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Monitoring of dopamine release in single cell using ultrasensitive ITO microsensors modified with carbon nanotubes

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ABSTRACT

The study of single cell dynamics has been greatly adapted in biological and medical research and applications. In this work a novel microfluidic electrochemical sensor with carbon nanotubes (CNTs) modified indium tin oxide (ITO) microelectrode was developed for single cells release monitoring. The sensitivity of the electrochemical sensor after CNTs surface modification was improved by 2.5–3 orders of magnitude. The developed CNTs modified ITO sensor was successfully employed to monitor the dopamine release from single living rat pheochromocytoma (PC 12) cells. Its ultrahigh sensitivity, transparency and need for fewer agents enable this smart electrochemical sensor to become a powerful tool in recording dynamic release from various living tissues and organs optically and electrically.

1. Introduction

Dynamic detection of biomolecular release from living cells has always been an area of great interest in biological and medical science as the study of single cell dynamics could help us better understand diverse processes such as neuronal communication, neurotransmitter transport and secretion, receptor-mediated signal transduction and voltage-gated ion channels in regulating cellular functions (Brajter-Toth and Chambers, 2002). Meanwhile the very small dimensions of microelectrodes help facilitate measurements in biological microenvironments well (Adams et al., 1976). As a result, microelectrodes have been used in large areas as amperometric sensors for detecting transmitter released from single cells recently (Wightman et al., 1991; Kennedy et al., 1996; Sun and Gillis, 2006; Amatore et al., 2007; Zhang et al., 2008).

Indium tin oxide (ITO) is an excellent photoelectric material because it displays both good photo-penetrability and high electrical conductivity. ITO has been widely used as working electrode for electrochemical analysis of biomolecules (Wang and Wang, 2004; Ruan et al., 2002; Kim et al., 2005). Although it can decrease electrical resistance when used as an electrode, the electroanalytical activity of ITO is relatively low when compared with several other electrodes such as carbon fiber electrodes (Zhang et al., 1996; Huang et al., 2001). Modifying the surface of ITO electrode would be one of the efficient ways to increase its electroanalytical activity (Lin et al., 2008).

Since the electrochemical properties of carbon nanotubes (CNTs) have been unveiled, their application in electrochemical sensors and biosensors has gained much attention (Lin et al., 2004; