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Head-to-Head Comparisons of Carbon Fiber Microelectrode Coatings for Sensitive and Selective Neurotransmitter Detection by Voltammetry

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Abstract

Voltammetry is widely used to investigate neurotransmission and other biological processes but is limited by poor chemical selectivity and fouling of commonly used carbon fiber microelectrodes (CFMs). We performed direct comparisons of three key coating materials purported to impart selectivity and fouling resistance to electrodes: Nafion, base-hydrolyzed cellulose acetate (BCA), and fibronectin. We systematically evaluated the impact on a range of electrode parameters. Fouling due to exposure to brain tissue was investigated using an approach that minimizes the use of animals while enabling evaluation of statistically significant populations of electrodes. We find that BCA is relatively fouling resistant. Moreover, detection at BCA-coated CFMs can be tuned by altering hydrolysis times to minimize the impact on sensitivity losses while maintaining fouling resistance. Fibronectin coating is associated with moderate losses in sensitivity after coating and fouling. Nafion imparts increased sensitivity for dopamine and norepinephrine but not serotonin, as well as the anticipated selectivity for cationic neurotransmitters over anionic metabolites. However, while Nafion has been suggested to resist fouling, both dip-coating and electro-deposition of Nafion are associated with substantial fouling, similar to levels observed at bare electrodes after exposure to brain tissue. Direct comparisons of these coatings identified unique electroanalytical properties of each that can be used to guide selection tailored to the goals and environment of specific studies.

Voltammetry is uniquely suited for the study of the dynamics of intercellular communication due to its high temporal and spatial resolution and excellent sensitivity. Voltammetry techniques are widely used for *in vivo* measurements in anesthetized^{1–3} and awake animals,^{4,5} *ex vivo* measurements in brain slices^{6–8} and synaptosomes,^{9–11} *in vitro* measurements from peripheral cells,^{12,13} and for interrogating neurotransmitter release from single cells.^{14,15} Moreover, voltammetric methods, including amperometry, have been used to investigate a variety of neurochemicals such as dopamine,^{4,16,17} norepinephrine,¹⁸

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SUPPORTING INFORMATION AVAILABLE

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serotonin,^{2,8,9,12,19,20} ascorbate,^{21,22} uric acid,²³ adenosine,^{24,25} choline and acetylcholine²⁶ with the aim of understanding chemical neurotransmission and its role in normal and altered brain function.

Measurements of neurotransmitters by voltammetry are predominantly carried out using carbon fiber microelectrodes (CFMs).^{27–30} These microelectrodes are widely used due to the commercial availability of small-diameter carbon fibers (5–30 μm) and readily accessible fabrication methods. Discrete measurements, even in highly heterogeneous tissues or distinct brain nuclei have been made using CFMs.^{1,2,16,31,32} Furthermore, small-diameter CFMs produce considerably less tissue damage than microdialysis probes having diameters >200 μm .^{33–35}

Nonetheless, the investigation of biogenic amines and other biological signaling molecules using CFMs suffers from problems associated with poor chemical selectivity and sensor fouling.^{12,36,37} Hydroxyl groups on carbon fiber surfaces, in combination with an sp^2 -hybridized composition, contribute to the adsorption of small polar neurotransmitters and metabolites, their oxidation products, and large biomolecules.^{38–41} Irreversible adsorption leads to fouling of electrode surfaces, which results in uncertainty in identifying and quantifying analytes.

To compensate for fouling, post-calibration of CFMs is often carried out.^{18,31,32,42} However, the trajectories of electrode responses over the course of experiments are unknown.^{2,43,44} Post calibration alone or averaging of pre- and post-calibration is likely to lead to over- or underestimation of analyte concentrations depending on the sensitivity of an electrode at the time of each measurement. Problems associated with precise and accurate detection are further compounded by low transmitter concentrations and interference by other high concentration electroactive compounds found in complex biological environments.^{45,46} Moreover, dopamine, norepinephrine, serotonin, ascorbate (ASC), and neurotransmitter metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA), all oxidize in a narrow potential window complicating selective neurotransmitter detection. Methods such as principal regression analysis have been used to distinguish some analytes among pairs of electroactive species.⁴⁷ However, this approach has not gained widespread use due to difficulties associated with evaluating complex calibration sets, *i.e.*, calibrations of different neurochemicals, and the probability that electrode sensitivities to and redox potentials of various components change during an experiment due to the adsorption of oxidation products^{20,48,49} and biofouling.

The most commonly used method for optimizing CFMs involves the application of selectively permeable electrode coatings that allow diffusion of analytes of interest to electrode surfaces, while simultaneously minimizing the adsorption/detection of interferents. Many different materials have been reported to enhance selectivity and to reduce fouling at CFMs. These include Nafion,⁴⁵ fibronectin,⁵⁰ base-hydrolyzed cellulose acetate (BCA),⁵¹ polypyrrole,⁵² chitosan,⁵³ and carbon nanotubes.^{2,54} Nafion is commonly used due to its ease of deposition on CFM surfaces and its cation selective permeability. Previous studies have also suggested that Nafion has fouling resistant properties.⁵⁵ Base-hydrolyzed cellulose has been used as a fouling resistant coating material for CFMs due to its ability to exclude large biomolecules.⁵¹ The highly biocompatible nature of fibronectin, in addition to its chemical conductivity, has resulted in its use as a coating material for biosensors,^{50,56} although fibronectin has not previously been investigated as a surface modification for CFMs.

Although many different coatings have been employed in voltammetry studies, there is little information directly comparing these materials and specifically with regard to monoamine

neurotransmitter detection. Consequently, we systematically investigated three CFM coatings: Nafion, base-hydrolyzed cellulose acetate, and fibronectin. We compared dip-coating and electro-deposition for the application of Nafion. We also investigated three different hydrolysis times for BCA. Our goals were to evaluate the effects of these different coating materials and protocols on CFM sensitivity, selectivity, and biofouling with the aim of determining performance in the context of monoamine neurotransmitter sensing by fast-cyclic voltammetry (FCV).

EXPERIMENTAL METHODS

Animals

Adult mice ($N=24$) were housed in groups of 2–4 same sex siblings per cage on a 12-h light/dark cycle with food and water *ad libitum*. To obtain brains for homogenate fouling studies, mice were euthanized by cervical dislocation. Brains were rapidly removed from the skulls and frozen at -70°C until use. In place of evaluating each electrode in a living animal, the use of brain tissue homogenates (*vide infra*) enabled fouling experiments to be carried out on many electrodes ($N>200$) using a comparatively small number of animals.

A small number of mice ($N=4$) were used for *in vivo* FCV to compare fouling in the intact brain with brain homogenates. Anesthesia was induced with 5% isoflurane. During surgery, the anesthetic plane was maintained using 2–3% isoflurane and monitored by observing breathing patterns and toe-pinch reflex. Mice were mounted on a stereotaxic frame. The skin over the skull was removed and a hole (~ 2 mm) was drilled in the skull over the caudate-putamen (A/P: +1.2 mm, M/L: -2.1 mm). Microelectrodes were lowered to D/V: -1.4 mm. A reference electrode was placed in the neck muscle. *In vivo* fouling was evaluated using two electrodes per animal at two slightly different locations. Animals were euthanized at the end of the procedure by cervical dislocation under anesthesia. All animal procedures were approved by the University of California, Los Angeles Animal Research Committee or the Pennsylvania State University Institutional Animal Care and Use Committee and were conducted in strict accordance with National Institutes of Health Animal Care Guidelines.

Electrode Fabrication

Carbon fiber microelectrodes were fabricated as described previously with minor modifications (see supplemental online materials for additional information).^{9,12} For reference electrodes, silver wires (Alfa Aesar, Ward Hill, MA) were immersed in 2 M KCl and the manufacturer's proprietary step-potential waveform varying from +8.0 V to -8.0 V was applied using an automatic chlorider (NPI Electronic GmbH, Tamm, Germany) to deposit AgCl.

Electrochemistry

Fast-cyclic voltammetry was performed using a Universal Electrochemistry Instrument (UEI) potentiostat and Tar Heel CV software (University of North Carolina, Chapel Hill, NC). Raw data were further analyzed using custom LabVIEW modules written in house. Experiments were carried out to monitor oxidation and reduction currents, background currents, rise times, noise, and half-wave potentials (ΔE_p). A 10 Hz triangular waveform was applied to working electrodes consisting of an oxidative sweep from -0.4 V to +1.2 V and a reductive sweep from +1.2 V to -0.4 V at 400 V/s. This waveform is often used to measure dopamine,^{2,31,32} serotonin,² and norepinephrine¹⁸ with minor variations in the potential limits.

Each working electrode was lowered into the inner compartment of a custom flow cell along the flow path, while a reference electrode was placed in the electrically connected outer

reservoir. Phosphate-buffered artificial cerebrospinal fluid (aCSF; 2.7 mM KCl, 140 mM NaCl, 1.2 mM MgCl₂, 1.2 mM CaCl₂, 10 mM Na₂HPO₄ and 1.76 mM KH₂PO₄, pH 7.4 modified from Mathews *et al.*⁴⁶) was flowed at 3 ml/min using a peristaltic pump (PeriPro, World Precision Instruments, Sarasota, FL). Neurotransmitters and metabolites were injected into the flow stream using a Rheodyne automated switching valve (Valco Instruments Company Inc., Houston, TX).

For sensitivity and fouling experiments, electrodes were calibrated against 1 μ M dopamine prior to and after coating, and then again after fouling. For selectivity experiments, each bare- or coated-electrode was challenged with 1 μ M dopamine, 1 μ M norepinephrine, 0.5 μ M serotonin, 100 μ M DOPAC, 20 μ M 5-HIAA, and 100 μ M ASC. Electrodes were exposed to neurotransmitters followed by metabolites in this order to minimize the effects of fouling due to higher concentrations of metabolites. Additional information on the electrochemical measurements can be found in the supplemental online material.

Development of Coating Protocols

Carbon fiber microelectrodes were first cleaned in 2-propanol for 10 min followed by air-drying for 10 min. Coating protocols were optimized to minimize sensitivity losses, while also minimizing fouling. Details on coating protocol development appear in the supplemental online material.

Chemicals

Neurotransmitters, metabolites, and chemicals for electrode coatings were purchased from Sigma-Aldrich (St. Louis, MO). Nafion was purchased from Ion Power (New Castle, DE).

Data Analysis and Statistics

One-way analysis of variance (ANOVA) was used in cases where more than two means were compared across a single independent variable. When two classes of variables were compared, two-way ANOVA with or without repeated measures was applied, depending on the experimental design. Individual group comparisons were by Tukey's *post hoc* tests. All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA). Values are expressed as means $\pm$ standard errors (SEMs) with differences of $P < 0.05$ considered statistically significant. Significant differences are denoted in the figures as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

RESULTS AND DISCUSSION

Bare Electrode Fouling

We developed a method that reproducibly causes CFM fouling by exposing electrodes to brain tissue homogenates to approximate *in vivo* and *ex vivo* conditions. For comparison, some CFMs were exposed to BSA, a commonly used fouling agent.⁵⁰ Fouling was assessed by measuring changes in electrode sensitivity to 1 μ M dopamine. Exposure of bare electrodes to BSA (2 h or overnight) produced reductions in current responses to dopamine on the order of 25–40% (Fig. 1). Larger magnitude decreases in electrode sensitivity (60–70%) were observed after exposing CFMs to brain tissue. One group of electrodes was cycled (–0.4 V to +1.2 V to –0.4 V at 400 V/s and 10 Hz) during 2 h of exposure to brain tissue to simulate typical experimental conditions. Even so, similar levels of fouling were observed with and without potential cycling. Moreover, the extent of electrode fouling after exposure to homogenized brain tissue was similar to that observed after 2 h of *in vivo* FCV measurements. Since there were no significant differences in fouling after 2 h vs 12 h of exposure to brain tissue, subsequent experiments on the fouling of coated CFMs were

carried out using electrodes calibrated prior to overnight brain tissue exposure, followed by recalibration the next day.

Similar levels of fouling were observed in response to 2-h vs overnight exposures of bare CFMs to brain tissue. These data suggest that the majority of biofouling occurs early in the time course of electrode exposure to tissue in agreement with what has been hypothesized from *in vivo* studies.^{44,55,59–62} If biological fouling occurs in the first few minutes of tissue exposure and electrode sensitivity remains relatively stable after that, then post-calibration would provide a fairly accurate estimate of monoamine concentrations. Even coating materials that show considerable decreases in sensitivity after tissue exposure but still provide higher sensitivity after fouling than bare electrodes are advantageous due to gains in sensitivity, provided that fouling proceeds quickly. By contrast, if fouling occurs relatively slowly, *i.e.*, over the timeframe of the experiment, then changes in sensitivity *during* an experiment become an important factor to consider with regard to quantification.⁶³

Evaluation of Electrode Coatings

Scanning electron microscopy was used to examine electrode coatings on disk-shaped CFM sensing surfaces. Representative images are shown in Figure S-1. Fibronectin does not appear to cover the carbon fiber surface completely (Fig. S-1B) and coverage varied slightly from electrode to electrode. By contrast, Nafion (Fig. S-1C) and BCA (Fig. S-1D) formed continuous layers on electrode sensing surfaces. Nafion-coating appeared to be relatively thin compared to the other materials investigated. Even when dip-coated, its presence was only detected by the absence of sharp edges on carbon fiber surfaces compared to bare electrodes. Base-hydrolyzed cellulose formed an even polymeric layer that was easily visualized in electron microscopy images.

Effects of Coating on Electrode Sensitivity

Responses to brief exposure to dopamine were characterized before and after the deposition of fibronectin, Nafion, or cellulose acetate. For comparison, a group of bare CFMs was calibrated against 1 μ M dopamine, stored in aCSF overnight, and recalibrated the next day. Representative voltammograms are shown in Figure S-2. Mean electrode responses are compared in Figure 2. Bare electrodes exposed to aCSF (no coating) showed no changes in mean current responses (Fig. 2A). Nafion films applied by either dip coating or electro-deposition were associated with increases in sensitivity to dopamine (Figs. 2C and 2D). By contrast, fibronectin and BCA (20 min hydrolysis) were associated with decreases in dopamine currents (Figs. 2B and 2E). Only BCA-coated CFMs with a 40 min hydrolysis time showed no change in electrode sensitivity to dopamine after coating (Fig. 2F).

We applied fibronectin directly to bare CFMs and observed a 30% decrease in the oxidation current due to 1 μ M dopamine (Fig. 2B). Ponsonnet and coworkers investigated $\text{Fe}(\text{CN})_6^{4-/3-}$ oxidation at gold electrodes first modified by self-assembly of alkanethiols, followed by coating with polystyrene and then fibronectin.⁶⁴ In this case, no differences in electrode sensitivity were reported after the application of the final fibronectin layer compared to gold electrodes coated with thiol/polystyrene. Notably, large decreases in electrode sensitivity had already occurred after self-assembly of the innermost alkanethiol layer, which likely precluded further losses in sensitivity associated with the addition of a fibronectin layer. O'Hare and colleagues applied fibronectin directly to gold electrodes for O_2 sensing and also reported a lack of effect on sensitivity.⁵⁰ However, fibronectin is expected to be more permeable to O_2 than dopamine. Different types of electrodes (gold vs carbon fiber), analytes, and/or the multi-material coatings sometimes used for gold electrodes might underlie differences in the effects of fibronectin on electrode sensitivity.

We observed a decrease in signal-to-background ratios (S/B) for fibronectin-coated CFMs compared to bare CFMs (Table S-1). However, comparison of background current values before and after coating did not reveal significant changes for any of the coatings tested indicating that the decrease in S/B associated with fibronectin is due to a decrease in dopamine current. No significant changes in noise or rise time were associated with fibronectin coating (Table S-1). We did observe small but significant increases in ΔE_p values, probably due to slower diffusion and mass transfer through the globular fibronectin layer (Table S-1).

Similar to early results by Gerhardt *et al.*,⁵⁷ and Rice and Nicholson,⁴⁵ we observed a significant increase in the dopamine oxidation current with FCV for CFMs coated with Nafion using either protocol compared to bare CFMs (Figs. 2C and 2D). We also observed a significant increase in S/B for Nafion dip-coated CFMs (Table S-1). Since there were no changes in background current values and thus, electrode surface areas, increases in signal (dopamine oxidation current) account for the increases in S/B for Nafion dip-coated CFMs. No changes in noise, ΔE_p or rise time were detected for CFMs modified using either Nafion protocol (Table S-1).

Cellulose coatings on electrode surfaces have been used for the detection of O₂,⁶⁵ NO,⁶⁶ and with enzyme-modified electrodes to detect glucose⁶⁷ and uric acid.⁶⁸ Wang and coworkers further developed BCA coating methods by demonstrating that hydrolysis of cellulose acetate increases film porosity by fragmenting cross-linked groups.^{69,70} Porosity can be controlled by altering hydrolysis times.⁶⁹ Responses to 1 μ M dopamine after BCA-coating with 40 min of hydrolysis were not significantly different from bare CFMs (Fig. 2F), while a 20 min hydrolysis time was associated with a small but statistically significant decrease (15%) in electrode sensitivity (Fig. 2E). This suggests that 40 min of hydrolysis produces a film that does not interfere with dopamine diffusion through BCA coatings on the disk-shaped CFMs used here. Similarly, coating with BCA (40 min hydrolysis) was not associated with changes in rise time (Fig. S-5F). By contrast, coating with BCA (20 min hydrolysis) resulted in a significant increase in rise time (Fig. S-5E), which can be attributed to lower diffusion and mass transport rates through the cellulose acetate coating. Differences in noise, background currents, or ΔE_p were not observed for electrodes coated with BCA followed by 20 min or 40 min of hydrolysis (Table S-1).

