Supplemental Information for:

Laser Treated Carbon Nanotube Yarn Microelectrodes for Rapid and Sensitive Detection of Dopamine in Vivo

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Supplemental Information includes additional text describing solutions, electrochemistry, surface characterization, in vivo measurements, and statistics; one figure showing the 4-hour and long term stability test of laser-treated CNTYMEs.

Supplemental Methods

Solutions

Dopamine hydrochloride and ascorbic acid were purchased from Sigma–Aldrich (St. Louis, MO). A 10 mM stock solution was prepared in $HClO_4$, and were diluted daily to the desired concentration in phosphate buffered saline (131.3 mM NaCl, 3.00 mM KCl, 10 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4, and 1.2 mM $CaCl_2$ with the pH adjusted to 7.4).

Electrochemistry

FSCV was performed with a ChemClamp potentiometer (Dagan, Minneapolis, MN, with 1 MOhm Headstage). The waveform was generated and the data was collected using a High Definition Cyclic Voltammetry (HDCV) breakout box, HDCV analysis software program (UNC Chemistry Department, Electronics Design Facility) and PCIe-6363 computer interface cards (National Instruments, Austin, TX). Electrodes were backfilled with 1 M KCl and a silver wire was inserted to connect the electrode to the potentiostat headstage. The typical triangular waveform swept the applied potential from –0.4 V to 1.3 V at 400 V/s versus an Ag/AgCl reference electrode, at a scan repetition frequency of 10 Hz. The repetition rate was varied for some experiments.

Electrodes were tested using a flow-injection system, as previously described. Analyte was injected for 5 seconds and current versus time traces were obtained by integrating the current in a 100 mV window centered at the oxidation peak for each cyclic voltammogram (CV). Background-subtracted CVs were calculated by subtracting the average of 10 background scans, taken before the compound was injected, from the average of five CVs recorded after the analyte bolus was injected.

Surface Characterization

Scanning electron microscope (SEM) images were taken on Merlin field emission SEM (Zeiss, Thornwood, NY) with a secondary electron detector using an accelerating voltage of 2 kV and a working distance of 5.0 mm. Raman spectroscopy measurements were performed

with a Renishaw 100 confocal micro-Raman system (Renishaw, Hoffman Estates, IL) with a 1800 lines/mm diffraction grating, 532 nm laser focused to a spot size of about 2 μm through a 100x objective, and a Peltier-cooled charge-coupled device detector. Three-dimensional laser (violet laser, 408 nm) scanning confocal microscopy (VK-X, Keyence, IL) was performed to measure the surface roughness.

In Vivo Measurements

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 rat was anesthetized with urethane (1.5 mg/kg i.p.), the scalp shaved, and 0.25 mL bupivicaine (0.25% solution) given subcutaneously. The working electrode was implanted in the caudate putamen (in mm from bregma: AP + 1.2, ML + 2.0, and DV – 4.5 to 5.0), the stimulating electrode in the substantia nigra (AP –5.4, ML + 1.2, and DV – 8.0), and the Ag/AgCl reference electrode in the contralateral side of the brain. The DV placement of the stimulating electrode was adjusted downward until a robust dopamine signal was measured. After implantation in the brain, the FSCV waveform was applied to the microelectrode for 30 min to allow the electrode to stabilize and the brain to recover. Stimulated release was electrically evoked using biphasic stimulation pulses (300 μA, 120 pulses, 60 Hz). For *in vivo* measurements, the scan repetition frequency was either 10 Hz or 50 Hz, which was chosen to avoid overlap with the electrical stimulation at 60 Hz.

Statistics

All values are given as mean ± standard error of the mean (SEM) for n number of electrodes and all error bars are SEM. Paired or unpaired t tests were performed to compare properties between two groups. A one-way ANOVA with Bonferonni post-tests was used to compare effects among multiple groups. All statistics were performed in GraphPad Prism6

(GraphPad Software,Inc., La Jolla, CA). Scanning electron microscopy images were processed using ImageJ (Rasband, W.S., National Institutes of Health, Bethesda, MD,).

Supplemental Figures

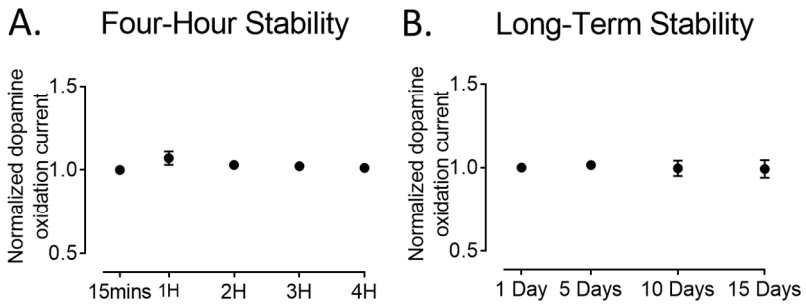

Figure S1. (A) Four hour (n=4) and (B) long term (n=7) stability test of laser treated CNTYMEs. Oxidation current to 1μM dopamine was normalized to the signal observed after 15 minutes equilibration. Electrochemical response was measured with scan rate of 400V/s and scan repetition frequency of 10Hz. Error bar is standard error of mean.