Journal of Electroanalytical Chemistry 848 (2019) 113295

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Highly sensitive and selective detection of dopamine using overoxidized polypyrrole/sodium dodecyl sulfate-modified carbon nanotube electrodes

Gayoung Eom[a,b,1], Chaerin Oh[a,1], Jeong Moon[c,d], Hyojung Kim[a], Mi Kyung Kim[a], Kiup Kim[a], Ji-Won Seo[a,b], Taejoon Kang[d,e], Hyunjoo J. Lee[a,*]

[a] School of Electrical Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea
[b] Information and Electronics Research Institute, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea
[c] Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea
[d] Bionanotechnology Research Center, Korea Research Institute of Bioscience & Biotechnology, Daejeon 34141, Republic of Korea
[e] Department of Nanobiotechnology, Korea Research Institute of Bioscience & Biotechnology School of Biotechnology, University of Science and Technology, Daejeon 34113, Republic of Korea

ARTICLE INFO

Keywords:
Dopamine
Polypyrrole
Carbon nanotubes
Overoxidation
Electrochemical sensor
In vitro monitoring

ABSTRACT

Dopamine (DA), an organic chemical neurotransmitter in the human brain, plays important roles in neuronal reward, motor control, and decision making. Thus, accurate quantification of DA concentration is essential for the investigation of various dopaminergic neural circuits and diagnosis of neurological diseases. Herein, we report an overoxidized polypyrrole/sodium dodecyl sulfate (SDS)-modified multi-walled carbon nanotube (OPPy/SDS-CNT) electrode which allows detection of DA with high resolution and selectivity. By using SDS as a dopant and sodium hydroxide (NaOH) as an oxidizing agent, highly sensitive detection of DA down to 5 nM with a detection limit of 136 pM is achieved. Moreover, due to the strong electrostatic interaction between the negatively-charged electrode and the positively-charged DA molecules, selective electrochemical detection of DA is successfully demonstrated in the presence of ascorbic acid (AA) and glucose (Glc). Lastly, by demonstrating *in vitro* detection of DA secreted from dopaminergic cells (PC12 cells) and examining biocompatibility of the electrode, we show the potential of our OPPy/SDS-CNT electrode as a promising candidate for a functional neural interface for *in vitro* and *in vivo* monitoring of DA concentrations.

1. Introduction

Dopamine (DA), a catecholamine, is an important neurotransmitter that is involved in multiple neural pathways in our central nerve system [1]. Dysregulation and deficiency of DA are often observed in a number of pathological conditions, such as Parkinson's disease, attention deficit hyperactivity disorder (ADHD), Huntington's disease, depression, and schizophrenia [2–4]. Thus, accurate quantification of DA concentration is of great significance in the clinical diagnosis, prognosis, and treatment of neurological disorders [3,5]. Since the basal concentration of DA in the extracellular fluid of brain is low in concentration (*i.e.*, 5–60 nM), high-performance analyzers with high sensitivity such as fluorescence microscopy, high-performance liquid chromatography, and spectrophotometry are often employed for quantification of neurotransmitters [6]. However, these methods are often expensive and require sophisticated equipment and long pretreatment steps.

Common detection methods for neurotransmitters suitable for low-cost miniaturized platforms include immunoassay and electrochemistry. Detection based on immunoassay exhibits high sensitivity and high selectivity due to strong affinities between analytes and receptors. However, immunoassay-based detection is often irreversible and requires several hours due to analyte incubation process [7]. In contrary, a biosensor based on electrochemical detection offers easy operation, cost-effectiveness, robustness, and real-time monitoring [8–10]. Specifically, electrochemistry technique provides sub-seconds measurements of redox signals and thus is suitable for the detection of DA released in milliseconds [11,12]. However, due to the similar window of oxidation potentials of neurotransmitters, electrochemical sensors often suffer from low selectivity. Therefore, it is still a challenge to develop a low-cost biosensor that is sufficiently sensitive and selective for real-time detection of low concentrations of DA.

One approach to enhance selectivity for electrochemical detection

* Corresponding author.
E-mail address: hyunjoo.lee@kaist.ac.kr (H.J. Lee).
[1] These authors contributed equally.

https://doi.org/10.1016/j.jelechem.2019.113295
Received 30 May 2019; Received in revised form 8 July 2019; Accepted 9 July 2019
Available online 10 July 2019
1572-6657/

of electropositive neurotransmitters is to use overoxidation of conductive polymer to endow electronegativity. For instance, when polypyrrole (PPy), a conductive polymer, is overoxidized, electronegative groups (−COOH, −C(=O)−) are formed on the PPy backbone, which attracts the electropositive groups of DA and significantly enhances the sensitivity [13,14]. Among numerous conducting polymers, PPy has been widely utilized in the detection of DA because an amine group on pyrrole ring induces the enhancement of biomolecular sensing [15]. In addition, PPy exhibits good conductivity, biocompatibility, redox properties, and environmental stability [16–18]. In conjunction with a conductive polymer, carbon nanotubes (CNTs) are often used due to its large surface areas (700–1000 m^2/g), high conductance, low impedance, and high charge transfer characteristics [15,19–22].

Thus, in this work, we developed an electrochemical biosensor based on a composite of overoxidized PPy (OPPy) and carbon nanotube (CNT) for selective and sensitive detection of DA. In contrast to other works on PPy/CNT modified electrodes, we further enhanced sensitivity by using sodium hydroxide (NaOH) as an oxidizing agent for overoxidation and enhanced selectivity by using sodium dodecyl sulfate (SDS) as a dopant. Using this protocol, we achieved a limit of detection (LOD) of 136 pM and demonstrated successful detection of DA in the presence of interferences such as ascorbic acid (AA) and glucose (Glc). Sensitive and selective sensing is attributed to strong electrostatic interaction between the negatively-charged OPPy/SDS-CNT electrode and the positively-charged DA molecules. Moreover, our OPPy/SDS-CNT electrode successfully measured DA released from dopaminergic cells (PC12 cells) in real time. Based on high sensitivity, selectivity, and biocompatibility, our proposed strategy for preparing OPPy/SDS-CNT electrode is a promising surface modification method for functional neural interfaces for *in vivo* monitoring of endogenous DA concentration.

2. Material and methods

2.1. Reagents and materials

Sodium hydroxide (NaOH, Reagent grade) and sodium dodecyl sulfate (SDS, BioReagent) were purchased from Sigma-Aldrich. Multi-walled carbon nanotubes (MWCNTs, purity ≥95 wt%, average diameter 20 nM, 20–100 μm, bulk density 0.04–0.06 g/cm^3) were purchased from CNT Co. (Republic of Korea). Pyrrole solution (Reagent grade), potassium ferricyanide (99%), and potassium ferrocyanide (ACS Reagent), dopamine hydrochloride (DA), L-ascorbic acid (AA, Reagent grade), sodium chloride (NaCl, ReagentPlus®), dimethyl sulfoxide (DMSO, Anhydrous) were purchased from Sigma-Aldrich. D(+)-glucose (Glc, ACS Reagent) was purchased from ACROS Organics. Phosphate buffered saline (PBS, pH 7.4) was purchased from Thermo Scientific. Sulfuric acid (6 N standardized solution) was purchased from Alfa Aesar. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Invitrogen™.

