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Ultrasensitive in-vitro monitoring of monoamine neurotransmitters from dopaminergic cells

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ABSTRACT

The design of biosensing assay of monoamine neurotransmitters (MANTs) such as epinephrine (Ep), norepinephrine (NE), and dopamine (DA), as well as the monitoring of these MANTs released from dopaminergic cells, are of particular interest. Electrochemical sensors based on the novel construction of nickel oxides (NiO) were fabricated and employed for electrochemical screening of MANTs. A novel NiO-lacy flower-like (NLF) geometrical structure with semi-spherical head surfaces connected with a trunk as an arm was achieved. The designed semi-spherical head associated with abundant and the well-dispersed tubular branches with needle-like open ends might lead to the creation of vascular vessels for facile diffusion and suitable accommodation of the released MANTs throughout active and wide-surface-area coverage, multi-diffusive pores, and caves with connective open macro-/meso-windows along the entire top-view nanoneedles of lacy flower head and trunk. These electrode surfaces possess high-index catalytic site facets associated with the formation of ridges/defects on {110}-top-cover surface dominants for strong binding, fast response, and signaling of MANTs. The NLF- modified electrode enabled high sensitivity for MANTs and a low limit of detection of 6 nM. Ultrasensitive in-vitro monitoring of DA released from dopaminergic cells (such as PC12) was realized. The NLF electrode was used to detect MANTs from its sources (PC12), and it could be used for clinical diagnosis.

1. Introduction

Monoamine neurotransmitters (MANTs), such as dopamine (DA), norepinephrine (NE), and epinephrine (Ep), play a key role in the endocrine and central nervous systems. Fluctuation levels of MANTs are a result of some neurological or immunological diseases, such as Parkinson's disease, human immunodeficiency virus infection, and schizophrenia [1–3]. DA acts as a precursor of Ep and NE, plays a key role in movements, and can be used as a cardiac stimulant and in most potent vasopressin drugs [1–3]. DA and Ep are used to treat some diseases, such as allergy, asthma, cardiac arrhythmias, and infarction [4]. MANTs have a similar chemical structure, and they are often present in biological samples. Therefore, fabrication of an efficient electrochemical biosensor to monitor and determine MANTs in their receptors or resources is clinically needed.

Several techniques such as chromatography [5,6], mass spectroscopy [7], fluorescence [8], and electrochemical approaches [9,10] have been used to detect MANTs. Most of these techniques have the following characteristics: i) need highly equipped machines, ii) time-consuming, iii) induce excessive costs, iv) required highly trained technicians, v) can be used in-vitro/in-vivo, and vi) adopt sophisticated procedures. By contrast, electrochemical methods are sensitive approaches for MANT detection [9–12]. Electrochemical techniques are more widely used for monitoring MANTs because they do not require pretreatment of samples, have a fast response, allow on-site detection, and may be performed in situ [13–17].

Several reports have shown the electrochemical responses of DA, such as modified electrodes with 3D nitrogen-doped graphene on Ni foam electrode, graphene nanosheet/SnO_2 nanocomposite, 3D interpenetrating graphene electrode,

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imprinted poly(nicotinamide)/CuO, graphene, N-doped carbon dots (CDs), N-doped graphene/MnO, cibacron blue/poly-1,5-diaminonaphthalene, and K^+-induced DA released from dopaminergic cells (PC12) by ethylenediaminetetraacetic acid (EDTA)-immobilized polydiaminonaphthalene (pDAN)/graphene oxide (GO)/gold nanoparticles (AuNPs) [11–18]. Electrochemical detection of Ep by using various modified electrodes, such as electrochemically pretreated glassy carbon electrodes (GCEs) [19], carbon fiber microelectrodes [20], polymer-film-modified GCEs [20–22], and other modified electrode based on functionalized substrate [23–25], has been reported. The sensitivities and linear ranges of all the methods using different modified electrodes are indicated in Table S1, and all of them have low comparable detection limits. Simultaneous detection of two or three types of MANTs is difficult because they exhibit the same oxidation-reduction mechanism.

Fabrication of modified electrodes based on metal-oxide nanomaterials, such as CuO, NiO, TiO_2 and Co_3O_4, is widely considered because of their good biological compatibility, large surface area, and special physical and chemical properties [26–34]. NiO is a transition metal oxide that has received considerable attention in many applications, such as catalysts, lithium-ion batteries, and fuel cells, because of its intrinsic properties considered as antiferromagnetic materials, easy fabrication, building of novel construction with abundant active sites, highly efficient electrocatalyst, and good electrochemical conduction [35–40]. Although many synthetic approaches can produce porous NiO and magnetism, achievement of a simple and feasible method for designing mesostructured NiO with a controlled morphology is highly desirable and remains a great challenge to material scientists.

