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O₂ Plasma Etching and Anti-Static Gun Surface Modifications for CNT Yarn Microelectrode Improve Sensitivity and Anti-Fouling Properties

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Abstract

Carbon nanotube (CNT) based microelectrodes exhibit rapid and selective detection of neurotransmitters. While different fabrication strategies and geometries of CNT microelectrodes have been characterized, relatively little research has investigated ways to selectively enhance their electrochemical properties. In this work, we introduce two simple, reproducible, low-cost, and efficient surface modification methods for carbon nanotube yarn microelectrodes (CNTYMEs): O₂ plasma etching and anti-static gun treatment. O₂ plasma etching was performed by a microwave plasma system with oxygen gas flow and the optimized time for treatment was 1 minute. The anti-static gun treatment flows ions by the electrode surface; two triggers of the anti-static gun was the optimized number on the CNTYME surface. Current for dopamine at CNTYMEs increased three-fold after O₂ plasma etching and four-fold after anti-static gun treatment. When the two treatments were combined, the current increased 12-fold, showing the two effects are due to independent mechanisms that tune the surface properties. O₂ plasma etching increased the sensitivity due to increased surface oxygen content but did not affect surface roughness while the anti-static gun treatment increased surface roughness but not oxygen content. The effect of tissue fouling on CNT yarns was studied for the first time, and the relatively hydrophilic surface after O₂ plasma etching provided better resistance to fouling than unmodified or anti-static gun treated CNTYMEs. Overall, O₂ plasma etching and anti-static gun treatment improve the sensitivity of CNTYMEs by different mechanisms, providing the possibility to tune the CNTYME surface and enhance sensitivity.

Graphical Abstract

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Supporting Information

Additional text describing solutions, electrochemistry, surface characterization, and statistics; five figures showing oxidation current change with time at anti-static gun treated CNTYMEs with 2 triggers, Raman spectra obtained at CNTYMEs with different surface modifications, comparison of the response to 1 μ M dopamine and background current at an unmodified CFME and the same electrode after anti-static gun treatment with different triggers, CVs and statistics of AA and UA measured at different CNTYMEs, oxidation current versus time traces for a flow injection analysis experiment at an unmodified CNTYME and the same electrode after combined treatment, and comparison of the response to dopamine at CNTYMEs before/after in tissue fouling; one table showing comparison of dopamine detection at anti-static gun treated CNTYMEs with different triggers.

Introduction

CNT yarns are macrostructured carbon materials directly spun from CNTs arrays.^{1,2} Schmidt et al. first used CNT yarn microelectrodes (CNTYMEs) for neurotransmitter monitoring with fast-scan cyclic voltammetry (FSCV) and demonstrated improved selectivity, sensitivity, and electron transfer kinetics compared to conventionally used carbon-fiber microelectrodes (CFMEs).³ The enhanced electrochemical properties of CNTYMEs are attributed to the alignment of the CNTs, which exposes the ends of the tubes that contain more oxygen functional groups known to promote adsorption of neurotransmitters such as dopamine.^{4–8} Moreover, the ability to fabricate CNT yarns directly into electrodes in a manner similar to CFMEs dramatically simplifies the electrode fabrication process and improves the reproducibility.^{9,10} One main advantage of CNTYMEs is that the dopamine signal is independent of the repetition rate of the applied voltage waveform for FSCV, a stark contrast to traditional CFMEs which dramatically lose sensitivity with increasing repetition rate.^{11,12} This frequency independence is due to the scale of the surface roughness, which matches the diffusion distance and leads to thin-layer cell effects.^{5,11} Since CNT yarns are a soft material, when they are cut with scalpel, the end of the yarn puffs out and the geometry and the homogeneity changes. Therefore, the polished disk geometry is preferred, but disk electrodes have a lower surface area which limits the sensitivity. Methods to increase sensitivity as well as improve resistance to fouling will facilitate CNTYME use *in vivo*.