2.2. Fabrication of OPPy/SDS-CNT electrodes

Gold (Au) electrodes were prepared by evaporating 150 nm of Au and 5 nm of Cr on a silicon wafer and dicing the wafer into 1 cm × 1.5 cm square pieces. Prior to electrochemical deposition of the OPPy/SDS-CNT composite, the Au electrodes were cleaned by cycling the potential from −0.2 V to 1.6 V in 0.5 M H_2SO_4. A CNT solution was prepared by adding 1 g/L MWCNT and 20 g/L SDS in 0.1 M NaCl. The solution was sonicated for 20 min. As-purchased pyrrole solution was added into the CNT solution where the final concentration of pyrrole was 0.5 M. For electrochemical deposition, this mixture was diluted in deionized (DI) water by three folds. Fabrication of the OPPy/SDS-CNT electrode consisted of two steps: electrodeposition of PPy/SDS-CNT and overoxidation. After immersing the cleaned Au electrode in the mixture of SDS-modified CNT and pyrrole, a constant potential of 0.7 V was applied for 5 s for electrochemical deposition of the PPy/SDS-CNT composite. Upon applying potential, electrochemical polymerization of the monomers of pyrrole was induced and SDS-modified CNTs in the mixture were co-deposited with PPy. The PPy/SDS-CNT electrode was dried for 2 min and rinsed in DI water. As the last step, the as-prepared PPy/SDS-CNT electrode was overoxidized in 0.1 M NaOH using cyclic voltammetry. The potentials were swept twice from 0 to 1 V. The OPPy/SDS-CNT electrode was dried and washed in DI water.

2.3. Electrochemical measurements

Electrochemical characteristics of our electrodes were measured using a potentiostat (Autolab PGSTAT302N, Metrohm AG, Switzerland). A 3-electrode system with Ag/AgCl (3 M NaCl) as a reference electrode and platinum (Pt) wire as a counter electrode was used. The cyclic voltammograms (CV) of dopamine was obtained by sweeping the potential from −0.2 V to 0.6 V at 50 mV/s in PBS (pH 7.4) solution. The differential potential voltammograms (DPV) of dopamine was obtained by sweeping the potential from −0.1 V to 0.35 V. The step and modulation amplitudes were 0.005 V and 0.025 V, respectively. The modulation time and interval time were 0.025 s and 0.5 s, respectively. The total measurement times required for the CV and DPV were 16 s and 45 s.

2.4. Cell culture

PC12 cell line was purchased from Korean Cell Line Bank and cultivated according to the supplier's protocol. We used a medium which consists of 85% RPMI1640 (11835-030, Thermo Scientific, USA), 10% heat-inactivated horse serum (H1138, Sigma-Aldrich, USA), and 5% heat-inactivated fetal bovine serum (FBS, 26140-079, Thermo Scientific, USA). Cells that were attached to cell culture flasks were incubated at 37 °C and 5% carbon dioxide (CO_2).

2.5. In vitro detection of dopamine

OPPy/SDS-CNT electrode was first cleaned by cycling the potential from −0.2 V to 1.6 V for three times in PBS. After the cleaning step, PC12 cells were carefully transferred on the OPPy/SDS-CNT electrode. By applying a constant potential of 0.18 V on the OPPy/SDS-CNT electrode, we continuously measured the oxidation current using the amperometry technique provided through the potentiostat. To stimulate the PC12 cells and to induce dopamine release, we used a syringe pump to inject 20 μL of high concentration potassium chloride solution (3 M) into the medium. Finally, the DA concentration released from PC12 cells was estimated by integrating the current peak.

2.6. In vitro toxicity test

Vybrant MTT cell proliferation assay kit was purchased from Invitrogen™. First, cells were cultured in a standard 96-well microplate. Cell medium was removed and replaced with 100 μL of fresh culture medium. For labeling the cells with MTT, MTT stock solution (12 mM, 10 μL) was added to each well and incubated at 37 °C for 4 h. Then, 85 μL of cell medium was removed and 50 μL of dimethylsulfoxide (DMSO) was added to each well. The medium was incubated at 37 °C for 10 min. The absorbance was read at 540 nm.

3. Results and discussions

3.1. Surface characteristics of OPPy/SDS-CNT electrodes

Our OPPy/SDS-CNT electrodes were successfully fabricated using three steps: (1) electrochemical cleaning of the Au electrodes, (2) electrochemical deposition of the PPy/SDS-CNT composite, and (3) electrochemical overoxidation (see details in Experimental Section)

