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Timed Electrodeposition of PEDOT:Nafion onto Carbon Fiber-Microelectrodes Enhances Dopamine Detection in Zebrafish Retina

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Carbon fiber-microelectrodes (CFMEs) are one of the standards for the detection of neurotransmitters such as dopamine (DA). In this study, we demonstrate that CFMEs electrodeposited with poly (3,4-ethylenedioxythiophene) (PEDOT) in the presence of Nafion exhibit enhanced sensitivity for DA detection. Scanning electron microscopy (SEM) revealed the smooth outer surface morphologies of polymer coatings, which filled in the ridges and grooves of the bare unmodified carbon electrode and energy-dispersive X-ray spectroscopy (EDX) confirmed PEDOT:Nafion incorporation. PEDOT:Nafion coated CMFEs exhibited a statistically enhanced two-fold increase in DA sensitivity compared to unmodified microelectrodes, with stability and integrity of the coated microelectrodes maintained for at least 4 h. A scan rate test revealed a linear relationship with peak DA oxidative current (5 μM), indicating adsorption control of DA to the surface of the PEDOT:Nafion electrode. As proof of principle, PEDOT:Nafion coated electrodes were used to detect potassium chloride (KCl)-induced DA release in zebrafish (*Danio rerio*) retinal tissue ex vivo, thus illustrating their applicability as biosensors.

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Brain neuronal activity occurs through the release of neurotransmitters into synaptic junctions. Chemical signaling molecules regulate postsynaptic cell activity in various ways depending on neurotransmitter species and the corresponding receptors.[1–3] This neurotransmission determines a wide range of physiological and behavioral processes and its dysregulation can lead to disorders, including Parkinson's disease, Alzheimer's disease, depression, and drug addiction.[4–9] Among different neurotransmitters, DA is of particular interesting for understanding sex,[10] learning, motivation, movement, Parkinson's disease,[11] and drug abuse,[12,13] as well as specific disorders.[14,15] To monitor neurotransmission, various electrochemical techniques are utilized, including amperometry, potential pulse methods, cyclic voltammetry,[16–18] and microdialysis, which causes some tissue damage and has relatively low spatial and temporal resolution.[19]

Fast scan cyclic voltammetry (FSCV) is a commonly used electrochemical technique to detect target neurotransmitters.[20] With FSCV, a triangular waveform is applied to microelectrodes to rapidly oxidize and reduce electroactive species at the electrode surface, resulting in a chemical-specific "fingerprint" that is used to identify and quantitate neurotransmitter levels.[17,21] Recordings with CFMEs have been utilized over the past several decades as the standard for detection of neurotransmitters such as DA[15,22] because of their high sensitivity, high spatiotemporal resolution, low cost, and ease of operation.[23] Novel materials, such as epoxy coated electrodes,[24,25] carbon nanospikes grown onto metal wires,[26] carbon nanotube fibers,[27,28] and carbon nanotube yarns,[29–31] and other materials[32] have been used to enhance CFMEs with increased chemical sensitivities. Thus, FSCV utilizing CFMEs provides enhanced selectivity and temporal resolution than conventional slow scan CV, allowing for the measurement of neurotransmitter release on a subsecond timescale.[33–36] However, enhancing the faradaic current and signal-to-noise ratio to improve analytical performance for in vivo DA detection still remains as a challenge.

Owing to their low-temperature processing, mixed ionic and electronic transport, and facile chemical tunability, conjugated polymers, such as poly(3,4-ethylenedioxythiophene) (PEDOT), polypyrrole, and polyaniline are attracting a great deal of attention for the development of biosensors.[37] Studies have shown that conductive polymers render increased conductivity, electroactive surface area, and electrocatalytic response of an electrode, leading to even greater increases in sensitivity.[38,39] Specifically, PEDOT has been gaining more interests due to its high environmental stability and in vivo stability associated with retaining its activity in a broad pH range.[40–43] The rigid, linear conformation of PEDOT chains facilitates charge transport and has the ability to tailor the surface properties depending on doping counterions leading to high sensing capabilities.[44–50] In particular, the introduction of Nafion onto the PEDOT polymer backbone provides additional benefits by creating a thin, uniform, and negatively charged electrode surface that will electrostatically attract the positively charged cationic DA.[51–53] Recently, locally evoked release and uptake of DA within the zebrafish brains were measured with FSCV and CFMEs.[33] Zebrafish are a desirable model for the study of neurotransmission[54,55] because their nervous system is more analogous to the human central nervous system than are invertebrates, and they are easier to genetically manipulate than rodents.[56–58] However, to the best of our knowledge, FSCV measurements of neurotransmitter release within the zebrafish retina have not yet been reported. This is of particular significance to understanding the neurochemistry of the vertical pathway and further applications such as understanding the effects of psychostimulants and diabetic retinopathy.

Here, we report the optimized electrodeposition time for PEDOT and Nafion onto CFMEs to generate enhanced microelectrode sensor for DA and other neurotransmitters. We optimize a moderate thickness to improve the sensitivity of PEDOT:Nafion coatings to maximize electrocatalytic effect while avoiding a sluggish electron transfer and interference of neurotransmitters adsorption (at longer deposition times) onto the electrode surface. Under these conditions, DA is adsorbed onto the surface of the CFME, and the electrodes were stable in the flow cell over four hours illustrating the integrity of the PEDOT:Nafion coating. Moreover, we show that electrodeposited PEDOT:Nafion coatings on CFMEs significantly improve the detection of released DA in zebrafish retinas, thus illustrating that these polymer-coated electrodes are a novel biosensor and assay for the detection of DA release ex vivo.