Surface modification methods can be used to improve carbon electrode performance.^{13,14} Acid treatment¹⁵, electrochemical activation¹⁶, and spark etching¹⁷ change the electrode surface properties. Polymer coating^{18–20} and metal nanoparticle coating^{21,22} improve sensitivity but are complicated to fabricate reproducibly and can cause slower electron transfer rates and temporal resolution. Recently, we reported laser treatment as a solvent-free, reproducible, and efficient approach to further improve the sensitivity of CNTYMEs, and applied the laser-treated CNTYMEs for *in vivo* neurotransmitters detection for the first time.¹¹ Similar to the previous work on laser-activated CFMEs^{23,24}, the enhanced sensitivity of CNTYMEs after laser treatment is due to the synergistic effect of both enhanced surface roughness and enhanced numbers of oxygen-containing functional groups. Laser etching,

however, requires an optical system set-up and expensive laser. Therefore, cheaper and more accessible surface treatments would be beneficial for enhancing CNTYME properties.

In this work, two surface modification methods for CNT yarn microelectrodes were optimized and compared: O₂ plasma etching and anti-static gun treatment. These surface treatment methods are simpler and lower cost than laser treatment. O₂ plasma etching is widely used for surface cleaning and enhancing surface oxide groups on a variety of materials.^{25–27} O₂ plasma etching enhanced the dopamine sensitivity of several carbon nanomaterial electrodes,^{28–31} but had not been evaluated with CNT yarns. Here, we use a microwave plasma system with oxygen gas flow and demonstrate dopamine currents improve by more than three-fold with FSCV while biofouling decreases. Anti-static gun treatment is a novel and easy electrode surface treatment that flows ions across the surface of the electrode and increases the dopamine current at the CNTYME four-fold. The surface characterization data indicate that dopamine current increases after these treatments due to 2 distinct mechanisms: (1) increased oxygen content by O₂ plasma etching and (2) increased surface roughness by anti-static gun treatment. Distinct mechanisms were confirmed by combining the two treatments to achieve a 12-fold increase in current. Overall, these new surface treatments allow the surface chemistry and roughness of the CNTYME to be tuned and could be simple, low-cost methods to increase sensitivity and reduce biofouling of other carbon nanomaterial-based electrodes.

Experimental Section

Carbon Nanotube Yarn Microelectrode Preparation

A 0.68 mm ID × 1.2 mm OD (A-M Systems, Carlsborg, WA) glass capillary was pulled into a glass pipet puller and cut to have an opening diameter of ~50 μm. A piece of commercially available CNTY (10–25 μm in diameter, 1–2 cm long, General Nano, LLC, Cincinnati, OH) was inserted into the glass pipette and epoxied in place with Epon Resin 828 (Miller-Stephenson, Danbury, CT) mixed with 14% (w/w) 1,3-phenylenediamine hardener (Sigma-Aldrich, St. Louis, MO) heated to 85°C. The epoxied electrodes were cured overnight at room temperature and then heated at 100°C for 2 h and at 150°C overnight. Electrodes were polished at a 45° angle on a fine diamond abrasive plate (Sutter Instruments model BV-10, Novato, CA) to create an elliptical active area. Cylindrical CFMEs were fabricated using 7-μm-diameter T-650 carbon fibers (Cytec Technologies, Woodland Park, NJ).¹²

O₂ Plasma Treatment

The polished disk CNT yarn microelectrodes were oxygen plasma etched using PVA-TePla Microwave Plasma System 400 H2 (PVA TEPLA, Corona, CA). About 20 – 30 CNTYMEs fixed on silicon chip were placed in a plasma generator and etched with igniting plasma power of 600 W, with the etching duration of 1 or 3 minutes at room temperature. The oxygen gas flow rate was 250 sccm to achieve a chamber oxygen pressure of about 970 mTorr.

Anti-static gun Treatment

Anti-static treatment was applied on CNT yarn microelectrode by simply trigger anti-static gun for several times (1, 2 or 4 triggers) in atmospheric environment. A Milty Zerostat 3 anti-static gun (Milty Co., UK) was used with a unit cost less than 100 US dollars. The anti-static gun incorporates a unique Piezo Crystal device which generates a positive electrical stream of ions with a gentle slow squeeze of the trigger and a negative stream of ions upon slow release of the trigger with a charge about 1.5 Coulombs. The tip of CNT yarn microelectrodes was placed approximately 5 cm away from the muzzle. A trigger was defined as squeezing the projecting tongue slowly for about two seconds, followed by a slow release for another two seconds. The anti-static gun treated CNTYMEs were left in the air for about 1 hour equilibration before electrochemical measurements.

Tissue Fouling

Male Sprague-Dawley rats (250–350 g) purchased from Charles River were housed in a vivarium and given food and water *ab libitum*. All experiments were approved by the Animal Care and Use Committee of the University of Virginia. The brains for fouling studies were obtained after *in vivo* experiments, in which the rat was anesthetized with urethane (1.5 mg/kg i.p.), the scalp shaved, and 0.25 mL bupivacaine (0.25% solution) given subcutaneously. In place of evaluating each electrode in a living animal, the use of brain tissue homogenates enabled fouling experiments to be carried out on many electrodes using a comparatively small number of animals. The microelectrodes were pre-calibrated for their response to 1 μ M dopamine and then placed in the tissue sample for two hours without potential cycling. After immersing in PBS solution for 15 minutes, their responses to 1 μ M dopamine were tested and compared to test the effects of fouling.

Results and Discussion

Parameter Optimization

O₂ plasma etched CNTYMEs were treated with oxygen gas flow for either 1 or 3 min. The response to 1 μ M dopamine was characterized using FSCV on the same CNTYME before/after treatment (Figure 1). Both the oxidation and reduction currents for dopamine increased about three-fold after O₂ plasma etching, and the improvement is larger with 3 min etching than with 1 min etching. However, the longer duration of etching causes more noise. 3 min O₂ plasma etched CNTYMEs have a worse limit of detection (LOD) (26 ± 9 nM, $n = 5$) than 1 min O₂ plasma etched CNTYMEs (11 ± 2 nM, $n = 6$, unpaired t-test, $p = 0.05$). The separation between the oxidation and reduction peak potentials (E_p) is larger after 3 min of O₂ plasma etching than for 1 min etching, indicating that longer etching slows the electron transfer rate. In addition, the temporal response after 3 min etching is slower and the response does not return to baseline (Fig. 1E). SEM images of the electrodes before and after O₂ plasma etching reveal that 1 min O₂ plasma etching did not cause obvious morphology changes but 3 min O₂ plasma etching changed the CNT yarn surface morphology (Fig. 2). Large amounts of broken CNT bundles were formed in 3 min, which likely causes the reduced electron transfer rate and slower temporal response (Fig. 1E). Therefore, 1 min O₂ plasma etching was chosen as optimal and used in all further experiments.

Anti-static gun treatment is a novel surface treatment for electrodes and is simply performed by applying triggers of an anti-static gun with the muzzle about 3 cm away from the microelectrode tip. The piezo crystal device in the anti-static gun generates a charged electrical air stream. To optimize the amount of triggers, CNTYMEs were treated with 1, 2, or 4 triggers. Figure 3 shows the example CVs and the oxidation and reduction currents for 1 μ M dopamine are largest with 2 triggers, a 4-fold increase, compared to about a two-fold increase after 1 or 4 triggers. Similarly, when multiple electrodes are averaged, CNTYMEs treated with 2 triggers had the largest signal improvement and best LOD compared to 1 or 4 triggers (Table S1). The temporal response did not change much after 1 and 2 triggers of anti-static gun treatment, but slowed after treatment with 4 triggers (Table S1). SEM images of a CNTYME with 1 trigger (Fig. 4B) do not show much difference compared with unmodified electrodes, which explains the lower signal enhancement. In comparison, the CNT bundles stand straight up after 2 triggers. After 4 triggers, the morphology changed significantly: a secondary structure of CNT bundles is observed, which introduces noise, increases the LOD, and slows the temporal response. Therefore, CNTYMEs with 2 triggers of anti-static gun treatment were chosen as optimal for further electrochemical characterizations.