Biofouling Associated with Different Coating Materials

Electrodes were calibrated before and after overnight exposure to brain tissue to ascertain how different coating materials performed in response to fouling. Voltammograms for representative electrode responses after fouling are shown in Figure S-2. Mean electrode responses are compared in Figure 2. After exposure to brain tissue (step iii), all groups of electrodes showed significant reductions in oxidative current responses compared to both pre-coating (step i) and post-coating (step ii) responses. Reductive currents showed similar patterns of attenuated responses (Fig. S-3).

The relative effectiveness of different coatings/protocols to inhibit fouling was further compared by calculating percent changes in pre- vs post-fouling responses. Decreases in sensitivity associated with fouling differed with regard to the different coating materials/protocols [$F(5,78)=15$; $P<0.001$]. Smaller decreases in sensitivity after fouling were observed at fibronectin- and BCA-coated CFMs compared to bare electrodes ($P<0.001$; bare (60% $\pm$ 4%) vs fibronectin (30% $\pm$ 8%), BCA 20 min (20% $\pm$ 7%), and BCA 40 min (20% $\pm$ 4%)). By contrast, decreases in sensitivity at Nafion dip-coated (70% $\pm$ 4%) and Nafion electro-deposited (60% $\pm$ 6%) CFMs were not significantly different from those at bare CFMs. Large decreases in Nafion-coated CFM responses attributable to fouling reversed the advantages associated with the increased sensitivity of Nafion-coated electrodes to

dopamine after coating (step ii vs step iii, Fig. 2). Nonetheless, after coating and fouling, CFM responses were highest at Nafion electro-deposited CFMs and BCA-coated CFMs (20 or 40 min hydrolysis times), where currents due to 1 μ M dopamine were two-fold higher than at bare electrodes (23 nA vs 14 nA; Table S-1).

Fouling of electrodes due to the adsorption of biological materials on sensing surfaces, in addition to decreasing sensitivity, can lead to slower diffusion of analytes to sensor surfaces and/or slower electron transfer kinetics, an effect that is reflected in increased half-wave potentials.⁷¹ The high degree of fouling at bare and Nafion-coated electrodes resulted in significant increases in half-wave potentials after exposure to brain tissue (Fig. S-4). Fibronectin-coated electrodes showed smaller but statistically significant increases in ΔE_p after coating and after fouling, likely due to slower diffusion across the fibronectin layer. By contrast, no changes in half-wave potentials after coating (step ii) or fouling (step iii) occurred at BCA-coated electrodes. Rise time, the time required for the oxidation current to increase from 10% to 90% of the maximal value, also provides information about electron and mass transfer rates at electrode surfaces. We observed no change in rise times associated with fouling (step ii to step iii) for fibronectin and BCA-coated CFMs as compared to significant increases in rise time for bare and Nafion-coated CFMs after fouling (Fig S-5). Increases in rise time at bare and Nafion-coated CFMs could diminish the ability to detect small but important changes in fast neurotransmitter release *in vivo*.

Recently, fibronectin coating of gold electrodes was reported to be associated with low fouling.⁵⁰ Although, we observed measurable fouling of fibronectin-coated CFMs after exposure to brain tissue, this occurred to a lesser extent than at bare CFMs. The fouling protocol used here exposes electrodes to intracellular and extracellular cell fragments and proteins, similar to what is expected to occur when an electrode disrupts cells as it is inserted into tissue. Fibronectin forms a globular layer on electrode surfaces (Fig. S-1B) and might reduce CFM surface contact with biomolecules that lead to fouling. Currents associated with 1 μ M dopamine at fibronectin-coated electrodes after coating and fouling (~17 nA) were somewhat higher than those at bare electrodes; however, they were lower than dopamine currents at Nafion electro-deposited CFMs and BCA-coated CFMs (Table S-1).

The present findings show that a high degree of biofouling after brain tissue exposure occurs at bare electrodes, as well as at Nafion dip-coated and Nafion electro-deposited CFMs. It is possible that the fouling protocol used here, which involves a long exposure to homogenized brain tissue, leads to a greater degree of fouling than what typically occurs during *in vivo* or *ex vivo* experiments lasting several hours. However, we did not find that shorter (2-h) exposures, with or without potential cycling, produced fouling that was different from overnight exposures to homogenized brain tissue at bare electrodes (Fig. 1). In a previous study with peripheral blood lymphocytes,¹² we observed a 35% loss of sensitivity of Nafion dip-coated cylindrical CFMs after 20 min of cell contact. Moreover, we observed a 60% loss of sensitivity due to fouling of Nafion dip-coated CFMs after 20 min of exposure to brain synaptosomes.⁹ Together, these data suggest that Nafion-coated electrodes sustain substantial fouling after exposure to brain tissue.