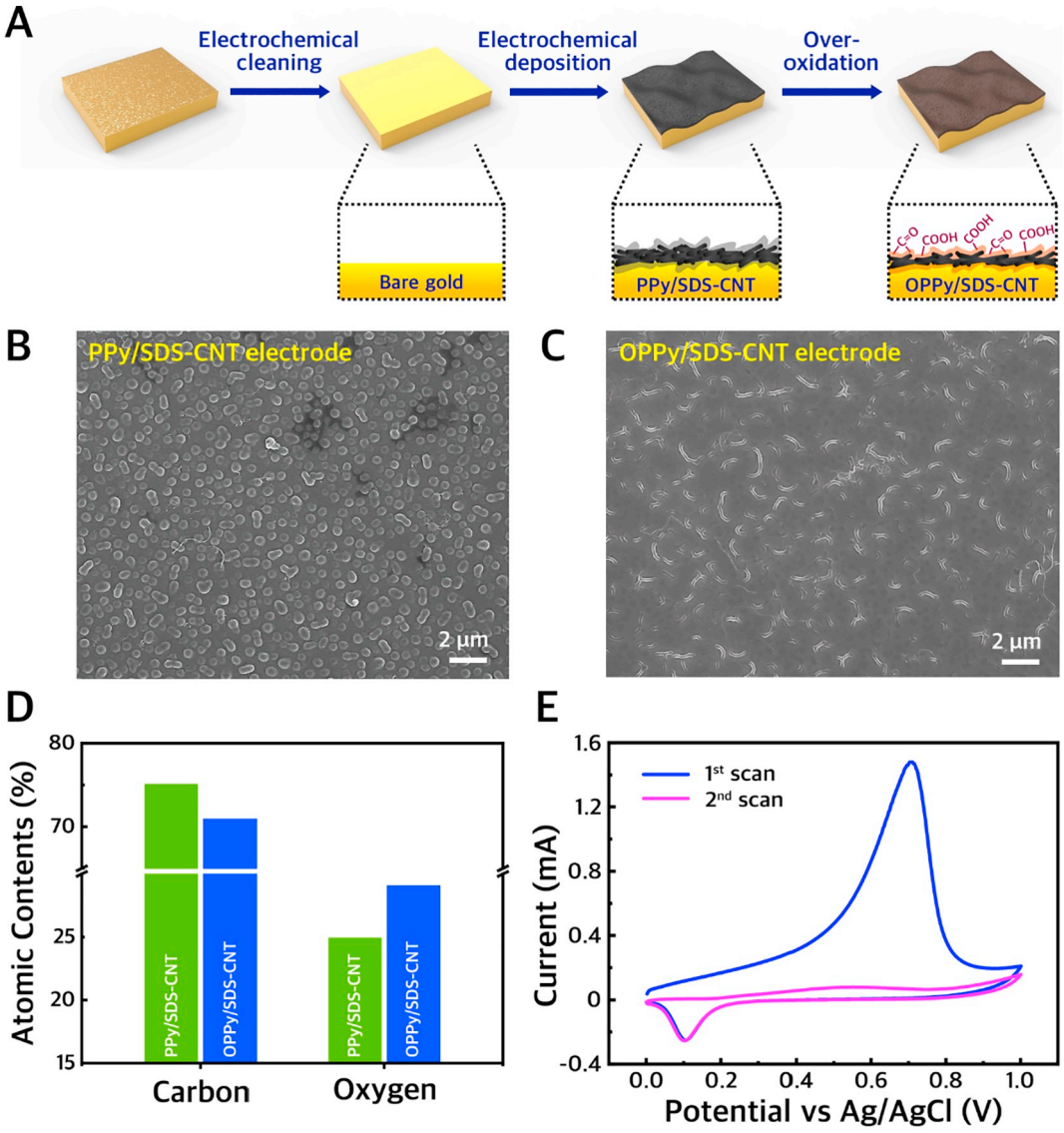

Fig. 1. (A) Schematic diagram of fabrication process of the OPPy/SDS-CNT electrode. (B) SEM image of the PPy/SDS-CNT electrode surface. (C) SEM image of the OPPy/SDS-CNT electrode surface. (D) Atomic percentage of carbon and oxygen of the PPy/SDS-CNT electrode (green) and the OPPy/SDS-CNT electrode (blue). (E) Cyclic voltammograms obtained during the overoxidation of the PPy/SDS-CNT electrode in 0.1 M NaOH. The potential was swept from 0 to 1 V twice with a scan rate of 50 mV/s. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 1A). First, the surface morphologies of PPy/SDS-CNT and OPPy/SDS-CNT electrodes were observed using a field emission scanning electron microscope (FEI Nova NanoSEM 230) (Fig. 1B and C). While the surface of the PPy/SDS-CNT electrode was rough due to the aggregates of the PPy/SDS-CNT composite, the surface of OPPy/SDS-CNT electrode was relatively more uniform as a result of surface etching during overoxidation. For the OPPy/SDS-CNT electrode, the similar shape of encapsulated CNTs was clearly observed throughout the whole electrode and the diameter of encapsulated CNTs was approximately 200 nm.

For a further investigation, carbon and oxygen contents on the surface of the PPy/SDS-CNT and OPPy/SDS-CNT electrodes were analyzed through an energy dispersive spectrometer (EDS, SU8200, Hitachi, Japan) (Fig. 1D). The atomic percentage of carbon decreased after overoxidation. In addition, the cross-sectional SEM images show that the surface was smoothed out and the thickness of OPPy/SDS-CNT electrode decreased from approximately ~53 nm to ~28 nm after overoxidation (Fig. S1). PPy/SDS-CNT composite, where PPy encloses the SDS-CNT micelles, forms a cauliflower-like structure rather than forming a uniform plane [23]. Thus, during the NaOH treatment, weakly bonded parts of the cauliflower-like structure were detached from the electrode surface. In contrary, the atomic percentage of oxygen increased after overoxidation. This increase indicates that the electron-rich oxygen-containing groups such as carboxylic and carbonyl groups were formed on the surface of the OPPy/SDS-CNT electrode. Lastly, we measured EDS to quantify the amount of carbon for samples with different thicknesses to investigate whether the overoxidation reaction was limited to the surface. The oxygen/carbon ratio did not show any special tendency to the thickness of the samples, which implies that the oxidation reaction was not limited to the surface and occurred inside the OPPy/SDS-CNT composite (Figs. S2 and S3).

3.2. Electrochemical characteristics of OPPy/SDS-CNT electrodes

To characterize the process of overoxidation, we obtained cyclic voltammograms during electrochemical overoxidation, which consisted of two sweeps from 0 to 1 V with a scan rate of 50 mV/s (Fig. 1E). While a clear oxidation peak of the PPy/SDS-CNT layer was observed in the first scan, a much smaller peak was observed in the second scan. This result indicates that most of the PPy/SDS-CNT surface was oxidized and

numerous carboxylic groups (−COOH) and carbonyl groups (−C(=O)−) were formed on the surface of PPy during the first scan. We propose that these negatively-charged groups would strongly attract the positively-charged DA molecules, which would not only increase the electrochemical signals during the detection but also endow selectivity against negatively-charged molecules.

It is also important to optimize surface activation of CNT in the OPPy/SDS-CNT composites for electrochemical detection of DA. Thus, we compared the effect of NaOH treatment of CNT to acid treatment by evaluating the sensing performance. First, CNT/Au electrodes were fabricated and treated with NaOH under the same condition as that of PPy/SDS-CNT electrodes (Fig. S4). The first oxidation peak observed for the OPPy/SDS-CNT electrode was not observed. The performance of CNT/Au electrodes before and after the NaOH treatment was also compared (Table S1). Since the CNT was surface-activated with oxygen containing group during the NaOH treatment process [24,25], the electrode performance was slightly enhanced after the treatment (Fig. S5). Next, since the DA detection performance could be also enhanced by the surface activation through the enrichment of the CNT surface with the carboxylic group, we tested the effect of acid treatment of CNT (functionalized CNT, fCNT) using the standard method [26] (Fig. S6). In contrast to the case of NaOH treatment, the sensing performance of OPPy/SDS-CNT and OPPy/SDS-fCNT was comparable (Fig. S7 and Table S2). Thus, the effect of NaOH treatment of CNT was more dominant than the effect of functionalization in electrochemical detection of DA (Fig. S8).

To confirm our hypothesis on the electrostatic interaction and to investigate the effect of the overoxidized pyrrole layer on the electrochemical activity, we measured cyclic voltammetry using the OPPy/SDS-CNT and PPy/SDS-CNT electrodes in the presence of 100 μM DA (Fig. 2A). The redox peaks of DA were more prominent for the OPPy/SDS-CNT electrode compared to that for the PPy/SDS-CNT electrode, which can be attributed to strong electrostatic interaction between OPPy/SDS-CNT and DA molecules. Since DA molecules exist as a cationic form at the physiological pH of 7.40, the negatively charged groups on the electrode electrostatically attract the positively-charged DA molecules [14,27,28]. To demonstrate the existence of the oxygen containing groups on the surface, Raman spectra of the OPPy/SDS-CNT electrode and PPy/SDS-CNT electrode were evaluated at the wavelength of 514 nm (Fig. S9). We observed a significant change near 1000–1500 cm^{-1}, which implied that the functional groups were attached to the surface after the overoxidation [29]. As a result, DA molecules accumulate at the surface of the OPPy/SDS-CNT electrode, which increases the redox signal of DA.

Moreover, a smaller charging current was observed for the OPPy/SDS-CNT electrode. For our electrodes, there are two possible factors that could influence electrochemical double layer capacitance (EDLC): surface area and oxygen-containing group. While an increase in the oxygen containing group on our OPPy/SDS-CNT electrode surface would increase EDLC [30], a decrease in the surface area would decrease EDLC [31]. Since a decrease in the charging current was observed for the OPPy/SDS-CNT electrode, the effect of reduction in the surface area was more dominant than the effect due to the presence of oxygen containing groups. Overall, overoxidation enhances the electrochemical detection of DA by increasing the electrochemical signals and decreasing the background charging current.