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Experimental

Materials.—Dopamine, serotonin, and adenosine (Sigma-Aldrich, Milwaukee, WI) were used to prepare stock solutions as received. Each 10 mM stock solution was prepared in 0.1 M perchloric acid and diluted with artificial cerebral spinal fluid buffer (aCSF; 145 mM NaCl, 2.68 mM KCl, 1.4 mM $CaCl_2$, 1.01 mM $MgSO_4$, 1.55 mM Na_2HPO_4, and 0.45 mM NaH_2PO_4 with pH adjusted to 7.4). Epon 828 Epoxy was obtained from Miller-Stephenson and diethylenetriamine hardener was obtained from Sigma Aldrich. All aqueous solutions were made with deionized water (Millipore, Billerica, MA).

Methods

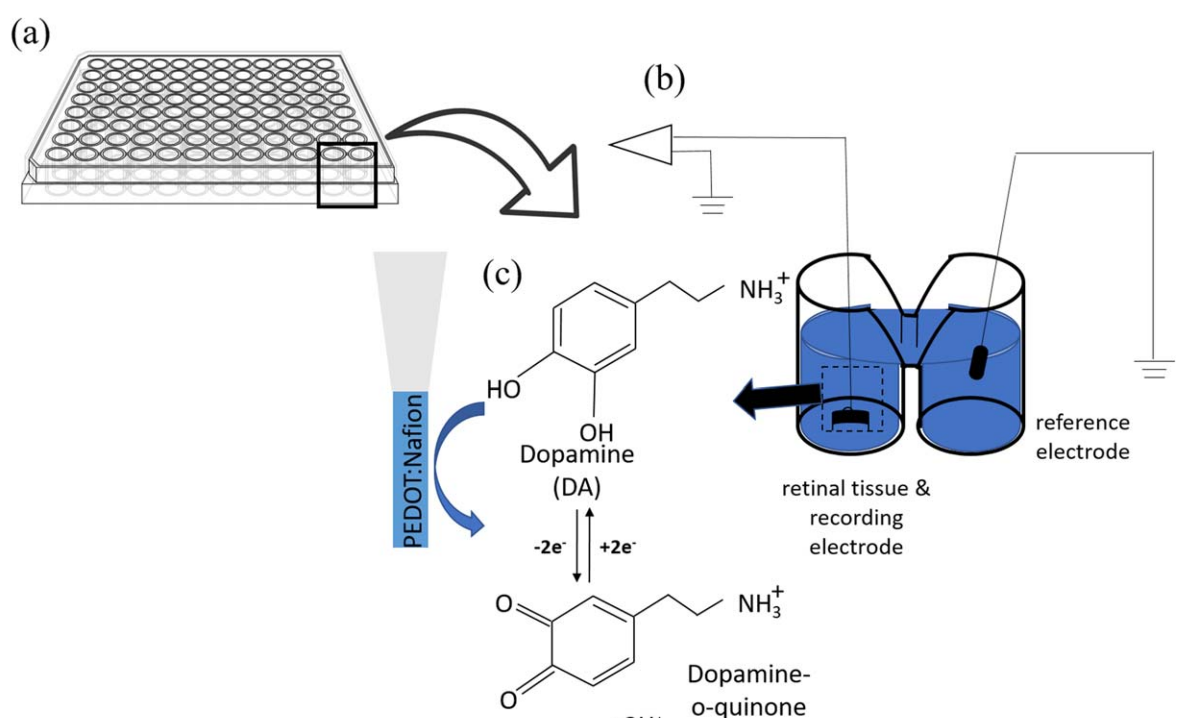
Carbon fibers (7 μm, Goodfellow, Huntingdon, England) were aspirated into cylindrical glass capillaries (1.2 mm by 0.68 mm, A-M Systems, Inc., Carlsborg, WA) using a vacuum pump (DOA-P704-AA, GAST, Benton Harbor, MI) to prepare CFMEs. Carbon fibers were pulled to form two electrodes on a vertical pipette puller (Narishige, model PC-100 and PE-22, Tokyo, Japan), followed by cutting the fiber to lengths of approximately 100–150 μm. Then, protruding carbon-fiber microelectrode tips were dipped in the epoxy hardener mixture (Epon 828 epoxy (Miller-Stephenson, Morton Grove, IL) and diethylenetriamine (Sigma Aldrich), 0.8% by mass resin, for approximately 15 s and then rinsed in acetone to wash away any excess residual epoxy.[15] The electrodes were cured in the oven for 4 h at 125 °C. Electrodeposition onto carbon fiber electrodes were then performed in a solution containing 3,4-ethylenedixoythiophene (EDOT) in the presence of Nafion as counterions by applying triangle waveform from +1.5 V to −0.8 V at 100 mV s^{-1} for 10, 20, 30, 40, and 60 s, respectively.

FSCV was performed with the WaveNeuro FSCV system with a 5 MΩ headstage (Pine Instruments, Durham, NC, USA). Data was collected using HDCV software (University of North Carolina Chapel Hill, Mark Wightman) and a computer interface board (National Instruments PC1e-6363, Austin, TX, USA). A triangle waveform was applied to the electrode from a holding potential of −0.4 V to 1.3 V and back at a scan rate of 400 V s^{-1} and a frequency of 10 Hz. The gain of the amplifiers were 200 and 1,000 nA V^{-1}. A silver–silver chloride (Ag/AgCl, 1.97 V) pellet (WPI, Sarasota, FL) was used as the reference electrode. Samples were tested in a flow injection analysis system (In Vitro/FSCV Microelectrode Flow Cell with xyz micromanipulator Translational Stage, Pine Instruments, Durham, NC). Buffer and samples were pumped through the flow cell at 1 ml min^{-1} using the NE-300 Just Infusion™ Syringe Pump (New Era Pump Systems, Farmingdale, NY). For the traditional waveform, the electrode was scanned from −0.4 to 1.3 V vs Ag/AgCl, reference electrode and back at a scan rate of 400 V s^{-1} and a wave application frequency of 10 Hz. Electrodes equilibrated for approximately 10 min in the flow cell to prevent the CFMEs from drifting at the waveform applied between each run. All data were background subtracted to remove any non-faradaic currents by averaging 10 CVs. Electrodes were tested at a flow rate of 1 ml min^{-1} using the aforementioned syringe pump.

Scanning electron microscopy images (SEM) images were obtained with a JEOL JSM-IT100 (JEOL, Tokyo, Japan). Bare or PEDOT polymer modified CFMEs were gold sputtered to prevent charging. The working distance was set to 10 mm and the accelerating voltage was 10 kV. Energy-dispersive X-ray spectroscopy (EDS/EDX) measurements were also performed to identify the chemical compositions of PEDOT:Nafion coatings on the surface of the CFME.

All data analysis was performed by using Graph Pad Prism 7. Statistical analysis was performed with a student's t-test, and significance was set to $p < 0.05$. All error bars are standard error of the mean (SEM) unless otherwise noted.

Ex vivo DA release in Zebrafish retina protocol.—Zebrafish (*Danio rerio*) adults were maintained at 28 °C–29 °C on a 14 h light: 10 h dark photoperiod in the Zebrafish Facility at American University.[54,55] On the day of an experiment, adult animals (mixed sex) were removed from stock tanks, anesthetized in 0.02% tricaine



Scheme 1. Ex vivo Measurements of Potassium Chloride (KCl) stimulated release of dopamine using CFMEs and FSCV. (a) 96-well plate showing placement of electrode. (b) Illustation of working and reference electrode placement in well and retinal tissue. (c) Mechanism of Dopamine Oxidation of PEDOT-coated CFME.

solution and decapitated. Eyes were then removed in preparation for FCSV. All animal procedures were approved by the Institutional Animal Care and Use Committee at American University.

Preparation of retinal tissue.—Excised eyes were placed on a dissection plate. Using forceps, the eye was placed cornea side-up and held in place. Two small radial incisions were made in the periphery of the cornea 180° apart with an ALCON I-knife-Mackool sideport knife. The edges of one incision was then retracted with forceps creating a tear across the cornea that connected the two initial incisions, and the lens was removed forming an eyecup. The eyecup was then inverted, so the retinal surface projected outward. This "inverted eyecup" was placed on a piece of 0.45 μm filter paper (Millipore) ~0.5 cm in length. For optimum recording and accessibility of the electrode, retinal tissue was positioned to be elevated as much as possible on the filter paper. Prepared retinas were then placed into a 96-well plate and anchored in place using a small drop of Vaseline. The well plate that was modified so two of the wells were directly connected. This set-up resulted in the retina + filter paper in one of the wells and the reference electrode in the adjacent well. Both wells were filled with 200 μl aCSF buffer, taking care that an equal volume of buffer was present in both wells and that the fluid was contiguous between the sides. The well plate was then stabilized on the recording stage using labeling tape.

FSCV recordings in retinal tissue.—The tip of a carbon fiber electrode was positioned in the retinal tissue using a xyz micromanipulator (Pine Instruments). A reference electrode was placed into the buffer of the adjoining well as depicted in Scheme 1. After 10 min of equilibration, 5 μl of saturated KCl (4 M) was injected into the well containing the retinal tissue, without touching the buffer meniscus so that the electrode and tissue were undisturbed. After a recording was taken, the solution was removed from the wells and fresh buffer added, so replicate recording from the same tissue could be taken. FSCV recordings from a given eyecup continued until the signal became too weak for detection.

Results and Discussion

PEDOT:Nafion coating.—To determine the efficacy of PEDOT:Nafion coating of electrodes, we performed three analyses: (1) SEM and EDX visualization of coated electrodes, (2) identification of optimal time for electrodeposition, and (3) determining DA sensitivity vs uncoated electrodes and vs other molecules that can be oxidized.

The morphology of bare and PEDOT:Nafion modified CFMEs was observed by SEM as represented in Fig. 1. The image of bare, unmodified CFMEs which are approximately 7 μm in diameter reveal deep groves and ridges. On the other hand, PEDOT:Nafion modified CFMEs have a thin coating over the entire surface after polymer electrodeposition. (Figs. 1b, 1c) The glass insulation of the CFMEs can easily be seen in Fig. 1b, while 1c is a higher magnification image of the microstructure of the coated electrode. The ridges and grooves of the bare unmodified carbon electrode have been filled in, thus creating smoother outer surface features at the meso-scale. EDS analysis identified the presence of sulfur and fluorine elements, relative to the presence of carbon, confirming the incorporation of PEDOT:Nafion onto CFMEs (Fig. S1 is available online at stacks.iop.org/JES/167/115501/mmedia). A deposition CV trace showing an oxidation current (+1.5 V) is attributed to the oxidation of EDOT to form PEDOT coatings, indicating successful deposition of PEDOT (Fig. S2).

To optimize DA detection sensitivity of the coated electrodes, we determined the effect of electrodeposition duration time (10, 20, 30, or 40 s) for PEDOT:Nafion coatings. The aforementioned deposition times were chosen, since longer deposition time (>60 s) and consequent thick polymer layers could hinder the adsorption of neurotransmitters onto the electrode and produce a sluggish electron transfer. Furthermore, the protruding carbon fiber was cut to a length at approximately 100 microns coupled with high gain amplifiers (1,000 nA V^{-1}) to assure that background current is under the maximum current threshold to prevent overloading of the FSCV system after subsequent conductive PEDOT:Nafion coatings. The sensitivity of bare and PEDOT:Nafion modified electrodes was then determined by the linear regression slope of calibration plot relating peak oxidative current as a function of DA concentration in the range of 1 to 5 μM DA as represented in Figs. 2a–2d and the corresponding t-tests. (Fig. S3) The same electrode was pre-calibrated and then used to compare the sensitivity of each electrode before and after PEDOT:Nafion deposition.

DA sensitivity for CFMEs increased with increasing PEDOT:Nafion deposition time up to 30 s. With this deposition time, we observed a significant increase in sensitivity from 10.5 ± 0.5 nA μM^{-1} for uncoated carbon fiber electrodes to 58.0 ± 2.3 nA μM^{-1} (approximately six-fold larger) for coated electrodes. A slightly lower enhancement of sensitivity of the 20 s deposition time compared to 10 s was observed. This was possibly caused by a subtle variation between each CFME and less polymer being electrodeposited at the surface of the electrode at the shorter time period. In other words, the 10–20 s deposition time period is not sufficiently long enough to allow for an appreciable amount of PEDOT:Nafion polymer to attach on the surface of the electrode. However, the sensitivity of electrodes that had a 40 s deposition time was slightly decreased, suggesting longer deposition duration decreases sensitivity towards DA. This moderate thickness of the PEDOT:Nafion at 30 s film enhances charge-transfer and augments adsorption sites, which correlates with increasing the electroactive surface area. Furthermore, it can also be attributed to electrocatalytic effect of the PEDOT:Nafion coatings[38,39] evidenced by a larger background charging current and higher peak oxidative CV response from our PEDOT:Nafion coated microelectrodes.

Bare, 10 s, and 20 s PEDOT:Nafion deposited electrodes had relatively square current vs time (I vs T) traces. On the other hand, coated electrodes formed after 40 s and 60 s deposition times had

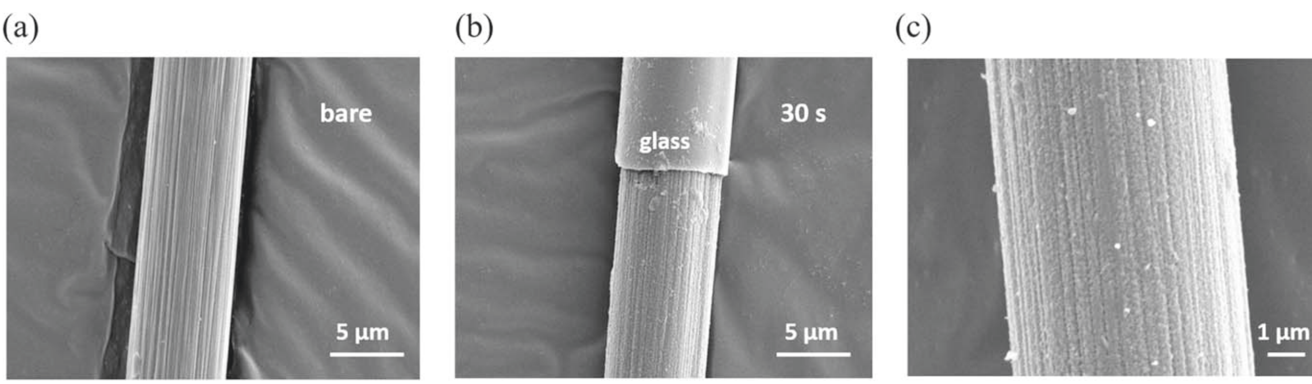


Figure 1. SEM images of (a) bare uncoatde carbon fiber electrode showing grooves and ridges, (b) carbon fiber electrode electrodeposited with PEDOT:Nafion for 30 s with the clear presence of the glass insulation at the top of the CFME, (c) zoomed-in view carbon fiber microstructure.