In this study, a modified electrode based on the new design of NiO for monitoring MANTs was assembled. The controlled NiO-lacy flower-like (NLF) morphology expressed the electrode design, which was similar to a lacy flower with a semi-round architecture connected by a trunk. The designed NLF surface was like a jagged surface with thick tubular pipes that spread with unity and homogeneity over the semi-round flower head surface. The designed NLF possessed intrinsic features, which play key roles in its electrocatalytic characteristics, such as active and wide-surface-area coverage, multi-diffusive pores, and caves with connective open macro-/meso-windows along the entire top-view nanoneedles of the NLF head and trunk. The actively catalytic surface of the modified electrode assembled with the designed NLF showed highly sensitive, selective, and fast responses of MANTs. The NLF-modified electrode provided highly sensitive detection of DA secreted from living cells under K^+ stimulation with low cytotoxicity and high biocompatibility. Sensitive monitoring of MANTs from dopaminergic cells enabled the potential use of our biosensing assay for clinical diagnosis of schizophrenia, Alzheimer's, and Parkinson's diseases.

2. Experimental

2.1. Synthesis of NLF

A new morphological NiO was synthesized by one-pot hydrothermal synthetic approach. ($Ni(NO_3)_2{\cdot}6H_2O$) (1 mM) was dissolved in a 100 mL volumetric flask containing 20 mL of deionized water and was stirred until it dissolved at room temperature. $(NH_4)_2HPO_4$ solution (15 mL of 1 mM) in Milli-Q water was added to the above solution dropwise with continuous stirring. The prepared solution was transferred into a 100-mL Teflon-lined stainless-steel autoclave, sealed, and maintained at 160 °C for 8 h. After the required time, a green precipitate was retrieved and rinsed with ultrapure water/absolute ethanol to remove soluble impurities. The NLF was dried in an oven at 80 °C and annealed at 400 °C for 4 h.

2.2. Working electrode designing and formation

The NLF was dispersed in Milli Q water with concentration 5 mg/ml at room temperature and sonicated for 4 h for complete dispersion and unit formations. Thin films of NLF were fabricated by uniformly spreading 20 μL solution of NLF onto the conducting surface of ITO glass substrate (2 cm × 1 cm). To control the exposed surface area of ITO substrate and for better electrical contact a definite area (1 cm × 1 cm) of the ITO electrode substrate were masked prior to the deposition. This process was repeated 3 times; the resulting electrode was dried overnight at room temperature, followed by washing with deionized water to remove any unbound particles.

2.3. Cell culture and in vitro study

The PC12 cell line was obtained from PC12 (ATCC® CRL1721™) and was cultured by incubation under 5% CO_2 at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), and 10% horse serum. The culture medium was replaced every 2–3 day.

For cells visualization experiments using (NLF), cells were seeded in 6 well-plate under 2×10^6Cells/ml. To enhance extracellular dopamine release, a desired amount of 20 mM KCl solution was added, kept in a humid chamber under 5% CO_2 at 37 °C for 15 min followed by thoroughly washing using Dulbecco's Phosphate Buffered Saline (DPBS). A desired amount of NLF (20 μg/ml) was added to each well, incubated in a humid chamber for additional 30 min under 5% CO_2 at 37 °C followed by thoroughly washing using DPBS. Nuclear counter-staining was carried out using 0.1 μg/ml of 4′, 6-diamidino-2-phenylindole (DAPI) in PBS for 10 min. Finally, confocal microscopy measurements were observed using Leica TCS SPE5 X machine

2.4. Cytotoxicity and cell viability

The cytotoxicity of NLF was investigated by Cell Counting Kit-8 (CCK-8) assay. The cells (5×10^3 cells/mL) were seeded onto 96-well microplates to a total volume of 100 μL/well and maintained at 37 °C in a 5% CO_2/95% air incubator for 24 h. Then, NLF (10 μL) with different concentrations were added (10, 20, 50 and 100 μg/ml), and incubated for 24 h. The CCK-8 solution (10 μL, 5 mg/ml) was added into each well and incubated for another 2–4 h in the CO_2 incubator. Then measure at absorbance of 450 nm using a microplate reader

3. Results and discussions

The controlled morphology of NiO architecture was synthesized by hydrothermal treatment after addition of $(NH_4)_2HPO_4$ as shown in Scheme S1. The PO_4^{3-} anions act as morphology directing agent for controlled designing of NiO-lacy flower like. The formation of NiO-lacy flower like depends on the presence of ammonia and water, producing OH^- anions, which directly reacts with a Ni^{2+} cation to form $Ni(OH)_2$. Phosphate anions and ammonium cations play the key role for the controlled design and enhanced the crystal growth of NiO. The self-directing formation of NLF was formed after prolonged time with the supporting of PO_4^{3-} anions. Controlled design of NiO which is like a lacy flower with semi-spherical dense head and connected by a trunk was formed.