Stability of the signal enhancement was investigated in a variety of experiments. First, the effect of time after antistatic gun treatment was tested. Immediately after modification, the signal is high and it decreases an hour after treatment and then reaches a plateau and does not change significantly up to five hours (Fig. S1, one-way ANOVA, Bartlett's test, $p > 0.5$, $n = 4$). Any extra charge on CNT yarn surface is dissipated after 1 hour so all future electrodes were run at least 1 h after anti-static gun treatment to ensure stable, reproducible signals. To test stability over the length of a typical experiment, electrochemical tests were performed over 5 hours with the potential being applied. Relative standard deviations (RSD) were 4.1% ($n = 5$) and 5.8% ($n = 5$) for O_2 plasma etched and anti-static gun treated CNTYMEs, respectively. In addition, to test shelf stability, electrodes were tested and the retested after 24 hours shelf storage after fabrication. The RSDs were 3.4 % for O_2 plasma etched and 2.6 % anti-static gun treated CNTYMEs. Thus, the electrode treatments produce electrodes with stable signals over the time course of an experiment.

Comparison of Electrochemical Properties of O_2 plasma etched and Anti-Static Gun Treated CNTYMEs

Electrochemical properties were compared to the optimized O_2 plasma etched (1 min) and anti-static gun treated (2 triggers) CNTYMEs. The CVs for 1 μ M dopamine for both treatments show an increased signal. The increase for anti-static gun treatment was a 4.3 ± 0.4 fold signal improvement, which is larger than the 3.0 ± 0.4 fold increase at O_2 plasma etched CNTYMEs (unpaired t-test, $p = 0.05$). E_p decreased after both treatments (Table 1, paired t-test, $p = 0.05$ for both comparison), indicating improved electron transfer rate.³² The CVs show that peak potentials shift positively for O_2 plasma etched electrodes but not for anti-static gun treated CNTYMEs. The possible reason is that O_2 plasma etching provides abundant oxygen-containing functional groups that are deprotonated and bring more negative charge to the surface at the physiological pH. Therefore, the extra positive charge is required for oxidation at the electrode, to compensate for the additional negative charge and

reach the point of zero charge of the electrode.³³ For anti-static gun treated CNTYMEs, the potentials did not shift positively (Fig. 3D, Table 1) and E_p significantly decreased (paired t-test, $p = 0.05$, $n = 8$).

The current vs time traces allow a comparison of the time response before and after treatment. After 1 min treatment O₂ plasma treatment, the average 10–90% signal rise time ($t_{10-90\%}$) was 1.2 ± 0.2 ($n = 4$), not significantly different (unpaired t-test, $p = 0.36$) than the pretreatment value of 1.1 ± 0.2 ($n = 4$). Similarly the anti-static gun treated electrode in Figure 3E had no delay in the response to a bolus of dopamine (unpaired t-test, $p = 0.70$), with the 10–90% signal rise time of 1.2 ± 0.3 ($n = 4$) compared to a pretreatment value for those electrodes of 1.2 ± 0.2 ($n = 4$). Therefore, both treatments allow the CNTYME maintain good temporal response while increasing sensitivity.