To further confirm the effect of endowed electronegativity from overoxidation, cyclic voltammogram of ferricyanide (FC, a mixture of ferricyanide and ferrocyanide) using the OPPy/SDS-CNT and PPy/SDS-CNT electrodes were compared (Fig. 2B). In contrast to positively-charged DA molecules, ferricyanide (FC) molecules are negatively charged. By comparing cyclic voltammograms of DA and FC, we aimed to confirm the electrostatic effect of the electrode on redox signals. While the FC peak was clearly shown for the PPy/SDS-CNT electrode, the FC peak was hardly observed for the OPPy/SDS-CNT electrode. Because of the repulsive force between the negatively-charged OPPy/SDS-CNT electrode and FC anions, FC molecules are repelled from the electrode surface. As a result, no redox peaks of FC are observed for the OPPy/SDS-CNT electrode. Thus, endowed electronegativity through overoxidation improves the selectivity of electrochemical detection of DA against negatively charged interference molecules.

Lastly, we also measured cyclic voltammetry of DA using the OPPy/SDS-CNT and OPPy electrodes to explore the effect of the presence of CNTs on the electrochemical detection (Fig. 2C). The OPPy/SDS-CNT electrode showed not only a higher charging current but also a higher oxidation peak current compared to that of the OPPy electrode. The higher charging current is attributed to the larger surface area due to the presence of CNTs. Since the larger surface area also induces numerous active sites to react with the target analyte [32,33], the peak current of DA is also increased. Thus, despite the higher background current, the presence of CNTs enhances the sensitivity of electrochemical detection of DA.

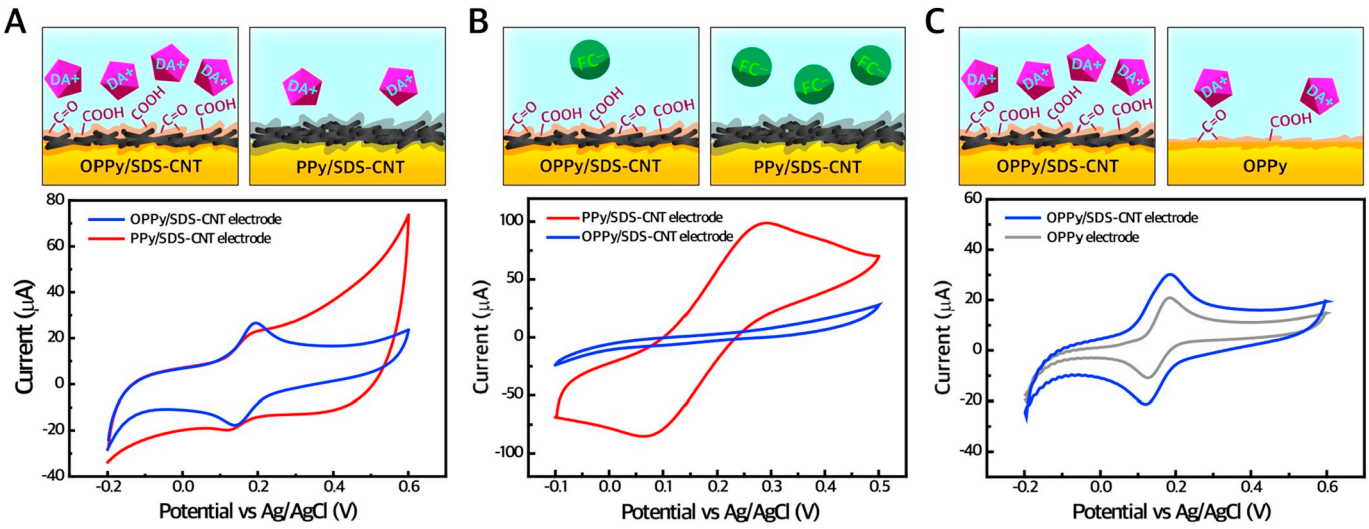

Fig. 2. (A) Cyclic voltammograms of the OPPy/SDS-CNT and PPy/SDS-CNT electrodes in PBS buffer (pH 7.4) containing 100 μM DA. (B) Cyclic voltammograms of the OPPy/SDS-CNT and PPy/SDS-CNT electrodes in PBS buffer (pH 7.4) containing 10 mM $[Fe(CN)_6]^{3-/4-}$. (C) Cyclic voltammograms of the OPPy/SDS-CNT and OPPy electrodes in PBS buffer (pH 7.4) containing 100 μM DA. The scan rate of 50 mV/s was used for all measurements. Schematics of surface interaction on each electrode are shown on top of the cyclic voltammograms.