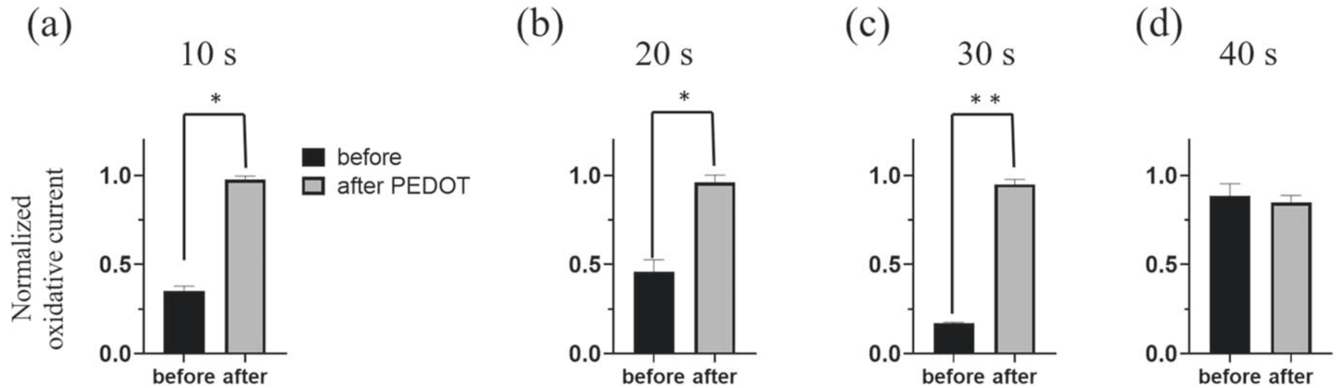


Figure 2. The effect of electrodeposition duration time on dopamine (DA) detection sensitivity at 5 μM DA, compared to the uncoated carbon fiber electrode after (a) 10 s (t-test, $p < 0.0001$), (b) 20 s (t-test, $p = 0.0004$), (c) 30 s (t-test, $p < 0.0001$), (d) 40 s (t-test, $p = 0.6645$). The data was normalized to the largest peak oxidative current after three measurements. All the measurements were carried out in artificial cerebral spinal fluid buffer (aCSF).

more curved I (current) vs T (time) trace (Fig. S4) denoting lower temporal resolution. These longer deposition times, past 40 s, lead to excessive polymer coatings on the surface of the electrode, which can slow electron transfer, increase response time, and lower sensitivity since the electron must diffuse through a larger surface area of polymer to reach to the electrode surface (Fig. S5). This relatively slower electron transfer and response times are shown by the deviation from the square I vs T trace.

Cyclic voltammetry electrodeposition of PEDOT:Nafion onto CFMEs may lead to an increased Nafion density at the electrode surface.[51] Consequently, Nafion groups can improve the affinity and enhance selectivity toward DA detection by electrostatically repelling negatively charged interferants such as DOPAC, ascorbic acid, or 5-hydroxyindoleacetic acid (5-HIAA).[59] When applying a potential, then, the waveform electrochemically etches the surface of the electrode and breaks the carbon-carbon bonds at the surface to functionalize it with negatively charged oxide groups, which electrostatically attracts and adsorbs dopamine and other cationic monoamines.[60] This also increases the electroactive surface area of the CFMEs, which can account for higher sensitivity for neurotransmitter measurements. Overall, these properties make our coated CFMEs well-suited to electrostatically attract and adsorb positively charged catecholamines (such as DA) to their surface by increasing negative charge and electroactive surface area.

Next, we compared the sensitivity of PEDOT:Nafion coated CFMEs for DA, serotonin, and adenosine detection with respect to the uncoated CFMEs. As shown in Fig. 3a and the example cyclic voltammogram of 5 μM DA (d), a 30 s PEDOT:Nafion coating significantly enhanced the sensitivity for DA detection ($p = 0.0018$, $n = 6$, t-test) compared to the uncoated bare CFMEs. At the physiological pH 7.4, the amine groups of DA are protonated, resulting in an overall positive charge. The negatively charged Nafion groups, then, facilitate the electrostatic interaction/adsorption of DA at the electrode surface, resulting in high sensitivities toward DA detection. It is also possible to detect serotonin and adenosine using PEDOT:Nafion electrodes as shown in normalized average (Figs. 3b, 3c). However, there is no statistically significant change in the peak oxidative currents for serotonin and adenosine for bare and PEDOT:Nafion coated microelectrodes at the time periods chosen. The corresponding cyclic voltammogram example of 5 μM serotonin, and adenosine are shown in Figs. 3e and 3f, respectively, using PEDOT:Nafion modified electrodes.

The shape of the cyclic voltammogram for adenosine detection with PEDOT:Nafion coated CFMEs suggests that the adsorption of adenosine onto the surface of the electrode is altered by the electrodeposition of PEDOT:Nafion. This could be due to the irreversible oxidation mechanism of adenosine, which differs from the quasi-reversible DA oxidation scheme. The bulky molecular structure of serotonin and adenosine (Fig. S6) may lead to a steric hindrance effect on the diffusion towards the electrode surface, and the resulting weak electrostatic interactions with Nafion groups. This selectivity observed for enhanced DA detection compared to serotonin and adenosine is also likely due to slightly tighter π–π stacking interactions between its phenyl group with PEDOT-rich coatings as well as a hexagonal lattice of the graphitic carbon electrode.[61] Taken together, PEDOT:Nafion coatings on CFMEs enhance the sensitivity and selectivity for dopamine detection with respect to other analytes.

Adsorption control of DA on PEDOT:Nafion CFMEs.—To determine whether 30 s PEDOT:Nafion coatings affected adsorption- or diffusion-control to the surface of microelectrodes with respect to the neurotransmitters and metabolites detection, we performed stability, scan rate, and concentration tests. As shown in Fig. 4a, there was no significant change in peak oxidative current over 4 h, which is the typical duration of an in vivo experiment with CFMEs. The current slightly rose in the first hour due to the electrochemical etching of the electrode surface upon applying the waveform, which increased surface area, roughness, and functionalization with negatively charged oxide groups to make the electrode more sensitive towards DA.[60] It is important to note that the polymer coating is not compromised or leached off of the electrode after 4 h of applying the triangle waveform as verified by SEM/EDS measurements (data not shown). Therefore, these electrode coatings have utility for longer in vivo and ex vivo measurements.