The background currents provide information about electrode surface area enhancements as well as surface functional group changes. The background current increases about 2 fold for both oxygen plasma etching (2.3 ± 0.4) and anti-static gun treatment (1.9 ± 0.2), which is lower than the increase in the Faradaic current for dopamine. Because the anti-static gun had the largest increase in dopamine signal and smallest background change, it has a larger signal to background current ratio (0.09 ± 0.01 , $n = 8$) compared to unmodified (0.05 ± 0.01 , $n = 6$) and O₂ plasma-etched CNTYMEs (0.05 ± 0.01 , $n = 8$). Background voltammetric features at about -0.1 V and 1.3 V were enhanced at CNTYMEs after O₂ plasma etching (Fig. 1C). These extra background features are likely due to increased oxygen functional groups after O₂ plasma etching.^{32–34} Moreover, the increase in oxygen functional groups would cause a more hydrophilic surface, which leads to a larger charging current as well.³³ In comparison, the charging current shape does not change much after anti-static gun treatment, indicating the improvement in current is more likely because of the increasing surface area. Surface changes were investigated further with surface techniques.

Physical Characterization of Treated CNTYMEs

The CNT yarn surfaces with O₂ plasma etching or anti-static gun treatment were physically characterized by SEM, three-dimensional laser scanning confocal microscopy, Raman spectroscopy, and energy-dispersive X-ray spectroscopy (EDS). SEM images reveal that the multi-walled CNTs bundles with a diameter about 30 nm did not change obviously after 1 min O₂ plasma etching (Fig. 2B) but that the bundles stand up straighter after anti-static gun treatment (Fig. 4C). Laser scanning confocal microscopy was applied to measure the surface roughness precisely. Table 2 summarizes the averaged mean roughness depth (R_z), defined as the average distance between the highest peak and lowest valley in each sampling length. The surface roughness did not change significantly after O₂ plasma etching (unpaired t-test, $p > 0.05$, $n = 4$). In contrast, CNTYMEs with anti-static gun treatment had a significantly larger surface roughness than both unmodified and O₂ plasma etched CNTYMEs (one-way ANOVA, Bartlett's test, $p = 0.05$, $n = 4$).

Raman spectroscopy and EDS were performed to characterize the surface defect to graphite carbon (D/G) ratio and oxygen content. The ratio of the D and G band area is widely used to evaluate the relative amount of sp³ and sp² hybridized carbon at carbon nanomaterials.^{35,36} The D/G ratio at O₂ plasma etched CNTYMEs was larger than either unmodified or anti-

static gun treated CNTYMEs (Fig. S2 and Table 2, one-way ANOVA, Bartlett's test, $p = 0.001$, $n = 4$) due to the carbon-carbon bonds broken by the plasma and the introduction of larger amounts of sp^2 hybridized carbon. The D/G ratio after anti-static gun treatment did not change from control (unpaired t-test, $p = 0.40$, $n = 4$), indicating there is likely no chemical change on the surface. The oxygen content measured by EDS significantly increased at O_2 plasma-etched electrodes compared to unmodified control (Table 2, one-way ANOVA, Bartlett's test, $p = 0.001$, $n = 4$) due to the increased amounts of oxygen functional groups. The anti-static gun treated CNTYMEs do not have a significantly different oxygen content than unmodified CNTYMEs (unpaired t-test, $p = 0.28$, $n = 4$).

Oxygen-containing functional groups, such as quinones, hydroxyl, and carboxylic acids are negatively charged at physiological pH and have electrostatic interactions with positively charged dopamine.⁴ O_2 plasma etching introduces more oxygen-containing functional groups, providing higher dopamine sensitivity and higher surface hydrophilicity, without changing the surface roughness. In contrast, anti-static gun treatment is a mild surface modification approach for CNTYMEs that does not change the D/G ratio or oxygen content. Instead, the dopamine sensitivity improvement is due only to the increased surface area and resultant diffusion pattern changes.¹¹ This increase in surface area must be due to nanostructures realigning, as treating CFMEs with the anti-static gun has no effect on the current for dopamine or background (Fig. S3). The mechanism of the anti-static gun treatment on CNT yarns is likely due to the repulsion of individual MWCNT bundles by the charge delivered from the anti-static gun. Since the CNT bundles in CNT yarns are long and well-aligned^{3,11,12}, electrons/charges are prone to transfer along the sp^2 hybridized carbon on each yarn, instead of passing a current through junctions between CNTs because of the high energy barrier.^{37,38}

