the electrode surface. In contrast, the oxidation current at PEI-

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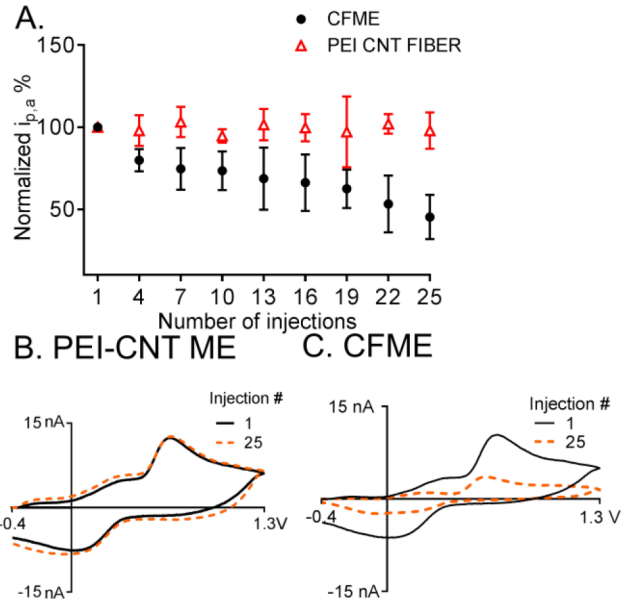

Figure 5. Serotonin fouling in vitro. (A) Serotonin solution (1 μM) was injected for 5 s every 15 s for 25 injections. The electrodes were scanned from −0.4 to 1.3 V at 400 V/sec at 10 Hz. There was no decrease in current for serotonin for PEI CNT fiber electrodes (red) as opposed to the 50% decrease for CFMEs (black). Both CFMEs and PEI CNT fibers were normalized to the first electrode to account for electrode to electrode differences. Error bars are SEM (n = 3). (B) Example cyclic voltammograms of 1 μM serotonin for PEI-CNT fiber microelectrodes (PEI-CNT MEs) for the 1st (solid black) and 25th injection (dashed orange), approximately 6.25 min apart. Serotonin fouling does not occur at the surface of the PEI-CNT fiber electrode. (C) Example cyclic voltammograms of 1 μM serotonin for CFMEs for the 1st and 25th injection, indicating serotonin fouling does occur at the surface of the CFME.

CNT microelectrodes remained at 100% throughout the experiment, demonstrating there is no signal decrease due to serotonin fouling. There is very little change in the serotonin CV between the first and last injection at PEI-CNT microelectrodes (Figure 5B), as opposed to CFMEs where there is a greater than 50% decrease (Figure 5C).

Characterization of 5-HIAA Fouling at PEI-CNT Fiber Microelectrodes. A recent study found that a metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), is the main cause of fouling at the CFME surface during in vivo serotonin detection.[33] 5-HIAA fouling is similar to serotonin fouling because it also blocks serotonin adsorption sites on the surface of the electrode and results in decreased sensitivity. Physiological concentrations of 5-HIAA are approximately 10 times greater than serotonin, and the fouling of 5-HIAA occurs even with the waveforms developed specifically to reduce serotonin fouling.[23] Physical or electrochemical deposition of Nafion coatings can be used to repel the negatively charged 5-HIAA, but the electrode preparation methods are time-consuming, and the thickness of the Nafion layer is difficult to control reproducibly.[33] Because both polymer coatings and electrochemical pretreatments are known to slow the time response of electrodes, it is advantageous to avoid them if possible.[33]

The effect of 5-HIAA fouling was tested at PEI-CNT fiber microelectrodes by comparing the response to 1 μM serotonin before and after the microelectrode was bathed in 10 μM 5-HIAA for 2 h while the serotonin waveform was applied.[23] At the PEI-CNT microelectrode, there was almost no change in oxidation or reduction current for serotonin before and after 5-HIAA immersion (Figure 6A). However, for CFMEs, both the