To measure the adsorption control of dopamine towards the electrode surface, we then varied the scan rates in a range from 50 to 750 V s^{-1} to detect 5 μM DA, which showed a linear relationship between scan rate and peak oxidative current. This confirmed its adsorption-controlled interaction (from the Randles-Sevcik equation) with the surface of the PEDOT:Nafion coated electrode (Fig. 4b) ($R^2 = 0.919$). Concentration testing revealed that a peak oxidative current was linear with respect to DA concentration up to 10 μM as shown in Fig. 4(c). The slope of PEDOT:Nafion coated electrode ($R^2 = 0.977$) is 24.06 ± 1.97 whereas that of bare electrode ($R^2 = 0.977$) is 11.71 ± 0.96, indicating two-fold increase of sensitivity for DA detection of PEDOT:Nafion coated microelectrodes. It appears that an asymptotic curve is displayed at higher concentrations, suggesting DA concentrations higher than 10 μM are saturated and all adsorption sites are occupied by excessive DA (Fig. 4d).

Measuring DA release in zebrafish retina.—To determine the utility of CFMEs electrodeposited with PEDOT:Nafion to make real-time measurements in biological tissue, we measured the KCl-stimulated release of DA in zebrafish retina. With these experiments, we are illustrating the DA release by amacrine and interplexiform

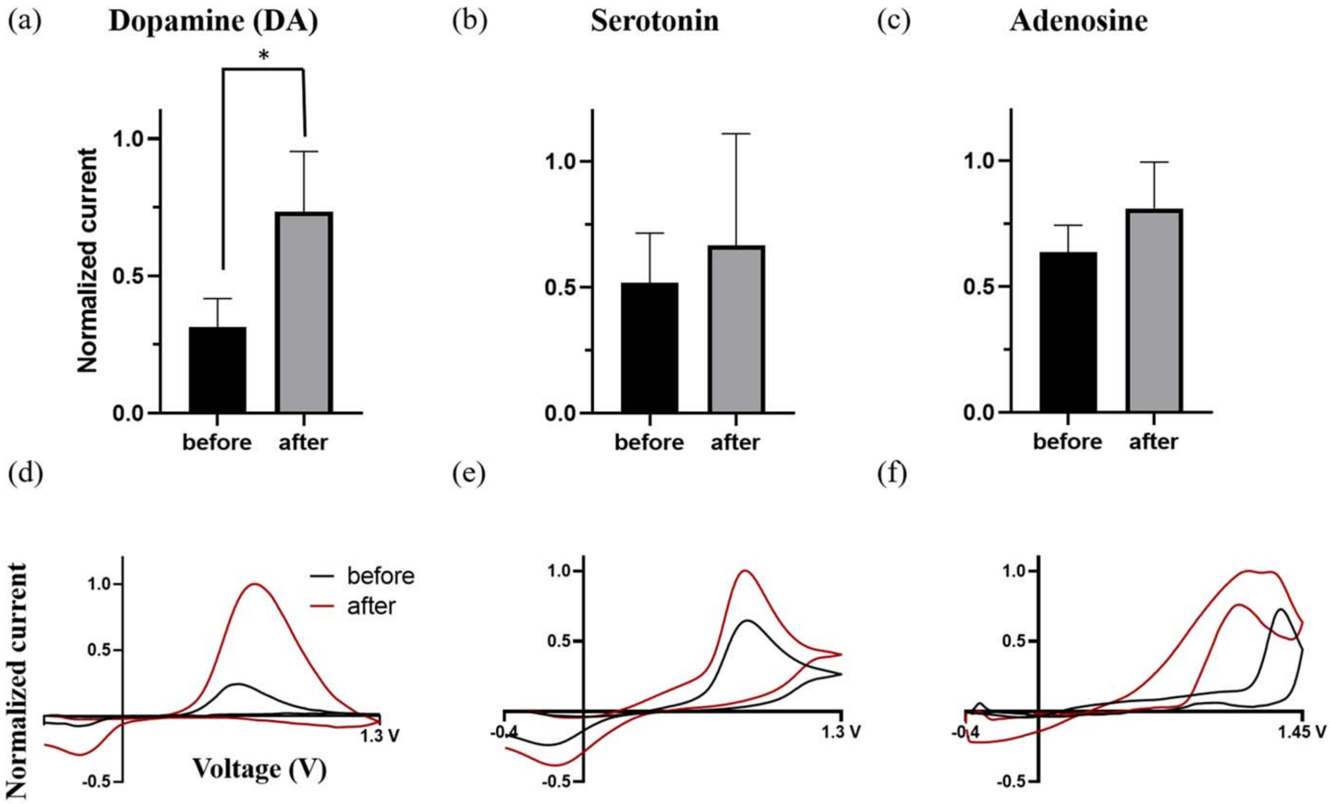


Figure 3. The effect of PEDOT:Nafion coatings in comparison to the bare unmodified electrodes on the sensitivity of (a) 5 μM dopamine (DA) detection ($p = 0.0018$, $n = 6$, t-test), (b) 5 μM serotonin detection, and (c) 5 μM adenosine detection with example cyclic voltammograms (d)–(f) of uncoated and Nafion coated CFMEs to the bottom.

cells in zebrafish retina. Retinal dopamine is a neuromodulator that affects neurotransmission in both inner and outer retina. The application of excessive KCl depolarizes retinal neurons, causing exocytosis of DA into the synapse and extracellular space, which is subsequently detected by the CFMEs.

