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Defect Sites Modulate Fouling Resistance on Carbon-Nanotube Fiber Electrodes

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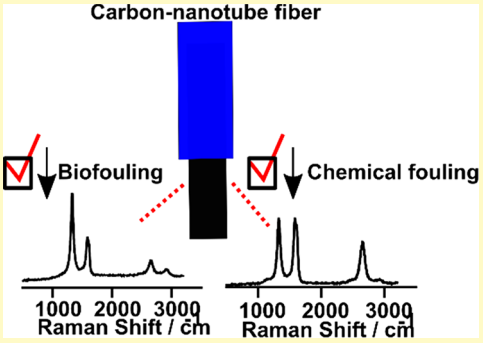
Supporting Information

ABSTRACT: Carbon nanotube (CNT) fiber electrodes have become increasingly popular electrode materials for neurotransmitter detection with fast-scan cyclic voltammetry (FSCV). The unique properties of CNT fiber electrodes like increased electron transfer, sensitivity, waveform application frequency independence, and resistance to fouling make them ideal biological sensors for FSCV. In particular, their resistance to fouling has been observed for several years, but the specific physical properties which aid in fouling resistance have been debated. Here, we investigate the extent to which the presence of defect sites on the surface attenuate both chemical and biological fouling with FSCV. We compared traditional carbon-fiber microelectrodes (CFMEs) to pristine CNTs and functionalized CNTs. CFMEs and functionalized CNTs are highly disordered with a great deal of defect sites on the surface. The pristine CNTs have fewer defects compared to the purposefully functionalized CNTs and CFMEs. All electrode surfaces were characterized by a combination of scanning electron microscopy (SEM), Raman spectroscopy, and energy dispersive spectroscopy (EDS). Chemical fouling was studied using serotonin, a popular neurotransmitter notoriously known for electrode fouling. To assess biological fouling, electrodes were implanted in brain tissue for 2 h. Defect sites on the carbon were shown to resist biofouling compared to pristine CNTs but were detrimental for serotonin detection. Overall, we provide insight into the extent to which the electrode surface dictates fouling resistance with FSCV. This work provides evidence that careful considerations of the surface of the CNT material are needed when designing sensors for fouling resistance.



KEYWORDS: *carbon nanotubes, carbon fiber, electrochemistry, serotonin, biofouling, fast-scan cyclic voltammetry*

Fast-scan cyclic voltammetry (FSCV) is a powerful electroanalytical tool most often used for millisecond detection of neurotransmission in the brain.[1−3] Carbon-fiber microelectrodes (CFME) have been the gold standard electrode for the past three decades;[3−6] however, carbon nanomaterials with FSCV detection have become increasingly popular recently.[7−11] Carbon nanotubes (CNTs) have been attached to the surface of carbon,[7,7,8,12−14] grown directly on metal wires,[11] and most recently spun into CNT fibers.[10,15−17] CNTs exhibit interesting properties with FSCV including increased electron transfer, sensitivity, application frequency independence, and resistance to both chemical and biological fouling.[9,11,15,18−20] Not all carbon nanomaterials resist fouling, and it has been hypothesized that specific surface properties and even waveform parameters are important for fouling resistance.[21] Here, we investigate the extent to which defect sites on the carbon modulate the resistance to both chemical and biological fouling with FSCV.

Resistance to chemical and biological fouling has been observed for CNT-based materials, but the specific physical properties which result in fouling resistance have been debated.[12,19,21−26,26−28] Similarly, differences in the mechanisms of chemical versus biological fouling resistance with CNTs has not been well investigated. Banks and Compton proposed NADH adsorption occurs at the edge plane sites on CNTs, and these sites are unsusceptible to electrode passivation. However, these studies were conducted on CNT-modified basal plane pyrolytic graphite which cannot totally eliminate effects from the graphite.[13] Zhang et al. demonstrated reduced ascorbate fouling by immobilizing CNTs on a glassy carbon electrode.[23] Likewise, Swamy and Venton observed serotonin fouling resistance with CFMEs modified with CNTs with FSCV detection. Despite these findings, producing a uniform layer of CNTs on a glassy carbon or carbon-fiber surface is difficult, which ultimately can result in interference from the underlying material.[7] Conversely, CNTs which were grown on quartz wafers and electrically connected to a gold band were shown to not resist fouling as well as polycrystalline boron-doped diamond.[21] Similarly, CNTs have been grown directly on carbon-fiber microelectrodes and have shown fouling resistance to both ascorbate and biofouling.[24] Eliminating the need to modify

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surfaces with CNTs could provide a means to more accurately assess antifouling without interference from other materials.