The electrostatic effects that drive dopamine signal improvement at oxygen plasma etched electrodes are also proven by examining anions, 200 μM ascorbic acid (AA) and 20 μM uric acid (UA), which are interferent anions in the extracellular environment¹⁴. The ratios of oxidative current for AA or UA to 1 μM DA are significantly smaller at O_2 plasma etched CNTYMEs than at unmodified and anti-static gun treated CNTYMEs (Fig. S4, one-way ANOVA Bonferroni post-test, $p = 0.01$, $n = 4$). The enhanced signal of dopamine cations and decreased selectivity to AA and UA anions on O_2 plasma etched CNTYMEs indicates electrostatic effects play an important role for the detection of charged analytes.

Since the mechanisms of signal enhancement for the two surface modification methods are different, combining the two treatments at CNTYMEs is expected to have a synergistic effect by both increasing the surface area and density of functional groups. To test this theory, we treated CNTYMEs with 2-triggers of the anti-static gun followed by 1 min oxygen plasma etching. An example CV obtained at a CNTYME with combined treatment to 1 μM dopamine (Fig. 5A) demonstrates combined features of both anti-static gun treatment (symmetric oxidation/reduction peak) and O_2 plasma etching (positively shifted oxidation/reduction potential). The signal for dopamine increased 12 ± 2 times (from 6.6 ± 0.9 nA to 92 ± 18 nA, $n = 6$). The 12-fold improvement is a multiplication of 4-fold and 3-fold signal increasing of anti-static gun treatment and oxygen plasma etching alone. Thus, these treatments do work by independent mechanisms and can be combined for to make an

electrode that has large current enhancements for dopamine. The downside of the combined treatment is that the electrodes were extremely porous (Fig. 5B), and so the temporal resolution is not as fast (Fig. S5). The average 10–90% signal rise time of 3.7 ± 0.3 s ($n = 6$) was significantly larger than the pretreatment value of 1.1 ± 0.3 s (paired t-test, $p = 0.001$, $n = 6$). Therefore, the increases with the combined treatment demonstrate the independent mechanisms of activation, but future work is needed to optimize a combined treatment to provide both high sensitivity and temporal resolution.

Effect of Bio-fouling

Microelectrode surface fouling is a major concern for *in vivo* experiments because adsorption of macromolecules can severely affect the sensitivity and reproducibility of electrochemical sensing *in vivo*.^{13,39} To investigate biofouling, we placed electrodes in tissue for 2 hours, following procedures by Singh et al.¹³ Table 3 shows the oxidation current, E_p , and background current before and after tissue fouling at unmodified, O₂ plasma etched, and anti-static gun treated CNTYMEs. Dopamine oxidation currents decreased for all electrodes after tissue fouling but the currents at both O₂ plasma etched and anti-static gun treated CNTYMEs are significantly larger than the unmodified CNTYMEs (unpaired t-test, $p = 0.0001$, $n = 6$, for both comparisons), indicating both surface modification methods improve the current even after tissue exposure. In addition, the E_p after either O₂ plasma etching or anti-static gun treatment is significantly smaller than unmodified CNTYMEs (unpaired t-test, $p = 0.01$ ($n = 6$), and $p = 0.0001$ ($n = 6$), respectively), indicating the electron transfer is faster at treated CNTYMEs. The background current did not change after fouling, consistent with previous work where background currents were stable at polymer coated electrodes.¹³ In addition, the temporal response was not significantly slowed at any electrode due to fouling (Fig. S6). The O₂ plasma etched CNTYMEs had the least fouling in terms of percentage signal decrease (unpaired t-test, $p = 0.05$, $n = 6$) but the anti-static gun treated electrodes had the largest absolute currents after fouling because the currents were higher to begin with (unpaired t-test, $p = 0.05$, $n = 6$). The current after fouling at anti-static gun treated CNTYMEs is the same as the current at unmodified CNTYMEs without any fouling.