The electrode was placed directly into retinal tissue (Fig. 5a, Scheme 1). After an application of 5 μl saturated KCl (4 M), the PEDOT:Nafion coated electrode detected the current changes due to DA release and oxidation on the electrode surface as shown in false 3D color plot (Fig. 5b). Two green traces represent the oxidation and release of DA by KCl, whereas dark blue spot is associated with the reduction of DA. An I vs T curve implies adequate temporal resolution and fast electron transfer kinetics between the electrode and the tissue to measure released DA as represented in Figs. 5c–5d.

Repeated FSCV recordings from a given eyecup continued until dopamine release was depleted and the signal became too weak for detection. It is likely that the repeated application of KCl depleted DA storage pools or that DA could be fouling the surface of the electrode, resulting in deceased responsiveness of the tissue over time. The PEDOT:Nafion coated microelectrodes detected released DA through four individual KCl stimulations of the same tissue, while uncoated bare CFMEs appeared to lack any typical DA shaped CV response (Figs. S7–S9). It demonstrates that PEDOT:Nafion coated microelectrodes are more sensitive and have lower limits of detection (LOD) for DA detection ex vivo in zebrafish compared to bare CFMEs. This can be attributed to the bare CFMEs having shorter (disc-like) carbon fiber tips, which also have lower sensitivities than longer cylinder-like microelectrodes. Subsequent PEDOT: Nafion coatings provide increased electroactive surface area, conductivity, and enhanced charge transport, which significantly improve both in vivo and ex vivo analytical performance compared to the bare uncoated microelectrodes.[62] Each electrode was pre- and post-calibrated using 1 μM DA solution before measuring the KCl-stimulated DA release in order to extrapolate released concentrations based on relative peak oxidative currents.[63,64] We measured average released DA concentrations of 0.51 ± 0.20 μM ($n = 3$) in zebrafish retina. These evoked DA concentrations in zebrafish retina are similar to levels in whole brain (0.49 ± 0.13 μM) and sagittal brain slices (0.59 ± 0.28 μM).[33] This shows proof of principle verification that PEDOT:Nafion coated CFMEs can be utilized to enhance and measure the stimulated release of neurotransmitters in zebrafish retina, a growing model system for neurochemical analysis. To our knowledge, this is the first measurement of neurotransmitter release from zebrafish retina with FSCV and polymer modified carbon electrodes.

Conclusions

This work has demonstrated the enhanced detection of DA by electrodepositing conductive polymer, poly (3,4-ethylenedioxythiophene) onto CFMEs in the presence of Nafion. Electropolymerization of PEDOT:Nafion for 30 s exhibited substantial enhancement of the sensitivity for DA detection compared to uncoated bare electrodes. Shorter electrodeposition times show less enhancement for DA detection, while electrodeposition longer than 30 s slowed electron transfer at the surface of the electrode due to the great variations of polymer applied. Stability, concentration, and scan rate experiments revealed DA to be adsorption controlled, thus the polymer did not alter the adsorption properties of DA onto the surface of the CFMEs. Proof of concept measurements of KCl stimulated DA release were performed in zebrafish retina with PEDOT:Nafion electrodeposited CFMEs. This is the first measurement of the stimulated release of DA in zebrafish retina with CFMEs and FSCV. The fast subsecond detection of neurochemicals in the retina could potentially provide greater understanding of neurological and ophthalmological disease states such as diabetic retinopathy and the neurotransmission of the vertical pathway.

