Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Monitoring the electrochemical responses of neurotransmitters through localized surface plasmon resonance using nanohole array

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ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form
17 August 2016
Accepted 29 August 2016

Keywords:
Coupled detection
Localized surface plasmon resonance (LSPR)
Cyclic voltammetry (CV)
Spectroelectrochemistry
Neurotransmitters

ABSTRACT

In this study, a novel spectroelectrochemical method was proposed for neurotransmitters detection. The central sensing device was a hybrid structure of nanohole array and gold nanoparticles, which demonstrated good conductivity and high localized surface plasmon resonance (LSPR) sensitivity. By utilizing such specially-designed nanoplasmonic sensor as working electrode, both electrical and spectral responses on the surface of the sensor could be simultaneously detected during the electrochemical process. Cyclic voltammetry was implemented to activate the oxidation and recovery of dopamine and serotonin, while transmission spectrum measurement was carried out to synchronously record to LSPR responses of the nanoplasmonic sensor. Coupling with electrochemistry, LSPR results indicated good integrity and linearity, along with promising accuracy in qualitative and quantitative detection even for mixed solution and in brain tissue homogenates. Also, the detection results of other negatively-charged neurotransmitters like acetylcholine demonstrated the selectivity of our detection method for transmitters with positive charge. When compared with traditional electrochemical signals, LSPR signals provided better signal-to-noise ratio and lower detection limits, along with immunity against interference factors like ascorbic acid. Taking the advantages of such robustness, the coupled detection method was proved to be a promising platform for point-of-care testing for neurotransmitters.

1. Introduction

Neurotransmitters are chemicals released by central and peripheral neural system that allow for signal transmission among neurons, and could be categorized into two types: excitatory neurotransmitters like dopamine, and inhibitory neurotransmitters like serotonin. These neurotransmitters are substantial in maintaining normal neurological functionalities, for example, dopamine in normal range allows the ordinary freedom of movement whereas insufficient dose of dopamine could trigger Parkinson's disease and schizophrenia (Arreguin et al., 2009). Serotonin, on the other hand, is responsible for balancing excessive excitatory neurotransmitters. Abnormal level of serotonin could trigger carcinoid syndrome (Wright-Honari et al., 1990) and depression (Mahar et al., 2014). Therefore, the accurate, real-time and on-spot measurement of these neurotransmitters are crucial not only in unraveling the mechanisms of neurological diseases but also in preventing possible neural disorders. Currently traditional detection methods are still widely used in neurotransmitters detection, such as capillary electrophoresis (Voegel et al., 1997; Zhou et al., 1995), fluorescence spectrometry (Clarke et al., 2008; Pérez-Ruiz et al., 2007; Wu et al., 2007) and liquid chromatography (Uutela et al., 2008). These detection techniques, however, are either time-consuming or expensive, requiring bulky detection equipment and complicated biomaterial binding process. To meet with the instantaneity and low-cost requirements of point-of-care (POC) monitoring neurotransmitters, several methods had been further developed, including electrochemical detection (Robinson et al., 2003) and nanoplasmonic sensing (Qin et al., 2015; Zheng et al., 2011).

Electrochemistry provides label-free methods to determine concentration and to yield energy data through redox potentials, and could be miniaturized using microelectronics technologies.

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http://dx.doi.org/10.1016/j.bios.2016.08.105
0956-5663/

Therefore it has been extensively utilized, especially for cyclic voltammetry, in rapid, on-spot determination of neurotransmitters, both in vivo (O'Neill, 1994) and in vitro (Mark et al., 1995). However, since the internal biological environments are extremely complicated, containing various kinds of interfering substances like ascorbic acid that always coexists with trace dopamine in organisms and shares a similar oxidation potential, therefore it is extremely difficult for direct examination using electrochemistry. Mostly in these electrochemical techniques, specially-designed electrodes were utilized in detection of neurotransmitters in order to both increase the sensitivity, selectivity as well as enhancing adsorption for trace transmitters (Ali et al., 2007; Sun et al., 2009). Unfortunately, the modification of electrode surface usually suffer from quick sensitivity loss and signal instability, like the electrochemically decorated electrodes reported in several publications (Gonon et al., 1981; Hafizi et al., 1990). In addition, one of the most commonly used electrochemical techniques, fast cyclic voltammetry, usually suffer from dramatic signal contortion and background noises, which increased the difficulty for accurate quantitative determination of neurotransmitters (Hermans et al., 2008).

The other promising detection method for neurotransmitters and other molecules is nanoplasmonic sensing. Plasmonic sensing is an enabling optical technique with capabilities of high-sensitive surface condition monitoring. Among nanoplasmonic sensing techniques, localized surface plasmon resonance (LSPR) sensing relies on localized field enhancement and confinement in close proximity to nanoparticles and therefore require very limited space for sensing (Willets and Van Duyne, 2007). In accordance with interface condition especially refractive index changes on the surface, LSPR could be triggered between the incident light and the surface plasmon, and resulting in corresponding change in transmission spectrum. Numerous effort have been placed on utilizing LSPR sensing methods to detect neurotransmitters, and most of these sensing methods are based on immunoassay in order to obtain specificity (Zhao et al., 2008). These methods, although generally granted with high sensitivity and promising selectivity, usually require complicated surface decoration process and therefore have limited repeatability because bioactive material are very fragile. Therefore, LSPR sensing are still limited in POC testing.