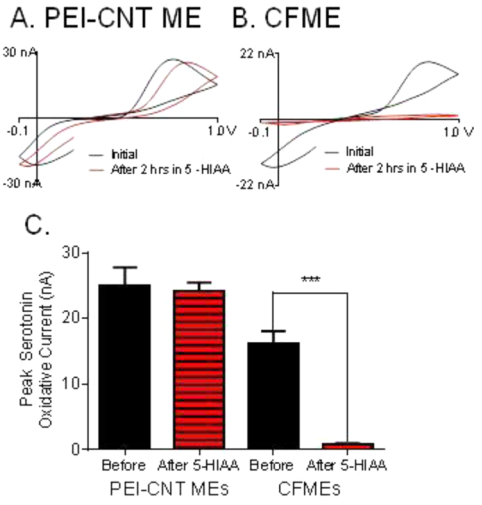

Figure 6. 5-HIAA fouling in vitro. One micromolar serotonin was detected at a PEI-CNT fiber electrode before and 2 h after it was bathed in 10 μM 5-HIAA while the waveform was applied. The applied waveform was the serotonin waveform (0.2 to 1.0 to −0.1 to 0.2 at 1000 V/sec). (A) The CVs are similar before and after 5-HIAA at PEI-CNT microelectrodes. (B) For CFMEs, after 2 h in 10 μM 5-HIAA, the oxidation peak of serotonin is no longer visible. (C) Bar graphs depicting the change in peak oxidative current for serotonin before and after exposure to 10 μM 5-HIAA for 2 h. The decrease in peak oxidative current is significant for CFMEs (n = 4, p < 0.0001, t-test, error bars SEM), while it is not significantly different for PEI-CNT fiber microelectrodes (n = 4, p = 0.6270, t-test, error bars SEM).

oxidation and reduction peaks of serotonin are distorted and barely visible after 2 h in 5-HIAA (Figure 6B). The serotonin oxidation peak is not significantly different for PEI-CNT fiber microelectrodes (n = 4, p = .6270, t-test) after 5-HIAA. For CFMEs, the signal decreases 95% after 5-HIAA (Figure 6C, n = 4, p < 0.0001, t-test).

The mechanism of the resistance to serotonin fouling to PEI-CNT fiber electrodes is not entirely understood. Not all CNT-based electrodes are antifouling for serotonin, and the extent of fouling is dependent on the applied waveform.[34] The Ewing group studied the fouling by large concentrations of dopamine at PVA-CNT fiber microelectrodes and found CNTs offer resistance to the first phase of fouling, the growth of the insulating layer from the polymerization products.[19] Resistance to fouling at CNT ends is often attributed to the higher density of edge plane sites. Indeed, some studies of edge-plane pyrolytic graphite electrodes have found that they have similar antifouling properties to CNT-based electrodes.[35] The CNT ends in an aligned PEI-CNT fiber might contain more edge plane sites that reduce fouling. Moreover, the mechanism of adsorption of serotonin or 5-HIAA products to the surface of the PEI-CNT fiber could differ from that of adsorption to a CFME. Studies of thin carbon films have found that adding oxide groups while maintaining a high sp^2 conjugation also helps with resistance to serotonin fouling.[36] Thus, electrochemical pretreatments such as extending the switching potential, that add oxide groups without significantly reducing the sp^2 hybridization, may also help with antifouling properties of PEI-CNT electrodes. Future studies are needed to tease out

dx.doi.org/10.1021/ac5003273 | Anal. Chem. 2014, 86, 8568−8575

this complex mechanism of the antifouling properties of CNT fibers.

Antifouling Properties Are Maintained in a Brain Slice Environment. To test whether PEI-CNT fiber microelectrodes maintained antifouling properties in tissue, repeated applications of exogenous serotonin were examined in rat brain slices of the caudate putamen. Serotonin (25 μM) was ejected 20−30 μm away from the electrode because large concentrations are needed due to diffusion and rapid uptake. Serotonin was puffed on every 2 min for 10 min (five total ejections). Figure 7A shows cyclic voltammograms of serotonin