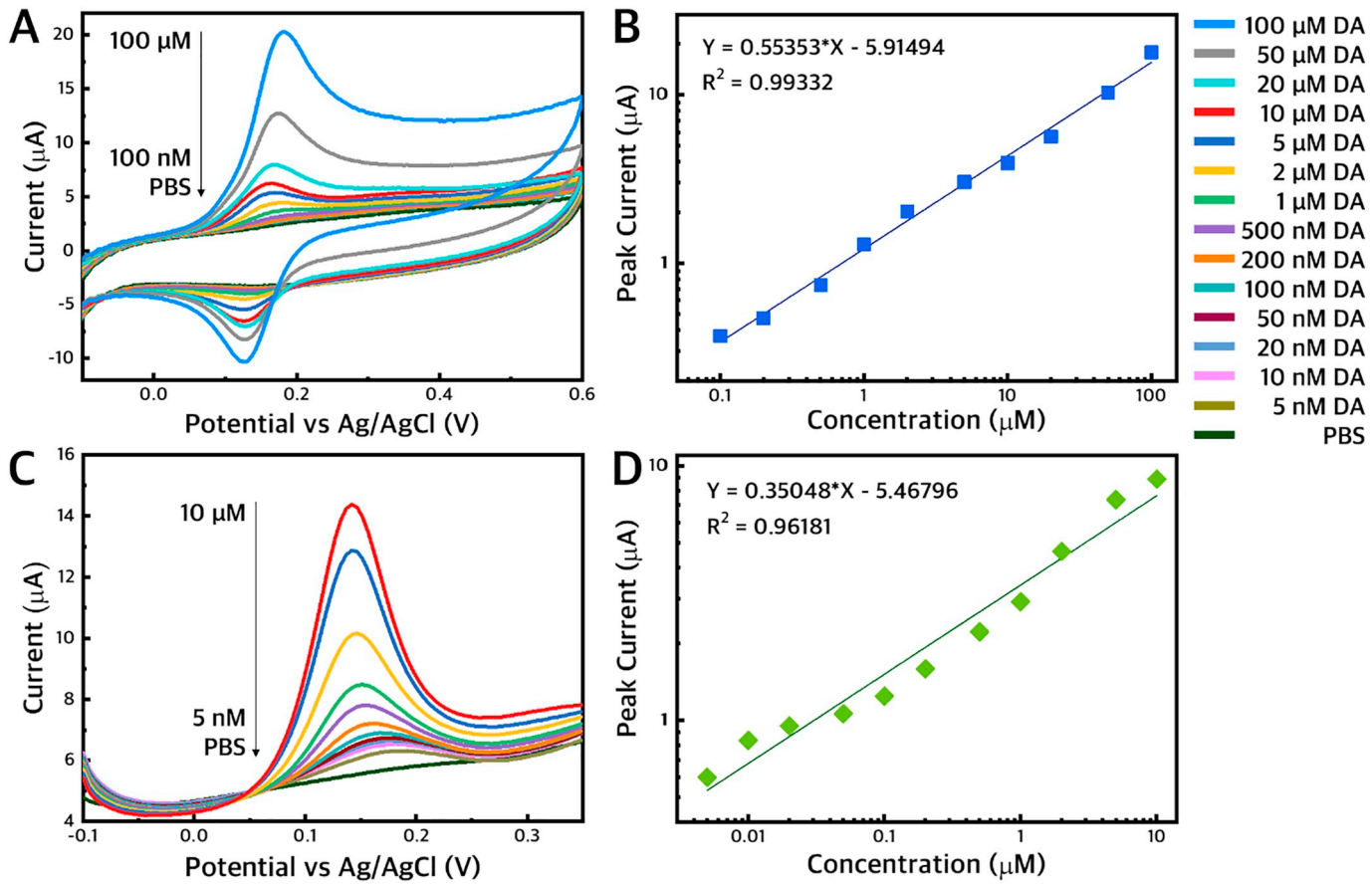

Fig. 3. (A) Cyclic voltammograms measured using the OPPy/SDS-CNT electrode in PBS buffer (pH 7.4) containing various concentrations of DA (100 nM–100 μM) with a scan rate of 50 mV/s. (B) Plot of oxidation peak currents observed at different DA concentrations. (C) Differential pulse voltammograms measured using the OPPy/SDS-CNT electrode in PBS buffer (pH 7.4) containing various concentrations of DA (5 nM–10 μM). (D) Plot of oxidation peak currents observed at different DA concentrations.

Table 1
Performance comparison of our electrode to that of other DA electrochemical sensors modified with PPy and CNTs.

Electrode		Detection method	Linear range (μM)	LOD (nM)	Reference
OPPy/CNT	OPPy/SDS-MWCNT	DPV	0.005–10	0.136	This work
	OPPy/MSA/MWCNT	DPV	0.001–2.87	0.4	Su et al. [40]
	OPPy/MWCNT	DPV	0.04–1.4	1.7	Tu et al. [14]
	OPPy/SWCNT	DPV	1–50	380	Li et al. [13]
PPy/CNT	PPy/Lac/MWCNT	DPV	0.5–4.75	140	Cesarino et al. [38]
	PPy/tyrosinase-SWCNT	Amperometry	5–50	5000	Min et al. [39]

3.3. Analytical characteristics as a DA sensor

While the basal concentration of DA in the extracellular fluid of brain ranges from 5 to 60 nM, DA transiently increases to 200–500 nM upon natural stimuli [34,35] or to 1 mM following evoked stimuli by implanted electrodes [36]. Thus, to monitor DA concentration in the brain real-time, biosensor with wide detection range and high sensitivity are essential. We measured both CV and DPV using the OPPy/SDS-CNT electrode at various concentrations of DA to evaluate the analytical characteristics of our electrode. For CV measurements, the oxidation and reduction peaks of DA in all concentrations (100 nM–100 μM) were clearly observed at 0.18 V and 0.13 V, respectively (Fig. 3A). The peak currents increased linearly as the concentration of DA increased from 100 nM to 100 μM (Fig. 3B). Limit of detection (LOD) is estimated to be approximately 70 nM.

To enhance resolution, we also performed DPV measurements at various concentrations of DA from 5 nM to 10 μM. Clear DA peaks at 0.14 V were observed in all concentrations. Similar to CV results, the peak currents of DPV also increased with increasing DA concentrations. A linear response was observed in the concentration range from 5 nM to 10 μM (Fig. 3D). LOD of DPV measurements is estimated to be approximately 136 pM. For a higher concentration range, however, the sensitivity of CV could be higher than that obtained from DPV because the peak current of CV contains the capacitive current [37]. Moreover, the sensing performance of OPPy/SDS-CNT electrodes of varying thicknesses was evaluated (Figs. S10 and S11), which showed that the optimal sensing performance was observed for the deposition time of 10 s.

We compared the analytical characteristics of our OPPy/SDS-CNT electrode to that of other DA electrochemical sensors modified with

polypyrrole layer and/or CNT layer (Table 1) [13,14,38–40]. The OPPy/SDS-CNT electrode proposed in this work exhibits the lowest LOD, which could be attributed to the synergistic effect of overoxidized polypyrrole and carbon nanotube. Moreover, the OPPy/SDS-CNT electrode in this work exhibits 10-fold lower LOD than that of other OPPy/CNT electrodes [13,14]. The OPPy/SDS-CNT electrode in this work was prepared by using sodium dodecyl sulfate (SDS) as a dopant and NaOH as an oxidation agent in contrast to the fabrication processes of OPPy/SDS-CNT electrodes in other reports. The negative charge of SDS and the overoxidation in NaOH enhance the sensitivity of the OPPy/SDS-CNT electrode as well as the permeability of DA to the electrode [41–44]. Thus, our OPPy/SDS-CNT electrode with high sensitivity, excellent LOD, and wide dynamic range is capable of monitoring the DA concentration in the brain at various states such as basal and stimulated states.

3.4. Selectivity of OPPy/SDS-CNT electrodes

Our cerebrospinal fluid contains numerous chemicals and neurotransmitters. Among these, ascorbic acid (AA) is often considered as a major interfering substance in the detection of DA due to a similar oxidation potential [45]. In addition, it is important to confirm the effect of glucose (Glc) on the detection of DA since Glc exists at mM levels as opposed to nM levels of DA [46]. In this work, we compared CVs and DPVs of DA, AA, and Glc using the OPPy/SDS-CNT electrode (Fig. 4A and B). For both CV and DPV measurements, while the DA peak was clearly observed, the AA and Glc peaks were hardly observed. The pKa of AA is 4.04, and thus AA exists as an anion under physiological condition (pH 7.4) [27,28]. The electronegative groups in the OPPy/SDS-CNT electrode repel the anionic AA. Thus, similar to FC molecules, no redox peaks of AA were shown. Moreover, the redox peaks of Glc were not observed, which indicates that our electrode possesses no electrocatalytic activity to oxidize Glc. When measuring CV and DPV in a mixture of DA, AA, and Glc, similar redox peaks to that of DA were observed. Moreover, amperometric responses to AA, DA, and Glc were explored using the OPPy/SDS-CNT electrode (Fig. S12). The OPPy/SDS-CNT electrode selectively and quantitatively detected DA also through the amperometric technique. These results indicate that the OPPy/SDS-CNT electrode is capable of selectively detecting DA even in the presence of major interference molecules.