More recently, CNT fibers have been used as a novel electrode material with cyclic voltammetry, FSCV, and amperometry.[10,18,22] CNT fiber electrodes eliminate the need to modify existing electrode surfaces, which ultimately allows for minimal interference from other materials. This is particularly important when studying specific electrochemical processes like electropolymerization of analytes onto the surface. A few studies have observed fouling resistance for both serotonin[19] and high concentrations of dopamine on CNT fiber electrodes.[22] Additionally, O_2 plasma-treated CNT fiber electrodes were shown to resist biofouling compared to pristine CNT fiber electrodes.[18] Despite these findings, to the best of our knowledge, a systematic investigation into the specific structural properties which modulate both chemical and biogenerated fouling behavior at CNT fiber electrodes with FSCV has not been explored.

Here, we show that increased defect sites are responsible for attenuating or propagating the effects of biological and chemical fouling, respectively. Carbon nanotube materials are known to resist fouling, particularly to serotonin (5-HT) polymerization with cyclic voltammetry (CV) and FSCV.[7,19,21] To investigate the antifouling of CNT materials for serotonin, we chose to compare the extent to which serotonin fouls on a "pristine" CNT fiber (denoted CNT-P) electrode to that on a purposefully functionalized CNT electrode (denoted CNT-F) by O_2 plasma. O_2 plasma functionalization allows the introduction of oxygen based functional groups such as hydroxyl, carbonyl, and carboxyl groups on the CNT surface.[29] One immediate result of this type of functionalization is the increase in wettability of the electrode surface, which can also be accomplished through wet chemical treatments with acids and oxidizing agents[30] and electrochemical methods.[31] Unlike other methods, plasma functionalization is fast, clean, and has been gaining popularity as plasma technology has evolved from low pressure, chamber methods to atmospheric pressure dielectric barrier discharge.[32−34] A comparison of pristine (not purposefully functionalized) to functionalized CNT fiber electrodes provides insight into the extent to which the defect sites modulate antifouling properties of CNTs. To assess biological fouling, electrodes were implanted in brain tissue for 2 h similar to previous reports.[18] Each electrode material surface was characterized using a combination of scanning electron microscopy (SEM), Raman spectroscopy, and energy dispersive spectroscopy (EDS). Overall, we show that defect sites are important for modulating the effects of fouling on carbon surfaces, and careful considerations of the surface are needed when designing sensors to resist fouling.

EXPERIMENTAL SECTION

Reagents. All reagents were purchased from Fisher Scientific (USA) unless otherwise noted. Dopamine (DA) and serotonin (5-HT) were dissolved in 0.1 M HCl to make 10 mM stock solutions and were stored at 4 °C. Stock solutions were diluted daily in Tris buffer for testing. The Tris buffer consists of 15 mM Tris (hydroxymethyl) aminomethane, 1.25 mM NaH_2PO_4, 2.0 mM Na_2SO_4, 3.25 mM KCl, 140 mM NaCl, 1.2 mM $CaCl_2$ dehydrate, and 1.2 mM $MgCl_2$ hexahydrate at pH 7.4. All aqueous solutions were made with deionized water (Milli-Q, Millipore, Billerica MA).