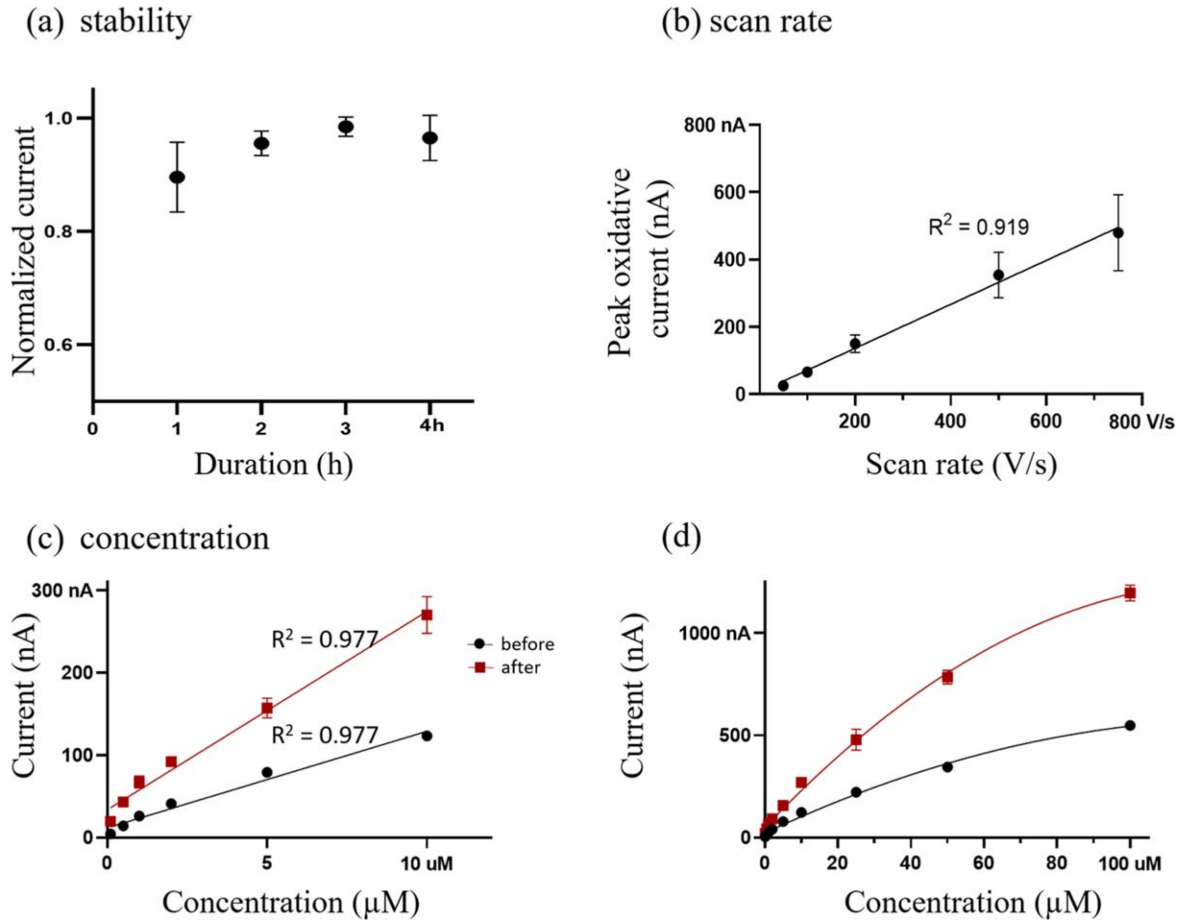


Figure 4. Adsorption control testing of PEDOT:Nafion coated electrodes (a) stability test showing a stability toward dopamine (DA) detection (peak oxidative current) for at least 4 h, (b) scan rate test showing a linear relationship between scan rate and peak oxidative current for DA (5 μM), (c) concentration showing a linear relationship between DA concentration and peak oxidative current between 100 nM abd 10 μM and (d) an asymphotic curve at higher concentration (10–100 μM).

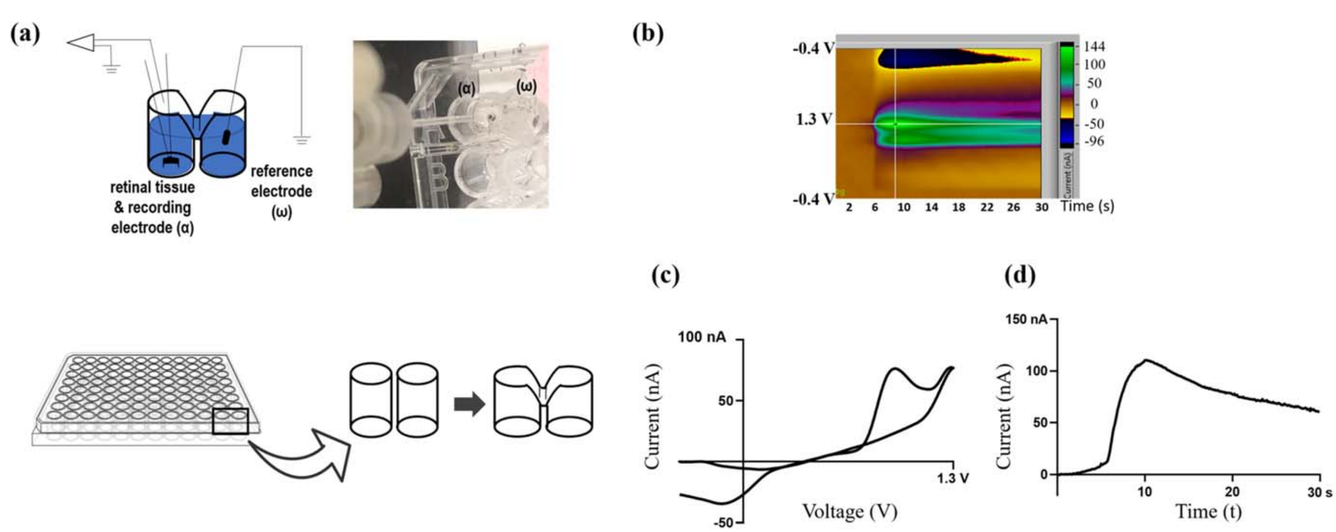


Figure 5. (a) Experimental Schematic. Retinal tissue (inverted eye cup) and working electrode (CFME) were placed in one well (α) and the reference electrode (Ag/AgCl) was placed into the adjacent well (ω). During recordings, both wells were filled with aCSF. The chamber for FSCV recording from zebrafish retinal tissue is a modified 96-well plate. The separation between two adjacent wells was cut, forming two connected chambers. Representative saturated potassium chloride (KCl) evoked dopamine (DA) release detection by PEDOT:Nafion coating (b) current response in false color plot (c) cyclie voltammogram (CV), and (d) I (current) vs T (time) trace.

Therefore, these electrodes show utility as enhanced neurotransmitter sensors in biological tissue. Future work could investigate the effect of the redox state of PEDOT on the sensitivity and selectivity of DA detection. These studies would pave the way for the rational design of PEDOT modified CFMEs in sensing neurotransmitters, namely by enhancing oxidized state and electron transfer.

Acknowledgments

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