Hereby we proposed a spectroelectrochemical sensing method for the detection of neurotransmitters, i.e., to combine cyclic voltammetry and LSPR measurement for studying the redox chemistry of neurotransmitters. The experiment was based on a previously-reported nanohole array sensing device (Gartia et al., 2013; Zhang et al., 2015), namely the Lycurgus nanocup array, which was utilized as working electrode. Oxidation states of dopamine and serotonin, at the presence of ascorbic acid, were changed electrochemically by addition or removal of electrons at the surface of the nanostructured electrode, while spectral measurement was simultaneously carried out to record the refractive index changes of the solution adjacent to the electrode. Results from both electrochemical and LSPR dimensions demonstrated good integrity, and LSPR results were shown to be immune against interference like pH value, buffer solution and ascorbic acid. The specificity of the coupled detection method was tested by analyzing mixed solution of dopamine, serotonin and ascorbic acid as well as acetylcholine and uric acid. Finally dopamine and serotonin was detected in rat brain tissue homogenates, and the accuracy of LSPR remained promising. Our robust detection system requires no biomaterial decoration and have the potential for miniaturization, therefore could potentially be further developed into a low-cost point-of-care platform for in vitro neurotransmitter monitoring.

2. Experiment

2.1. Reagents and materials

Tetrachloroauric(III) acid hydrate, potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), dopamine hydrochloride, serotonin hydrochloride, ascorbic acid, acetylcholine, uric acid and artificial cerebrospinal fluid (aCSF) were purchased directly from Sigma-Aldrich (Oakville, ON). All other chemicals were of analytical grade and were used as received. Sprague-Dawley rats with weights of 250 g to 300 g purchased from the Laboratory of Animal Research Center (Zhejiang Province, China).

Stock solutions of dopamine and serotonin were prepared by dissolving transmitters in the phosphate buffer saline (PBS, pH=7.2), and were diluted to desired concentrations before use. Preparation of rat brain tissue homogenates was include in Appendix A. All solutions were stored at 4 °C and protected from light.

2.2. Nanosensor fabrication

Replica molding technique was utilized in this study to reduce fabrication cost. The master nanocone array (nanocone height was 500 nm, and pitch 350 nm, periodicity 350 nm) was fabricated on the glass substrate using the laser interference lithography patterning and reaction ion etching technique, followed by cleaning and silanizing with dimethyl dichlorosilane solution for 30 min and rinsing in ethanol and deionized water. Then, as indicated in Fig. 1a, the two-dimensional square lattice was transferred to a flexible and optically transparent polydimethylsiloxane (PMDS) film using nanoreplica molding process: 5 μL of UV-curable polymer (NOA-61) was evenly spread on the top of the nanocone master and a PET sheet of 250 μm thick was carefully put on top of the polymer, to avoid the bubble formation and to act as a substrate. The UV polymer was cured with a UV light-curing flood lamp system (Dymax EC-Series) with average power density of 105 mW/cm^2 for 60 s at room temperature, after which the nanopatterned PET substrate was peeled off carefully from the master mold (Fig. 1b). In order to make the structure surface plasmon active, a thin adhesive layer of Titanium (5 nm) was deposited and followed by 90 nm of metal layer of gold (Fig. 1c).

As seen in Fig. 1d, in order to enhance the optical and electrochemical properties of the electrode, AuNPs were galvanized onto the nanostructured surface by applying cyclic voltammetry (scope −0.6 V −1.5 V, scan rate=0.1 V/s) in chloroauric acid (1 wt%) in the standard three-electrode system where the gold working electrode was replaced with the fabricated nanostructured sensor. Scanning cycles of CV was optimized as 1 cycle according to the optical responses of the galvanized electrode against dopamine (Appendix B depicted the LSPR responses of the nanosensor with different cycles of galvanization CV). Finally the fabricated nanohole array was trimmed into 2 mm × 4 mm strips and employed as working electrodes.

2.3. Detection system

The coupled measuring system is comprised of two components, i.e., the LSPR monitoring system and the electrochemical measuring system.

For the LSPR monitoring system, a halogen cold light source (DT-MINI-2, Ocean Optics Inc., Dunedin, USA) was utilized as light source, whilst a spectrophotometer receptor (USB2000+, Ocean Optics Inc., Dunedin, USA) was used to record the transmission spectrum of the nanostructured electrode. A light emitting probe and a light receptor probe were connected to the light source and the spectrophotometer respectively by fiber bundles and allowed

Please cite this article as: Li, N., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.08.105