Fast-Scan Cyclic Voltammetry Experiments. Fast-scan cyclic voltammograms were collected using the WaveNeuro with a 5 MΩ headstage (Pine Instruments, Durham NC) with HDCV software (UNC-Chapel Hill, Mark Wightman) and a National Instruments PC1e-6363 computer interface board (Austin, TX). The electrode was scanned from −0.4 to 1.3 V (vs Ag/AgCl) and back with a 400 V/s scan rate and a repetition rate of 10 Hz. For electrochemical pretreatment of CNT-pristine electrodes, the electrode was scanned from −0.8 to 1.8 V and back at 1000 V/s and a frequency of 10 Hz for 10 min. The electrode was then equilibrated at the traditional waveform for 10 min following electrochemical pretreatment. All data were background subtracted to remove non-Faradaic currents. Electrodes were calibrated using flow injection analysis as previously reported[35] with a flow rate of 1 mL/min using a Fusion 200 Two-Channel Chemyx Syringe pump (Stafford, TX).

Carbon-Fiber Microelectrode Fabrication. Cylindrical carbon-fiber microelectrodes were fabricated from 7-μm T-650 carbon fibers (Gift from Mitsubishi Chemical Carbon Fiber and Composites Inc., Sacramento CA). Carbon fibers were vacuum aspirated into a glass capillary with dimensions of 1.2 × 0.68 mm (A&M Systems, Sequim WA). Glass capillaries were pulled in two using a vertical Narishige PE-22 Electrode Puller (Tokyo, Japan). Extended carbon fibers were cut 50 μm from the glass seal using a scalpel under a microscope (Fisher Education). Electrodes were soaked in isopropyl alcohol for at least 10 min prior to use and backfilled with 1 M KCl.

Carbon-Nanotube Fiber Electrode Fabrication. Carbon nanotube fiber electrodes were fabricated similar to previous reports.[9,18] In summary, glass capillaries (1.2 × 0.68 mm) were pulled in two using the Narishige puller. Pulled glass capillaries were placed under a microscope, and the tip was cut off using a scalpel to reveal an approximately 50-μm diameter opening. A 1−2 cm strand of 15-μm in diameter CNT fiber was vacuum aspirated into the 50-μm opening. Electrodes were sealed with Epon Resin 828 (Miller-Stephenson, Danbury, CT) with 14% (w/w) 1,3-phenylenediamine hardener (Sigma-Aldrich). Electrodes were cured in an oven set to 150 °C overnight. Extended CNT electrodes were cut at the glass seal and polished at a 45° angle on a fine diamond abrasive plate (Sutter Instruments model BV-10, Novato, CA) to create a disk electrode. "CNT-P" indicates "pristine" CNT fibers without purposeful functionalization, and "CNT-F" indicates functionalized CNT fibers by O_2 plasma. See the Supporting Information for details on how the CNT fibers were synthesized and functionalized.

Surface Characterization Experiments. Scanning electron micrographs (SEM) and elemental characterization were done on a FEI XL30 SEM coupled to an EDAX detector for energy dispersive spectroscopy (EDS). Imaging was collected at an accelerating voltage of 5.00 kV, 6−10 mm away. Raman spectra of the sides of the fibers were collected using a Renishaw InVia Raman microscope (Guocestershire, UK) excited by a 633 nm Ar-ion laser at 10% power.

Tissue Fouling Experiments. All animal work was approved by the Institutional Animal Care and Use Committee at the University of Cincinnati. Female C57BL/6 mice between the ages of 6 and 8 weeks old (Jackson Laboratories) were housed in a vivarium and given food and water *ab libitum*. Mice were anesthetized with isoflurane (Henry Shrein, Melville, NY, USA) and euthanized by decapitation on the day of the experiment. The brain was removed and placed in ice-cold oxygenated (95% O_2 and 5% CO_2) DPBS for approximately 2 min. To slice, the brain was mounted onto the stage using glue, and 400-μm thick coronal slices of the caudate putamen were prepared using a Leica VT1000 S vibratome (Chicago, IL, USA). Slices were kept in oxygenated DPBS maintained at 37 °C. For the experiment, electrodes were inserted into the brain slice tissue, without electrochemical cycling, for 2 h. This method of biofouling has been used previously with brain tissue homogenates.[18] Electrodes were pretested and post-tested with 1 μM dopamine. Post-test current was normalized to pretest current, and the percent change was estimated.