The present and many previous studies suggest that regardless of its magnitude, fouling at bare- and Nafion-coated electrodes occurs quickly after exposure to brain tissue.^{9,20,44,55,60–62,72,73} Furthermore, once electrode responses are stabilized, stimulated and behaviorally evoked dopamine release at bare- or Nafion-coated electrodes are reported to vary little over the course of *in vivo* experiments,^{55,61} even months after implantation.⁶⁰ Similarly, Daws and colleagues reported that following initial stabilization, reproducible signals due to pressure-ejected serotonin were detected using Nafion dip-coated CFMs and chronoamperometry *in vivo*.⁷⁴ Capella *et al.* showed that the sensitivity of Nafion-coated

CFMs for norepinephrine decays rapidly to 50% of initial sensitivity in the first 2 h after electrode implantation and then remains stable up to 12 h.⁶² In a preliminary experiment, we have observed a 55% decrease in electro-deposited Nafion-coated CFM sensitivity to 1 μ M DA after 5 min of exposure to homogenized brain tissue ($N=7$ electrodes). Therefore, even though Nafion-coated CFMs show the greatest loss of sensitivity in response to fouling in this study (with the exception of bare electrodes), they likely sustain these changes rapidly and retain sufficient sensitivity for *in vivo* voltammetry applications.

When compared to bare electrodes, BCA-coated CFMs with 20 or 40 min hydrolysis times showed significantly reduced fouling (20%) (Figs. 2E and 2F). Marinesco and coworkers observed that non-hydrolyzed cellulose acetate-coated cylindrical CFMs were fouling resistant but insensitive to dopamine and serotonin. Fouling resistance gradually decreased, while sensitivity increased with increasing hydrolysis times from 10 min to 30 min.⁵¹ For the 30- μ m disk-shaped CFMs used here, coating with BCA followed by a 10 min hydrolysis time resulted in significant sensitivity losses (Fig. 3). By contrast, BCA-coating followed by a 40 min hydrolysis time resulted in no change in electrode sensitivity to dopamine, norepinephrine, or serotonin (Fig. 2F and Fig. 3). Together, the findings on BCA-coated CFMs suggest that hydrolysis times need to be tuned for different electrode geometries or other experimental conditions to yield optimal diffusion of analytes to sensing surfaces while still maintaining fouling resistance.

Comparative Sensitivities to Monoamine Neurotransmitters and Metabolites

In a separate set of experiments, responses to dopamine, norepinephrine, and serotonin, as well as a number of electroactive metabolites found at high concentrations in the brain, were determined at bare and coated CFMs (Fig. 3). These data are also compared in Table S-2. Here, sensitivity is defined as the current response for a particular species at each type of electrode relative to its response at bare electrodes (i/i_{bare}). Selectivity is defined as the current response for each species at a particular type of electrode relative to the dopamine response at the same type of CFM (i/i_{DA}).

Each CFM was challenged with 1 μ M dopamine, 1 μ M norepinephrine, 0.5 μ M serotonin, 100 μ M DOPAC, 20 μ M 5-HIAA, and 100 μ M ASC. Neurotransmitter concentrations were based on levels released in response to stimulation in various brain regions^{18,75} and were adjusted to keep current responses at bare electrodes roughly equal. Oxidation and reduction peak potentials of metabolites are very close to those of monoamine neurotransmitters. However, in FCV, voltammograms are background subtracted; therefore, contributions due to steady-state metabolite levels are subtracted with the background. Only *changes* in metabolite levels during the measurement window (~15–30 s) contribute to background-subtracted current responses and possibly, to inaccurate estimates of neurotransmitter concentrations. Extracellular concentrations of DOPAC, 5-HIAA, and ASC are approximately 1000 μ M, 200 μ M, and 200–500 μ M, respectively.^{22,46,76} For the present experiments, we selected concentrations of metabolites that were 10% of their extracellular levels since we do not expect metabolite concentrations to change greatly during the short duration of FCV recordings.

Monoamine Selectivity of Coated Electrodes—Fibronectin- and BCA-coated (10 min hydrolysis) CFMs were associated with significant decreases in electrode responses to dopamine compared to bare CFMs (Fig. 3A). By contrast, Nafion-coated CFMs showed significant increases in oxidation currents for dopamine. BCA-coated CFMs (20 and 40 min hydrolysis times) showed no differences in electrode responses to dopamine compared to bare CFMs. These data comparing the changes in the responses of different groups of CFMs

to dopamine are in agreement with those shown in Fig. 2C, where current measurements were carried out at the same electrode before and after coating.