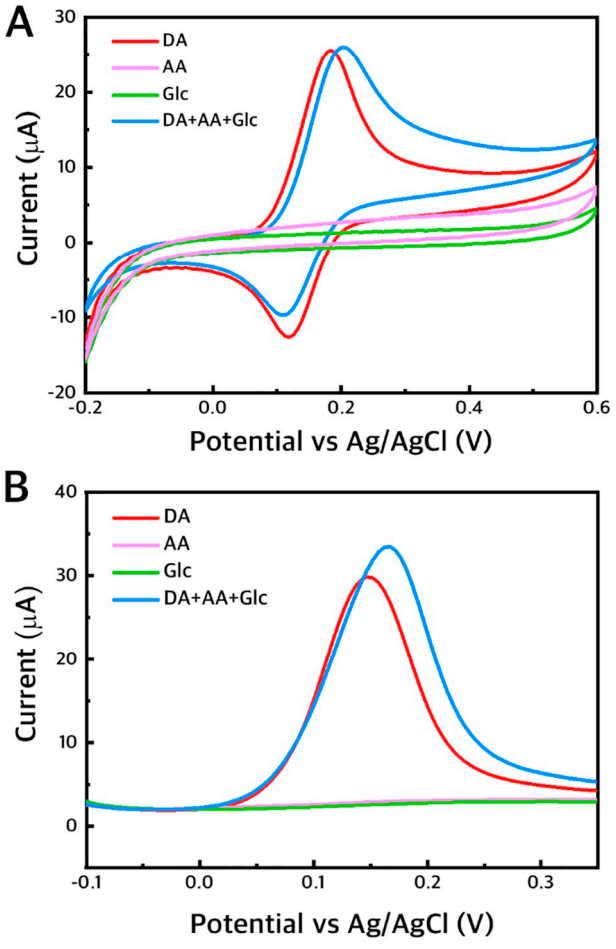

Fig. 4. (A) Cyclic voltammograms obtained using the OPPy/SDS-CNT electrode in PBS (pH 7.4) containing 100 μM DA, 160 μM AA, 10 mM Glu, and a mixture of 100 μM DA, 160 μM AA, and 10 mM Glc. The scan rate was 50 mV/s. (B) DPVs measured using the OPPy/SDS-CNT electrode in PBS (pH 7.4) containing 100 μM DA, 160 μM AA, 10 mM Glc, and a mixture of 100 μM DA, 160 μM AA, and 10 mM Glc.

3.5. In vitro detection of DA

To evaluate the real-time detection capability of our electrode, we conducted *in vitro* experiments using dopaminergic cells. A custom-designed electrochemical cell was fabricated using poly (dimethylsiloxane) (Fig. 5A). For a 3-electrode system, our OPPy/SDS-CNT electrode, an Ag/AgCl electrode and a Pt wire were used as working, reference, and counter electrodes, respectively. Dopaminergic cells (PC12) were incubated on the OPPy/SDS-CNT electrode to achieve direct contact with the electrode and real-time measurement of released DA (Fig. 5B). Since potassium ions depolarize cells and induce DA release by exocytosis [47], we chose a high concentration of KCL solution as a stimulant. By applying 0.18 V on the OPPy/SDS-CNT electrode, we continuously measured the oxidation current using the potentiostat (Fig. 5C). 20 μL of a 3 M KCl solution was injected into a cell medium at 71 s to stimulate PC12 cells. The final concentration of KCl was estimated to be 58 mM. The oxidation of DA molecules appeared immediately after the injection of KCl, which indicated that PC12 cells were successfully stimulated by KCl solution and released DA molecules (blue curve). In contrast, the oxidation of DA was hardly observed when PBS solution was injected as a control (green curve). In addition, there was no oxidation peak when KCl solution was injected into the cell medium without PC12 cells (dark gray curve).

Moreover, DA released from different concentrations of PC12 cells (1.04×10^5 and 4.16×10^5 cells/mL) was measured (Fig. S13). The amount of DA released should be larger for a larger number of cells. As predicted, the oxidation peak increased for the case with a higher cell concentration. By integrating the oxidation peaks, moles of released DA were estimated to be 1.7×10^{-12} mol for 1.04×10^5 cells/mL and 3.24×10^{-12} mol for 4.16×10^5 cells/mL (Table S3). MTT assay was performed to evaluate cytotoxicity of our OPPy/SDS-CNT electrode (Fig. 5D) on three samples. The first two samples contained PC 12 cells with and without the OPPy/SDS-CNT electrode which were cultured for 24 h. The last sampled contained only a media solution as a negative control. Absorbance intensities exhibited no significant differences between cells incubated with and without the OPPy/SDS-CNT electrode, which indicates that OPPy/SDS-CNT electrode has sufficient biocompatibility.

4. Conclusions

We proposed and demonstrated a new OPPy/SDS-CNT electrode which enables sensitive and selective detection of DA. By using SDS as a dopant and sodium hydroxide (NaOH) as an oxidizing agent, our electrode exhibited a lower LOD of 136 pM compared to the previously reported electrodes with a similar composite. In addition, a higher redox signal of DA and a lower charging current were observed for our OPPy/SDS-CNT electrode compared to that of PPy/SDS-CNT electrodes. Moreover, due to the endowed electronegativity, our electrode exhibited high selectivity to DA in the presence of interfering compounds

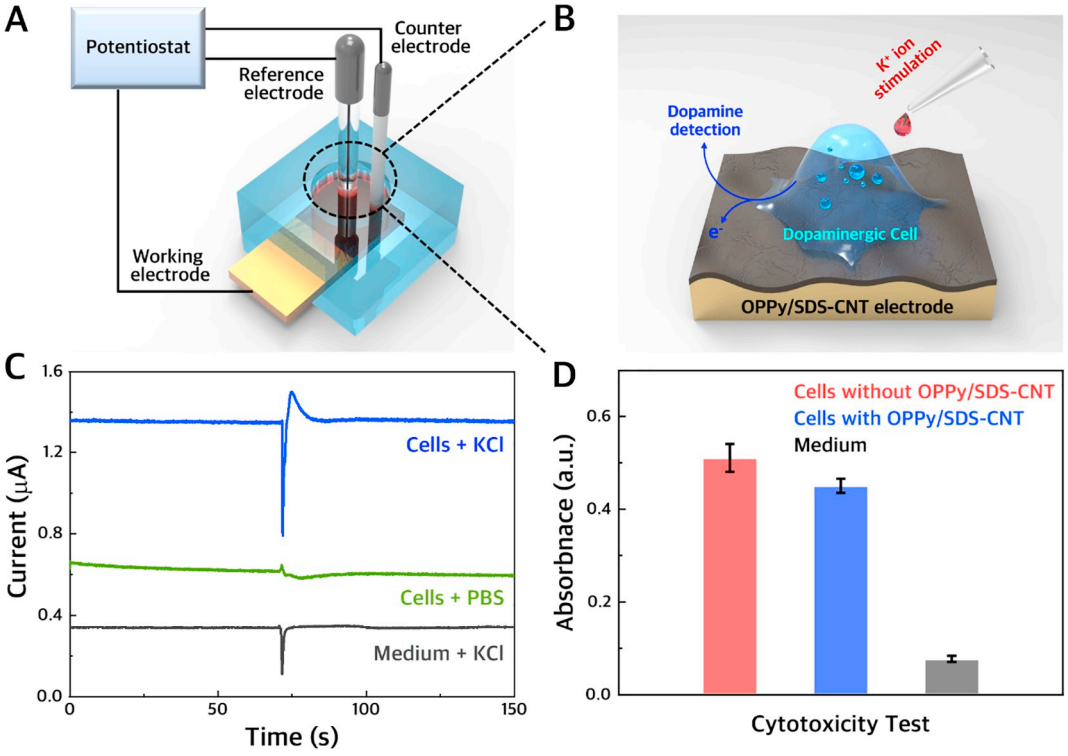

Fig. 5. (A) Schematic illustration of the custom-designed 3-electrode system for *in vitro* experiments. (B) Schematic illustration of electrochemical detection of dopamine released from dopaminergic cells (PC12 cells) using the OPPy/SDS-CNT electrode. (C) Transient plot of amperometric currents of the OPPy/SDS-CNT electrode measured when (1) cells were stimulated with KCl, (2) cells were stimulated with PBS, and (3) medium that contained no cells was stimulated with KCl. (D) Quantification of MTT assay evaluating the *in vitro* cytotoxicity of the OPPy/SDS-CNT electrode. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