Statistics. All statistics were performed in GraphPad Prism 8 (GraphPad Software Inc., La Jolla CA). Statistical p values were considered significant at the 95% confidence level ($p < 0.05$). Values reported are mean ± standard error of the mean (SEM) with n representative of the number of electrodes.

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RESULTS AND DISCUSSION

We investigate the extent to which defect sites on carbon-fiber and CNT fiber electrodes facilitate or resist fouling. The waveform used for all experiments scanned from a −0.4 V holding potential to 1.3 V switching potential and back at a rate of 400 V/s and frequency of 10 Hz. This waveform is the traditional waveform used to detect dopamine (DA), and holding at negative potentials has been shown to facilitate serotonin (5-HT) polymerization at the electrode.[7,36] Surface characterization using a combination of SEM, Raman, and EDS confirmed the differences between these electrode materials.

Serotonin Fouling at Carbon Materials. Serotonin is an important bioactive analyte and is a therapeutic target in several psychiatric and immune-related disorders.[37−40] FSCV has been used extensively to study 5-HT due to its rapid signaling mode in the brain.[41−43] CFME is the normal working electrode used with FSCV; however, serotonin polymerization products stick or "foul" to the surface of this electrode, which results in a rapid decrease in sensitivity (Figure 1A,B).[36] A

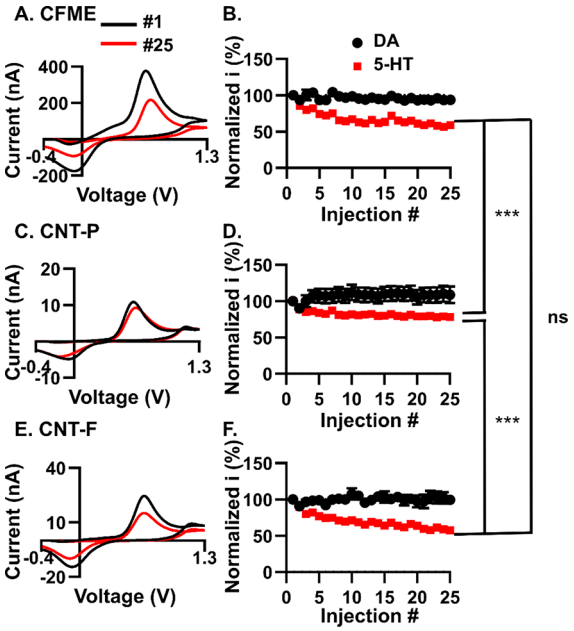

Figure 1. Facilitatation of serotonin fouling by defect sites on the carbon. Example CVs for the first injection and the last injection of 5 μM 5-HT (A,C,E). Serotonin (red rectangle) current decreased on average by 41 ± 1% by the 25th injection for CFME (B, n = 4), 22 ± 2.3% for CNT-P (D, n = 14), and 43 ± 4% for CNT-F (F, n = 11). The decrease in 5-HT current was significantly different between CFME and CNT-P and also between CNT-F and CNT-P (unpaired t test of decrease at 25th injection, $p < 0.001$); however, there was no significant difference between CFME and CNT-F (unpaired t test, $p > 0.05$). Dopamine (5 μM, black circle) does not foul at low concentrations and was used as a control.

novel waveform was developed to control the degree of fouling for serotonin; however, this waveform is limited in its ability to codetect with other important neurotransmitters like dopamine.[36,41] Carbon nanotube materials have been explored in recent years as an antifouling material for serotonin detection.[7,19] Despite their usage, the cause of their antifouling properties is not well understood.