For norepinephrine, we observed similar decreases in oxidation current values for CFMs coated with fibronectin or BCA with a 10 min hydrolysis time compared to bare CFMs (Fig. 3B). Conversely, Nafion dip-coating and electro-deposition were associated with significantly enhanced oxidation current values for norepinephrine compared to bare electrodes. Similar to dopamine, no significant differences in electrode responses to norepinephrine were observed for CFMs coated with BCA using the longer hydrolysis times.

For serotonin, decreased oxidation current values were associated with fibronectin coating and BCA-coated CFMs with 10 or 20 min hydrolysis times compared to bare CFMs (Fig. 3C). However, in contrast to dopamine and norepinephrine, neither Nafion coating protocol was associated with increased sensitivity to serotonin. Base-hydrolyzed cellulose acetate with a 40 min hydrolysis time did not significantly alter electrode responses to serotonin in comparison to bare CFM responses.

Fibronectin-coated CFMs and BCA-coated CFMs with 10 min hydrolysis showed significant decreases in electrode sensitivity for all three monoamine neurotransmitters compared to bare CFMs. Reduced electrode sensitivity associated with fibronectin coating could be due to a decrease in monoamine permeability through the fibronectin layer. Reduced electrode sensitivity of BCA-coated CFMs with short hydrolysis times might similarly be due to low porosity of the polymer films.⁷⁰ While electrode responses for dopamine and norepinephrine were similar at BCA-coated CFMs with 20 and 40 min hydrolysis times compared to bare CFMs, detection of serotonin appears to require BCA longer hydrolysis to maintain electrode sensitivity comparable to bare CFMs.

Interestingly, while others have reported increased sensitivity of Nafion dip-coated²⁰ and Nafion electro-deposited⁴⁹ electrodes to serotonin, we did not observe similar effects here or in a previous study.¹² Other studies used a faster scan rate (1000 V/s) to detect serotonin than the one used here (400 V/s). Fast scan rates are hypothesized to “outrun” the formation of serotonin oxidation products that can foul electrodes. Fouling of Nafion-coated electrodes at the slower scan rates used here might offset gains in sensitivity due to the concentration of serotonin in Nafion films. Additional work will be needed to understand the specific factors contributing to differences in the sensitivity of Nafion-coated electrodes to serotonin.

Electrode Responses to Metabolites—All coating materials/protocols were associated with significant decreases in electrode responses to DOPAC and ASC, while most of the coatings, with the exception of BCA-coated CFMs with 40 min of hydrolysis, demonstrated lower oxidation current responses for 5-HIAA compared to bare CFMs (Figs. 3D–3F). Most *in vivo* and *ex vivo* voltammetric studies focus on neurotransmitters, while metabolites are regarded as interferents. Therefore, it is preferable for sensors to show high sensitivity to neurotransmitters combined with low sensitivity to metabolites. Nafion coatings are associated with the highest oxidation current ratios of dopamine to all three metabolites (Table S-2). The anion-excluding properties of Nafion increase selectivity for monoamine neurotransmitters by reducing interference from negatively charged monoamine metabolites.^{77,78} Fibronectin-coated electrodes showed modest selectivity for dopamine over DOPAC and ASC at the concentrations tested (Table S-2). Base-hydrolyzed cellulose acetate-coated CFMs showed the least selectivity in terms of metabolite exclusion.

CONCLUSIONS AND FUTURE PROSPECTS

The major findings of this study illustrate that electrode responses to different coating processes and fouling are complex, with some coatings better suited for sensitivity and selectivity (*i.e.*, Nafion), while others are better at preventing fouling (*i.e.*, BCA or fibronectin). Surface modifications that enable the most accurate and precise measurements will be those associated with responses that change the least over the duration of an experiment while retaining high sensitivity and selectivity. None of the coatings investigated here met all of these criteria. Base-hydrolyzed cellulose-coated CFMs with optimized hydrolysis times showed negligible losses in sensitivity after coating and relatively high fouling resistance. However, BCA-coated CFMs appear to be better geared for applications where electroactive metabolites do not come into play since this coating material did not impart appreciable selectivity for monoamine neurotransmitters over metabolites. By contrast, if fouling occurs in the first few minutes after implantation and thereafter, sensitivity remains fairly constant, then Nafion-coated CFMs appear to be better *in vivo* sensors due to their high selectivity for monoamine neurotransmitters over metabolites and high current sensitivities, particularly for dopamine and norepinephrine. Fibronectin-coated CFMs did not show a high degree of fouling compared to bare electrodes; however, this was at the expense of reduced sensitivity to neurotransmitters.