The reduced fouling at treated electrodes is likely due to the more hydrophilic nature of the surface.^{40,41} Hydrophobic surfaces promote adsorption of hydrophobic proteins, such as albumin, that are typically irreversible in aqueous conditions.³² In contrast, fouling caused by hydrophilic interactions tends to be more reversible.⁴² O₂ plasma etched electrodes have a higher density of oxygen-containing functional groups which are hydrophilic and had the least fouling of any electrode tested. While anti-static gun treated electrodes did not have a higher density of functional groups (as evidenced by the Table 2 surface data that shows similar percentages of oxygen on the surface to unmodified), the absolute amount of surface functional groups increased because the surface area increased. Other anti-fouling treatments, such as PEDOT:Nafion coatings,⁴³ have been developed and O₂ plasma etching and anti-static gun treatments have the potential to be combined with polymer or other anti-fouling strategies to improve the fouling resistance on carbon based electrodes.

Thus, this research has demonstrated that these two surface treatments, O₂ plasma etching and anti-static gun treatment, are simple and efficient modification methods that increase the sensitivity and reduce tissue fouling of CNTYMEs. Used separately, the treatments allow the tuning of surface chemistry and surface area, which could be used to enhance signals to different neurotransmitters in the future. Used together, the effects of the treatments are additive and provide an electrode with extremely high currents for dopamine.

Conclusions

In this work, we optimized two simple, rapid, reproducible, and efficient surface modification methods for CNT yarn microelectrodes: O₂ plasma etching and anti-static gun treatment. O₂ plasma etching enhances the surface oxide groups, increasing the sensitivity for dopamine around three-fold and significantly decreasing surface fouling by making the electrode more hydrophilic. The anti-static gun treatment is novel and low-cost, and enhances the signal four-fold by causing the CNTs in the yarn to stand up and separate, increasing the surface area. Because the two surface treatments work by independent mechanisms, they can be combined to enhance the currents for dopamine by 12-fold. O₂ plasma etching and anti-static gun treatment can be used to tune the surface properties of carbon nanomaterial microelectrodes, providing an opportunity to study the fundamental interactions of neurotransmitters with the electrode surface and the opportunity to create electrodes that might have beneficial properties for future *in vivo* studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Comparison of the response to 1 μ M dopamine at an unmodified disk CNTYME (black line) and the same electrode after O₂ plasma etching (red line) for 1 min (A–C) or 3 mins (D–F). The waveform was –0.4 V to 1.3 V and back at a scan rate of 400 V/s and scan repetition frequency of 10 Hz. (A, D) Background subtracted cyclic voltammograms of 1 μ M dopamine, (B, E) oxidation current versus time traces for a flow injection analysis experiment (dopamine was injected at 5 s and removed at 10 s), and (C, F) background charging currents in PBS buffer.

Figure 2. SEM images of (A) an unmodified CNTYME and electrodes after (B) 1 min and (C) 3 mins O₂ plasma etching. Scale bar: 500 nm.

Figure 3.

Comparison of the response to 1 μM dopamine at an unmodified disk CNTYME (black line) and the same electrode after treatment (red line) of 1 trigger (A–C), 2 triggers (D–F), and 4 triggers of anti-static gun treatments (G–I). The applied waveform had a scan rate of 400 V/s and scan repetition frequency of 10 Hz. (A, D, G) Background subtracted cyclic voltammograms of 1 μM dopamine, (B, E, H) measured oxidation current versus time for a flow injection analysis experiment (5 s injection between 5 and 10 s), and (C, F, I) background currents in PBS buffer.