We compared the extent to which serotonin fouling occurs on CFME, CNT-P, and CNT-F electrodes (Figure 1). In these experiments, a 5 μM bolus of serotonin was delivered to the electrode repeatedly for 25 consecutive injections, similar to previous reports.[7,19] Dopamine can polymerize and foul the electrode surface; however, dopamine fouling is believed to be concentration dependent.[22] At low micromolar concentrations and on the time scale of a few minutes, fouling is not evident at carbon-fiber microelectrodes with FSCV. Therefore, 5 μM dopamine was used as a stable analyte for comparison to serotonin (Figure 1). Current was normalized to the first injection. Deviation from 100% indicates the signal is changing over time. On average, a loss of 41 ± 1% in signal by the 25th injection was observed at a CFME (Figure 1A,B, n = 4). This result is comparable to similar experiments at a CFME.[7,19] Pristine CNTs were more resistant to 5-HT fouling (Figure 1C,D, n = 14), with only a 22 ± 2.3% loss in signal. Although these materials do not completely eliminate sensitivity loss due to fouling, they show a marked improvement over the traditional CFME. To test whether the polishing procedure disrupted the surface enough to result in the observed fouling, a subset of CNT-P fiber electrodes was left unpolished and tested for serotonin fouling (Figure S1, n = 6). On average, the unpolished CNT-P electrodes resulted in a 21 ± 4.5% loss in serotonin current after the 25th injection. This loss in current was not significantly different than for the polished CNT-P electrodes (unpaired t test, $p > 0.05$). Likewise, CNT-F electrodes, which have a higher degree of defect sites, showed a similar signal loss compared to CFME with a 43 ± 4% decrease in signal (Figure 1E,F, n = 11). The loss in signal due to fouling was not significantly different between the CFME and CNT-F electrodes (unpaired t test, $p > 0.05$), which suggests that 5-HT polymerization products interact similarly at these materials and that an increase in defect sites is important for passivation at the electrode. The loss of signal was significantly different between CNT-P and CFME and between CNT-P and CNT-F (unpaired t test, $p < 0.001$). A reduction in defects or functionality on the surface helps restore the antifouling properties that are often attributed to CNT materials. It is unclear specifically how functional groups facilitate chemical fouling, but it could be due to a combination of electrostatic interaction and hydrogen bonding between the polymerization products and the oxide groups on the surface of the carbon. This provides evidence that when designing new electrode materials for serotonin detection with FSCV, care must be taken to avoid materials which are disordered. To confirm this finding, surface characterization of these three electrode materials was assessed.

Surface Characterization. Scanning electron microscopy (SEM) was used to compare qualitatively the surface of the electrodes used in the analysis. Figure 2 shows SEM images of CFME (Figure 2A), CNT-P (Figure 2B), and CNT-F (Figure 2C) fibers prior to serotonin fouling. SEM images were taken of five to seven electrodes per group, and Figure 2 shows examples for each condition, which best represented the overall findings. Topographical differences are evident between the traditional CFME and the CNT fibers. CFMEs are smoother than CNT fibers and do not exhibit the tangling bundles of individual fibers. Yang et al. previously compared the surface topology of these materials using laser scanning confocal microscopy, and the mean roughness depth was significantly greater for CNT electrodes compared to CFME.[9] SEM images were taken of a separate set of electrodes after

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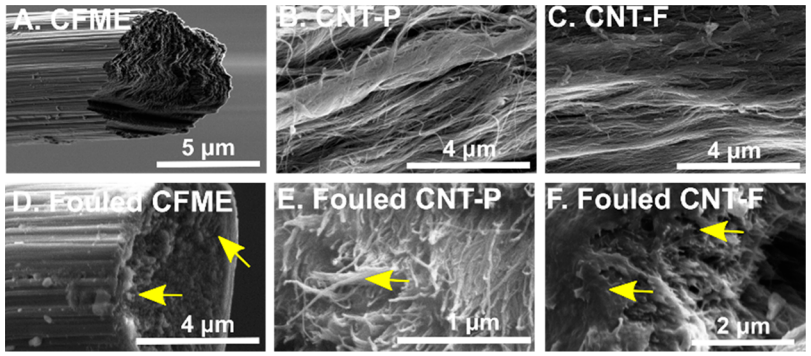