Future studies will be aimed at investigating fouling trajectories, particularly with respect to short tissue exposure times and different coating materials. Future work will also be directed at comparing CFM coating materials such as carbon nanotubes, chitosan, and polypyrrole. Additional head-to-head evaluations of different coatings will be important for understanding which materials are most advantageous based on the goals of specific experimental designs. In addition to surface modifications, regeneration of electrode surfaces during the course of an experiment⁷⁹ or alternate electrode materials are also being explored. For example, boron-doped diamond (BDD) microelectrodes have been used for some applications due to their excellent fouling resistant properties.^{12,80} However, BDD microelectrodes are limited by the larger diameters at which they are currently fabricated (40–100 μm) and their complex fabrication process.⁸¹ Notably, recent advances in fabrication techniques such as hot-filament chemical vapor deposition to generate BDD nanorod forest electrodes⁸² and focused ion beam milling to fabricate BDD ultra-microelectrodes with well defined geometries,⁸³ suggest the use of BDD electrodes for *in vivo* sensing might not be far off. Nevertheless, a primary focus for voltammetric sensing of biological analytes is likely to continue to rely, at least in part, on the optimization of CFM coating materials and protocols.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Bare carbon fiber microelectrode responses to fouling

Electrodes were calibrated in a flow cell against 1 μ M dopamine before and after exposure to fouling protocols involving different exposure times to bovine serum albumin (BSA), brain tissue homogenates, or *in vivo*. One group of electrodes was exposed to brain tissue for 2 h while the potential was cycled (-0.4 V to $+1.2$ V to -0.4 V at 400 V/s and 10 Hz), while another group of electrodes was cycled *in vivo* in anesthetized mice for 2 h. Data represent means of the percent changes in sensitivity for individual electrodes $\pm$ SEMs ($N=7-17$ electrodes per protocol). One-way analysis of variance (ANOVA) indicated that electrode responses differed with respect to fouling protocols [$F(6,59)=22$; $P<0.001$]. Carbon fiber microelectrodes calibrated after overnight exposure to artificial cerebrospinal fluid (aCSF) showed no change in sensitivity compared to pre-calibration (before= 25 ± 5 nA; after= 24 ± 5 nA). * $P<0.05$ and *** $P<0.001$ vs aCSF overnight.

Figure 2. Oxidative current responses to coating and fouling

Oxidative currents produced in response to 1 μ M dopamine are shown for (A) bare, (B) fibronectin-coated, (C) Nafion dip-coated, (D) Nafion electro-deposited, (E) BCA-coated (20 min hydrolysis), and (F) BCA-coated (40 min hydrolysis) CFMs. The data represent mean current values $\pm$ SEMs recorded after fabrication of bare electrodes (step i), after coating (step ii), and after fouling (step iii) for $N=12-17$ electrodes per group. Repeated measures two-way ANOVA revealed a significant interaction between coating protocol and step [$F(10,156)=8.7$; $P<0.001$] indicating that responses at each step differed with respect to the various coating protocols. Data for individual coatings were analyzed by repeated measures one-way ANOVA and Tukey's *post hoc* tests. Changes in oxidation currents with

respect to step were significant for all of the coating protocols. Individual group comparisons are indicated by $*P<0.05$, $**P<0.01$, and $***P<0.001$ before coating (step i) vs after coating (step ii) or after fouling (step iii) and $\dagger P<0.05$, $\dagger\dagger P<0.01$, and $\dagger\dagger\dagger P<0.001$ after coating (step ii) vs after fouling (step iii).

Figure 3. Comparative sensitivities of coated electrodes to monoamine neurotransmitters and metabolites

Oxidation currents produced at bare- and coated-CFMs for (A) 1 μ M dopamine, (B) 1 μ M norepinephrine, (C) 0.5 μ M serotonin, (D) 100 μ M DOPAC, (E) 20 μ M 5-HIAA, and (F) 100 μ M ascorbate (ASC). The data represent mean oxidation current values $\pm$ SEMs ($N=10-19$ electrodes for each coating material). Two-way ANOVA revealed a significant interaction between coating material and neurochemical [$F(30,552)=7.2$, $P<0.001$] indicating that different coatings were associated with different response patterns to various analytes. Data for the responses of each type of coated electrode to analytes were further analyzed by one-way ANOVA and Tukey's *post hoc* tests. A significant effect of analyte

was observed for all electrode types. Individual group comparisons are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. bare electrodes.