Figure 4. SEM images of (A) an unmodified CNTYME and the same electrode after anti-static gun treatments with (B) 1 trigger, (C) 2 triggers, and (D) 4 triggers. Scale bar: 500 nm.

Figure 5. Anti-static gun/O₂ treated CNTYME. (A) Comparison of the response to 1 μM dopamine at an unmodified CNTYME (black line) and the same electrode after combined (anti-static gun treatment followed by O₂ plasma etching) treatment (red line). (B) SEM images of an anti-static gun/O₂ plasma etching treated CNTYME.

Table 1Comparison of dopamine detection at unmodified, O₂ plasma etched, and anti-static gun treated CNTYMEs

	Oxidation Current/nA	Background Current/nA	E _p	LOD/nM
O ₂ Plasma Etching				
Unmodified (n=6)	5.5 ± 0.9	122 ± 25	0.67 ± 0.02	21 ± 4
O ₂ Plasma Etching (1min, n=6)	16 ± 3 ^{**}	298 ± 35 ^{***}	0.607 ± 0.003 [*]	11 ± 2 [*]
Treated/unmodified Ratio	3.0 ± 0.4	2.3 ± 0.4	0.91 ± 0.02	0.7 ± 0.1
Anti-Static Gun Treatment				
Unmodified (n=8)	5.2 ± 0.3	130 ± 19	0.67 ± 0.02	24 ± 5
Anti-Static Gun Treatment(2 triggers, n=8)	22 ± 1 ^{****}	252 ± 36 ^{***}	0.61 ± 0.01 [*]	12 ± 2 [*]
Treated/unmodified Ratio	4.3 ± 0.4	1.9 ± 0.2	0.93 ± 0.01	0.6 ± 0.2

Significantly different than unmodified:

^{*} paired t test, p = 0.05,^{**} paired t test, p = 0.01,^{***} paired t test, p = 0.001,^{****} paired t test, p = 0.0001.

Table 2Surface properties of CNTYMEs with different surface modifications^a

	Surface Roughness R_z /nm	Raman D/G Ratio	Oxygen Content %
Unmodified CNTYME (n=4)	1540 ± 160	0.72 ± 0.01	10 ± 1
O ₂ Plasma Etched CNTYME (n=4)	1800 ± 212	$1.02 \pm 0.03^{***}$	$19 \pm 1^{***}$
Anti-static gun treated CNTYME (n=4)	$2600 \pm 400^*$	0.73 ± 0.01	10.0 ± 0.5

^a Mean roughness depth (R_z), D/G ratio from Raman spectra, and relative oxygen content at unmodified, O₂ plasma etched, and anti-static gun treated CNTYMEs. Significant difference from unmodified:

* one-way ANOVA, Bartlett's test, $p = 0.05$,

*** one-way ANOVA, Bartlett's test, $p = 0.001$

Table 3Comparison of dopamine detection at CNTYMEs before/after in tissue fouling^a

	Initial $I_{p,a}/nA$	$I_{p,a}$ after Fouling/nA	$I_{p,a}$ Ratio(Post/Pre Fouling)/%	E_p after Fouling/V	Background Current Ratio(Post/Pre Fouling)/%
Unmodified CNTYME (n=6)	5.3 ± 0.5	0.95 ± 0.05	18 ± 3	0.923 ± 0.003	103 ± 4
O ₂ Plasma Etched CNTYME (n=6)	15 ± 2	4.5 ± 0.2 ****	30 ± 3 *	0.86 ± 0.01 **	99 ± 3
Anti-static gun treated CNTYME (n=6)	21 ± 3	5.3 ± 0.4 ****	25 ± 3	0.860 ± 0.006 ****	96 ± 5

^a Oxidation current before and after 2H fouling, the $I_{p,a}$ post/pre-fouling ratio, E_p value after fouling, and background current post/pre-fouling ratio. Significantly different than unmodified:

* paired t-test, p 0.05,

** paired t-test, p 0.01,

**** paired t-test, p 0.0001.