such as Glc and AA. Successful *in vitro* detection of DA released from dopaminergic cells (PC12 cells) implies good biocompatibility. Based on this excellent sensing performance, the proposed OPPy/SDS-CNT electrode is an excellent candidate for functional neural electrodes for *in vivo* monitoring of DA.

Acknowledgements

This work was supported by the Engineering Research Center of Excellence (ERC) Program supported by National Research Foundation of Korea (NRF), Korean Ministry of Science & ICT (MSIT) (Grant No. NRF-2017R1A5A1014708), Multi-Ministry Collaborative R&D Program (Development of Techniques for Identification and Analysis of Gas Molecules to Protect Against Toxic Substances) through the National Research Foundation of Korea (NRF) funded by KNPA, MSIT, MOTIE, ME, NFA (2017M3D9A073509), and KRIBB Initiative Research Program.

Author contributions

Gayoung Eom: Conceptualization, Methodology, Roles/Writing - original draft. **Chaerin Oh**: Conceptualization, Formal analysis, Methodology, Investigation, Roles/Writing - original draft. **Jeong Moon**: Formal analysis. **Hyojung Kim**: Formal analysis, Data curation. **Mi Kyung Kim**: Data curation. **Kiup Kim**: Data curation. **Ji-Won Seo**: Data curation, Writing - review & editing. **Taejoon Kang**: Formal analysis, Resources, Supervision. **Hyunjoo J. Lee**: Funding acquisition, Project administration, Supervision; Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2019.113295.

References

[1] T. Palomäki, E. Peltola, S. Sainio, N. Wester, O. Pitkänen, K. Kordas, J. Koskinen, T. Laurila, Unmodified and multi-walled carbon nanotube modified tetrahedral amorphous carbon (ta-C) films as in vivo sensor materials for sensitive and selective detection of dopamine, Biosens. Bioelectron. 118 (2018) 23–30.
[2] K. Blum, A.L.-C. Chen, E.R. Braverman, D.E. Comings, T.J. Chen, V. Arcuri, S.H. Blum, B.W. Downs, R.L. Waite, A. Notaro, Attention-deficit-hyperactivity disorder and reward deficiency syndrome, Neuropsychiatr. Dis. Treat. 4 (5) (2008) 893–917.
[3] D.C. Javitt, K.M. Spencer, G.K. Thaker, G. Winterer, M. Hajós, Neurophysiological biomarkers for drug development in schizophrenia, Nat. Rev. Drug Discov. 7 (2008) 68.
[4] A. Vázquez-Guardado, S. Barkam, M. Peppler, A. Biswas, W. Dennis, S. Das, S. Seal, D. Chanda, Enzyme-free plasmonic biosensor for direct detection of neurotransmitter dopamine from whole blood, Nano Lett. 19 (2018) 449–454.
[5] F.N. Emamzadeh, A. Surguchov, Parkinson's disease: biomarkers, treatment, and risk factors, Front. Neurosci. 12 (2018) 612.
[6] R.M. Wightman, L.J. May, A.C. Michael, Detection of dopamine dynamics in the brain, Anal. Chem. 60 (1988) 769A–793A.
[7] J.H. An, D.-K. Choi, K.-J. Lee, J.-W. Choi, Surface-enhanced Raman spectroscopy detection of dopamine by DNA Targeting amplification assay in Parkisons's model, Biosens. Bioelectron. 67 (2015) 739–746.
[8] J.-M. Moon, N. Thapliyal, K.K. Hussain, R.N. Goyal, Y.-B. Shim, Conducting polymer-based electrochemical biosensors for neurotransmitters: a review, Biosens. Bioelectron. 102 (2018) 540–552.
[9] D.P. Li, X.Y. Liu, R. Yi, J.X. Zhang, Z.Q. Su, G. Wei, Electrochemical sensor based on novel two-dimensional nanohybrids: MoS2 nanosheets conjugated with organic copper nanowires for simultaneous detection of hydrogen peroxide and ascorbic acid, Inorg Chem Front 5 (2018) 112–119.
[10] P. Li, M.F. Zhang, X.Y. Liu, Z.Q. Su, G. Wei, Electrostatic assembly of platinum nanoparticles along electrospun polymeric nanofibers for high performance electrochemical sensors, Nanomaterials-Basel 7 (2017).
[11] J.A. Johnson, N.T. Rodeberg, R.M. Wightman, Measurement of basal