Figure 2. Scanning electron microscopy (SEM) images of carbon materials. Scale bar shown on each figure. SEM image of (A) CFME, (B) CNT-P, and (C) CNT-F fibers untreated. Polymer coating is more evident on (D) CFME and (F) CNT-F electrodes after fouling with 5 μM 5-HT than on (E) CNT-P. Yellow arrow indicates presence of polymer.

serotonin electropolymerization on the surface (Figure 2D–F). A hazy polymer film is evident on a large area of both the CFME (Figure 2D) and CNT-F electrode (Figure 2F); however, minimal polymer was observed on the CNT-P after electropolymerization (Figure 2E). Despite these subtle observations, a more detailed surface characterization was needed to investigate these electrode materials. Additionally, evidence of functionalization is impossible to ascertain using SEM imaging, so Raman spectroscopy was used to compare the structural characteristics of the electrode surface in the following section.

Raman spectroscopy is frequently used to analyze the degree of defect sites present on carbon-based materials.[44−46] Specifically, the ratio of surface defects (D band, ~1350 cm^{-1}) to graphitic carbon (G band, ~1590 cm^{-1}) is used to evaluate the amount of sp^3 and sp^2 hybridized carbon. The larger the D/G ratio, the more "defective" or increase in sp^3 hybridized carbon on the surface. Carbon fibers are known to have high D/G ratios due to many sp^3 sites on the surface. Spectral traces for CFME, CNT-P, and CNT-F electrodes differed in the average D/G ratio (Figure 3A–C, Table 1). The D/G ratio for CFME and CNT-F was significantly higher than for CNT-P (one-way ANOVA, Bonferroni post-test, $p < 0.001$, $n = 6–7$), supporting that the functionalized CNT fibers have more defect sites than the pristine CNTs. The D/G ratio was not significantly different between CFME and CNT-F (one-way Anova, Bonferroni post-test, $p > 0.05$, $n = 6–7$), which suggests that the degree of defect sites between these two electrode materials is similar. The CNT-P electrodes have a higher degree of sp^2 hybridization as compared to the CFME and CNT-F even though the CNTs employed on the CNT-P assembly still have considerable amount of sp^3 sites. We attribute this to the few-walled nature of CNTs, which typically have higher sp^3 sites compared to single-walled CNTs. CNT-P fiber electrodes that were not polished were also analyzed using Raman spectroscopy to validate that our polishing procedure does not significantly disrupt the surface (Figure S2). No significant changes in D/G ratios were observed on polished vs unpolished CNT-P electrodes, confirming that the polishing procedure was not significantly altering the surface. As a side note, a peak at 2800 cm^{-1} (G' peak) was observed for both CNT fibers. This peak represents one or more layers of graphene or graphite, where the frequency and number of peaks are a result of both curvature-induced strain and quantum confinement of their vibrational and electronic structures.[47−49] The G' band has been used for differentiating single and double layer graphene and for investigating the electronic structure of SWCNTs.[50] However, a direct correlation to the degree of disorder is not easily interpretable.

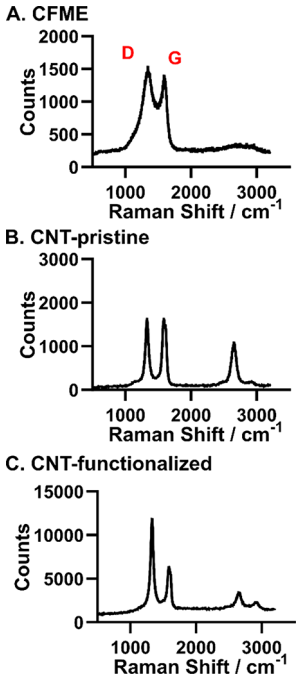

Figure 3. Example Raman spectra of (A) CFME, (B) CNT-P, and (C) CNT-F electrodes. The D (disorder peak, 1350 cm^{-1}) and G (graphite peak, 1580–1590 cm^{-1}) peaks are labeled in red in A. See Table 1 for average D/G ratios.