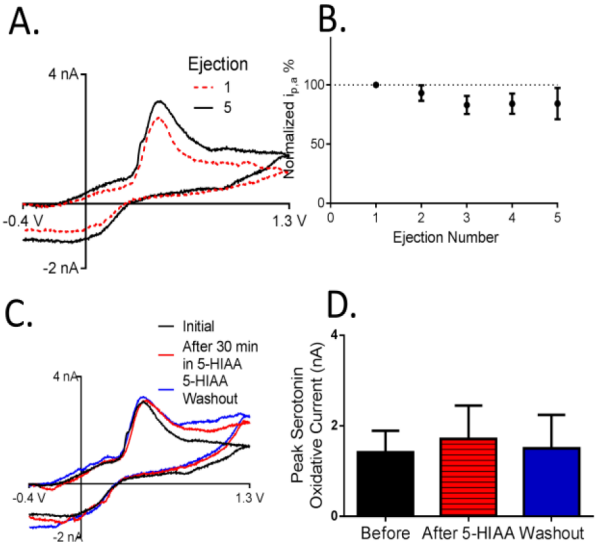

Figure 7. PEI-CNT fiber microelectrodes maintain antifouling properties in brain slices. (A,B) Serotonin (25 μM) was exogenously applied near a PEI-CNT fiber electrode in a brain slices every 2 min for 10 min. (A) The black trace shows the initial serotonin CV, and the dashed red trace shows the fifth serotonin CV. The CVs are similar. (B) Serotonin current does not significantly change with ejection number (repeated measures one-way ANOVA, p = 0.21, n = 6, error bars SEM). (C,D) Exogenous serotonin was applied, the slice bathed in 10 μM 5-HIAA and serotonin applied again after 30 min, and then the 5-HIAA was washed out and exogenous serotonin applied again. (C) Serotonin CVs before (black trace), after 30 min perfusion of 5-HIAA (red trace), and after 5-HIAA washout (blue trace) are similar. (D) The peak serotonin oxidative current did not significantly change in the presence of 5-HIAA (repeated measures one-way ANOVA, p = 0.46, n = 6, error bars SEM).

comparing ejection 1 (black trace) to ejection 5 (red dashed trace) and no change in peak shape or current is shown. On average, current for serotonin is not significantly dependent on ejection number in a brain slice (Figure 7B, repeated measures one-way ANOVA, p = 0.2086, n = 6). Likewise, no decrease in current for exogenous serotonin was observed upon bathing the slice in 10 μM 5-HIAA for 30 min. Figure 7C shows an example cyclic voltammogram for serotonin exogenously applied before the addition of 5-HIAA (black trace), after 30 min of 5-HIAA perfusion (red trace), and after the 5-HIAA was washed out (blue trace). The peak oxidative serotonin current did not significantly change in the presence of 5-HIAA or after 5-HIAA washout (Figure 7D, repeated measures one-way ANOVA, p = 0.4604, n = 6, error bars SEM). This experiment provides proof of principle that the antifouling properties of PEI-CNT fiber microelectrodes are maintained in tissue.

One primary advantage of PEI-CNT fiber microelectrodes is that they do not become passivated by either 5-HT or 5-HIAA, even in tissue. Thus, PEI-CNT fiber microelectrodes could be used in vivo for detection of serotonin without any modification, an advantage over carbon-fiber microelectrodes that require Nafion coating. With PEI-CNT fiber microelectrodes, the dopamine waveform can be used for serotonin detection without fouling, which is not possible at CFMEs.[33] The advantage of using the dopamine waveform is that dopamine and serotonin can both be detected by using their reduction peaks, which is not possible with the serotonin waveform because it does not show the reduction peak of dopamine. Co-detection of a mixture of both 2 μM dopamine and 2 μM serotonin is shown in Figure S1. The two molecules have an oxidation peak at the same potential, but they can be differentiated by their reduction peak. The results are similar to CFMEs that are dip coated in CNTs.[6] Combined with principal components analysis to analyze the shapes,[37] PEI-CNT microelectrodes could be explored in the future for codetection of dopamine and serotonin.

CONCLUSION

PEI-CNT fiber microelectrodes provide attractive properties for neurotransmitter detection: high sensitivity and resistance to fouling. The PEI-CNT fiber microelectrodes have improved electrochemical properties compared to PVA-CNT fiber microelectrodes, as well as lower limits of detection than traditional CFMEs. Dopamine detection is adsorption controlled at PEI-CNT fiber microelectrodes, and the electrodes have a linear range comparable to CFMEs. In contrast to CFMEs, PEI-CNT fibers can be used with high scan repetition frequencies without any loss of dopamine signal. In addition, PEI-CNT fiber microelectrodes are stable over long periods of measurement and are resistant to surface fouling by both serotonin and 5-HIAA in brain slices. Thus, PEI-CNT fiber microelectrodes have increased sensitivity for dopamine and serotonin and resistance to fouling with serotonin that would be beneficial for use as future in vivo neurotransmitter sensors.

ASSOCIATED CONTENT

Supporting Information

In the Supporting Information, we depict the codetection of 2 μM dopamine and 2 μM serotonin using polyethylenimine (PEI) CNT fiber microelectrodes. The two biomolecules are codetected on the basis of the different shape and position of their reduction peaks. This material is available free of charge via the Internet at http://pubs.acs.org/

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by NSF (CHE0645587522) and NIH (R01 MH085159, R21 DA037584, R01 NS076875).

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