neurotransmitter levels using convolution-based nonfaradaic current removal, Anal. Chem. 90 (2018) 7181–7189.
[12] A.C. Schmidt, X. Wang, Y. Zhu, L.A. Sombers, Carbon nanotube yarn electrodes for enhanced detection of neurotransmitter dynamics in live brain tissue, ACS Nano 7 (2013) 7864–7873.
[13] Y. Li, P. Wang, L. Wang, X. Lin, Overoxidized polypyrrole film directed single-walled carbon nanotubes immobilization on glassy carbon electrode and its sensing applications, Biosens. Bioelectron. 22 (2007) 3120–3125.
[14] X. Tu, Q. Xie, S. Jiang, S. Yao, Electrochemical quartz crystal impedance study on the overoxidation of polypyrrole–carbon nanotubes composite film for amperometric detection of dopamine, Biosens. Bioelectron. 22 (2007) 2819–2826.
[15] T. Qian, C. Yu, X. Zhou, P. Ma, S. Wu, L. Xu, J. Shen, Ultrasensitive dopamine sensor based on novel molecularly imprinted polypyrrole coated carbon nanotubes, Biosens. Bioelectron. 58 (2014) 237–241.
[16] X. Jiang, Y. Marois, A. Traoré, D. Tessier, L.H. Dao, R. Guidoin, Z. Zhang, Tissue reaction to polypyrrole-coated polyester fabrics: an in vivo study in rats, Tissue Eng. 8 (2002) 635–647.
[17] A. Ramanaviciene, A. Kausaite, S. Tautkus, A. Ramanavicius, Biocompatibility of polypyrrole particles: an in-vivo study in mice, J. Pharm. Pharmacol. 59 (2007) 311–315.
[18] T.-C. Tsai, H.-Z. Han, C.-C. Cheng, L.-C. Chen, H.-C. Chang, J.-J.J. Chen, Modification of platinum microelectrode with molecularly imprinted over-oxidized polypyrrole for dopamine measurement in rat striatum, Sensors Actuators B Chem. 171 (2012) 93–101.
[19] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56.
[20] E.W. Keefer, B.R. Botterman, M.I. Romero, A.F. Rossi, G.W. Gross, Carbon nanotube coating improves neuronal recordings, Nat. Nanotechnol. 3 (2008) 434.
[21] Y. Lu, T. Li, X. Zhao, M. Li, Y. Cao, H. Yang, Y.Y. Duan, Electrodeposited polypyrrole/carbon nanotubes composite films electrodes for neural interfaces, Biomaterials 31 (2010) 5169–5181.
[22] X.Q. Yu, W.S. Zhang, P.P. Zhang, Z.Q. Su, Fabrication technologies and sensing applications of graphene-based composite films: advances and challenges, Biosens. Bioelectron. 89 (2017) 72–84.
[23] H. Shiigi, K. Okamura, D. Kijima, B. Deore, U. Sree, T. Nagaoka, An overoxidized polypyrrole dodecylsulfonate micelle composite film for amperometric serotonin sensing, J. Electrochem. Soc. 150 (2003) H119–H123.
[24] A. Doepke, C. Han, T. Back, W.D. Cho, D.D. Dionysiou, V. Shanov, H.B. Halsall, W.R. Heineman, Analysis of the electrochemical oxidation of multiwalled carbon nanotube tower electrodes in sodium hydroxide, Electroanalysis 24 (2012) 1501–1508.
[25] Z.J. Zhuang, J.Y. Li, R.A. Xu, D. Xiao, Electrochemical detection of dopamine in the presence of ascorbic acid using overoxidized polypyrrole/graphene modified electrodes, Int J Electrochem Sc 6 (2011) 2149–2161.
[26] H.H. Cho, B.A. Smith, J.D. Wnuk, D.H. Fairbrother, W.P. Ball, Influence of surface oxides on the adsorption of naphthalene onto multiwalled carbon nanotubes, Environ Sci Technol 42 (2008) 2899–2905.
[27] A. Babaei, A.R. Taheri, Nafion/Ni(OH)$_2$ nanoparticles-carbon nanotube composite modified glassy carbon electrode as a sensor for simultaneous determination of dopamine and serotonin in the presence of ascorbic acid, Sensors Actuators B Chem. 176 (2013) 543–551.
[28] J. Zhang, X.-h. Song, S. Ma, X. Wang, W.-c. Wang, Z.-d. Chen, A novel sodium dodecyl benzene sulfonate modified expanded graphite paste electrode for sensitive and selective determination of dopamine in the presence of ascorbic acid and uric acid, J. Electroanal. Chem. 795 (2017) 10–16.
[29] C. Debiemme-Chouvy, T.T.M. Tran, An insight into the overoxidation of polypyrrole materials, Electrochem. Commun. 10 (2008) 947–950.
[30] Y.T. He, Y.H. Zhang, X.F. Li, Z. Lv, X.J. Wang, Z.G. Liu, X.Q. Huang, Capacitive mechanism of oxygen functional groups on carbon surface in supercapacitors, Electrochim. Acta 282 (2018) 618–625.
[31] P. May, M. Clegg, T. Silva, H. Zanin, O. Fatibello-Filho, V. Celorrio, D. Fermin, C. Welch, G. Hazell, L. Fisher, Diamond-coated 'black silicon' as a promising material for high-surface-area electrochemical electrodes and antibacterial surfaces, J. Mater. Chem. B 4 (2016) 5737–5746.
[32] H. Song, X. Zhang, Y. Liu, Z. Su, Developing graphene-based nanohybrids for electrochemical sensing, Chem. Rec. 19 (2019) 534–549.
[33] T.J. Liu, Y.Q. Guo, Z.F. Zhang, Z.C. Miao, X.Y. Zhang, Z.Q. Su, Fabrication of hollow CuO/PANI hybrid nanofibers for non-enzymatic electrochemical detection of H2O2 and glucose, Sensors Actuators B Chem. 286 (2019) 370–376.
[34] D.L. Robinson, M.L. Heien, R.M. Wightman, Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics, J. Neurosci. 22 (2002) 10477–10486.
[35] D.L. Robinson, P.E. Phillips, E.A. Budygin, B.J. Trafton, P.A. Garris, R.M. Wightman, Sub-second changes in accumbal dopamine during sexual behavior in male rats, Neuroreport 12 (2001) 2549–2552.
[36] C.P. Ford, S.C. Gantz, P.E. Phillips, J.T. Williams, Control of extracellular dopamine at dendrite and axon terminals, J. Neurosci. 30 (2010) 6975–6983.
[37] A.P. Brown, F.C. Anson, Cyclic and differential pulse voltammetric behavior of reactants confined to electrode surface, Anal. Chem. 49 (1977) 1589–1595.
[38] I. Cesarino, H.V. Galesco, F.C. Moraes, M.R. Lanza, S.A. Machado, Biosensor based on electrocodeposition of carbon nanotubes/polypyrrole/laccase for neurotransmitter detection, Electroanalysis 25 (2013) 394–400.
[39] K. Min, Y.J. Yoo, Amperometric detection of dopamine based on tyrosinase–SWNTs–Ppy composite electrode, Talanta 80 (2009) 1007–1011.
[40] Z. Su, Y. Liu, Q. Xie, L. Chen, Y. Zhang, Y. Meng, Y. Li, Y. Fu, M. Ma, S. Yao, Preparation of thiolated polymeric nanocomposite for sensitive electroanalysis of dopamine, Biosens. Bioelectron. 36 (2012) 154–160.
[41] Z. Gao, Z. Minxian, C. Beshen, The influence of overoxidation treatment on the permeability of polypyrrole films, J. Electroanal. Chem. 373 (1994) 141–148.
[42] J. Zhang, Z. Zhu, J. Zhu, K. Li, S. Hua, Selective determination of dopamine, ascorbic acid and uric acid at SDS-MWCNTs modified glassy carbon electrode, Int. J. Electrochem. Sci. 9 (2014) 1264–1272.
[43] J. Zheng, X. Zhou, Sodium dodecyl sulfate-modified carbon paste electrodes for selective determination of dopamine in the presence of ascorbic acid, Bioelectrochemistry 70 (2007) 408–415.
[44] X. Zhang, J. Zhang, Z. Liu, Conducting polymer/carbon nanotube composite films made by in situ electropolymerization using an ionic surfactant as the supporting electrolyte, Carbon 43 (2005) 2186–2191.
[45] Y. Wang, Y. Huang, B. Wang, T. Fang, J. Chen, C. Liang, Three-dimensional porous graphene for simultaneous detection of dopamine and uric acid in the presence of ascorbic acid, J. Electroanal. Chem. 782 (2016) 76–83.
[46] A. Spanos, F.E. Harrell, D.T. Durack, Differential diagnosis of acute meningitis: an analysis of the predictive value of initial observations, JAMA 262 (1989) 2700–2707.
[47] H. Abe, K. Ino, C.-Z. Li, Y. Kanno, K.Y. Inoue, A. Suda, R. Kunikata, M. Matsudaira, Y. Takahashi, H. Shiku, Electrochemical imaging of dopamine release from three-dimensional-cultured PC12 cells using large-scale integration-based amperometric sensors, Anal. Chem. 87 (2015) 6364–6370.