Table 1. Surface Properties of CFME, CNT-P, and CNT-F Microelectrodes

	Raman D/G ratio	C %	N %	O %
	(n = 6–7)		(n = 5–7)	
CFME	1.9 ± 0.05****[a]	96 ± 0.5	2.2 ± 0.5	2.5 ± 0.9
CNT-P	1.1 ± 0.06	98 ± 1		2.4 ± 0.9
CNT-F	1.7 ± 0.1***[b]	95 ± 0.8	0.12 ± 0.1	5 ± 0.8

[a]Significant difference between the D/G ratio of CFME and CNT-P (one-way ANOVA Bonferroni post-test, $p < 0.0001$). [b]Significant difference between CNT-F and CNT-P (one-way ANOVA Bonferroni post-test, $p < 0.001$).

Energy dispersive spectroscopy (EDS) was used to analyze the elemental components on the electrode surface (Table 1).

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The materials which have the least amount of carbon, but an increase in other elemental components such as oxygen or nitrogen, provide evidence of functional groups on the surface. On average, CNT-P electrodes had a higher percentage of carbon than both the CFME and CNT-F. CNT-P electrodes had a minimal amount of oxygen on the surface, which suggests, along with the Raman data, that these materials are not completely pristine. This could explain why an approximately 20% loss in signal after repeated injections is still evident (Figure 1D). Both CFME and CNT-F had similar carbon content, with approximately 5% of their surface occupied by either oxygen or nitrogen (Table 1). The oxygen content was highest on the CNT-F electrodes with limited nitrogen content, whereas the nitrogen and oxygen contents were evenly distributed on the CFME. These data support that these materials are functionalized. Additionally, the type of functionalization, whether nitrogen- or oxygen-containing groups, does not appear to affect the degree of fouling observed on the electrode surface. Both CFME and CNT-F resulted in a similar loss in signal after electropolymerization, despite their differences in elemental composition.

Electrochemical Pretreatment Increases Fouling at CNT-P Electrodes. Electrochemical pretreatment has been shown to functionalize the surface of carbon with oxygen-containing functional groups.[51,52] To further support the claim that functionalization reduces fouling resistance at carbon nanomaterials, we tested the extent to which electrochemical pretreatment reverses antifouling properties of CNT-P electrodes. CNT-P electrodes were electrochemically pretreated with an extended waveform at high scan rates to rapidly etch the carbon surface for 10 min (−0.8 to 1.8 V at a scan rate of 1000 V/s, 10 Hz). This pretreatment time is not long enough to significantly reduce the electrode size, so decreases in current are not attributed to the loss in electrode area.[53] The electrode was then equilibrated at the traditional waveform for 10 min, and 5 μM 5-HT was repeatedly injected at the electrode as before. CNT-P electrodes that had been electrochemically preconditioned resulted in a 51 ± 10% loss in signal, which is similar to the CFME and CNT-F electrodes (Figure 4B). Changes in the shape of the background current after electrochemical pretreatment provide additional evidence of oxygen functionalization (Figure 4A).[51,52] This provides evidence that carbon nanomaterials must have higher sp^2 hybridization in order to maintain resistance to chemical fouling.

Functionalization Affects Biofouling. Surface fouling due to adsorption of proteins in tissue can also reduce sensitivity to electrochemical recording in vivo.[54] Each electrode type (CFME, CNT-P, CNT-F) was placed in brain tissue for 2 h without waveform application (similar to previous reports[18,55]) to determine the extent to which defect sites modulate biofouling. Electrodes were tested with 1 μM dopamine prior to and after tissue exposure, and the percent ratio of post-test current to pretest current was plotted as a function of electrode material (Figure 5). On average, a 31 ± 15% reduction in current was detected for CFME, whereas a 76 ± 4.6% and 42 ± 7.2% reduction in current was detected for CNT-P and CNT-F electrodes respectively (n = 5−6). The signal decrease after biofouling for both CFME and CNT-F was not as large as the CNT-P electrodes (one-way ANOVA with Bonferroni post-test, $p < 0.05$). Previously, Yang et al. showed that O_2 plasma etched CNT fiber electrodes were more resistant to biofouling than unmodified CNT fiber

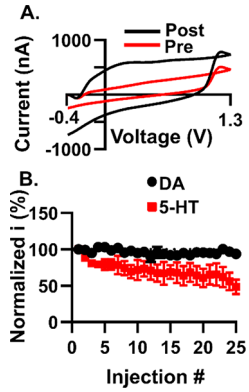

Figure 4. Decrease in resistance to fouling via electrochemical pretreatment of CNT-P electrodes. CNT-P electrodes were pretreated with an extended waveform to induce oxygen-containing functional groups on the surface (−0.8 to 1.8 V and back at 1000 V/s, 10 Hz) for 10 min prior to repeated injection experiments at the traditional waveform (−0.4 to 1.3 V and back at 400 V/s). (A) Background current increases after electrochemical pretreatment and evidence for oxygen-containing groups was observed. (B) Current was normalized to the first injection for repeated injections of both 5 μM dopamine and 5 μM 5-HT. On average, a 51 ± 10% decrease in signal was observed by the 25th injection, indicating passivation of polymer products on the electrode (n = 3).

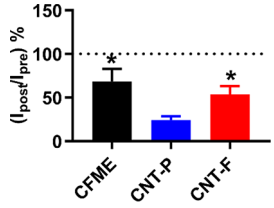

Figure 5. Resistance to biofouling aided by defect sites. CFME, CNT-P, and CNT-F electrodes were pretested with 1 μM dopamine prior to a 2-h implantation in brain tissue without cycling. All electrodes were post-tested with 1 μM dopamine to analyze the loss of sensitivity after tissue exposure. The ratio of the post- to pretest current was plotted as a percentage for each electrode. On average, CNT-P electrodes had the greatest loss in signal ,and it was significantly lower than both the CFME and CNT-F (one-way ANOVA, Bonferroni post-test, $p < 0.05$, n = 5−6). Asterisks indicate a significant difference when compared to CNT-P electrodes.

electrodes and CNT fiber electrodes treated with an antistatic gun.[18] This observation was attributed to the hydrophilic nature of the surface, which promotes repulsion of hydrophobic proteins. Similar results are reported here which suggest that although defect sites are detrimental for chemical fouling, they are useful for minimizing biofouling in tissue. Therefore, differences observed for fouling resistance in the literature may be in part due to (1) the degree of functionalization of the carbon nanomaterials, (2) surface roughness, and (3) the type of fouling being investigated.

SUMMARY AND CONCLUSIONS

Carbon nanomaterials offer excellent properties for biologically targeted sensors. The resistance to fouling is particularly important for doing long-term recording. Here, we have provided evidence that the degree of disorder and functionality on the CNT surface is what modulates the antifouling properties. Functionalization of a pristine CNT surface resulted in 5-HT polymerization products sticking to the

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electrode, similar to CFME. Nitrogen vs oxygen functionalization does not appear to affect the extent to which the surface will foul via 5-HT. However, functionalization can be useful when aiming to minimize biofouling. These results indicate that a careful consideration of the surface characteristics is necessary when optimizing a sensor to resist fouling. Carbon-based sensors designed for biofouling reduction should be functionalized with hydrophilic groups, whereas carbon-based sensors which are designed to reduce chemical fouling should be pristine, or relatively unfunctionalized. Additionally, we provide evidence that when aiming to sense serotonin in vivo, careful consideration in the electrode surface is needed, and a CNT electrode alone may not be optimal. A combinatorial approach is likely best to detect serotonin in vivo with FSCV, including either a CFME or CNT-F electrode for reduced biofouling, combined with the "serotonin waveform," which was previously developed to reduce serotonin fouling.[41] Understanding how disorder on carbon surfaces affects sensor resistance to fouling will hopefully eliminate "trial-and-error" type experiments when designing new sensors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.9b00161.

Supplemental methods describing carbon nanotube yarn synthesis and functionalization, serotonin fouling experiment comparing polished vs unpolished CNT-P fiber electrodes (Figure S1), and Raman spectra comparing a polished vs an unpolished CNT-P fiber electrode (Figure S2) (PDF)

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The authors declare no competing financial interest.

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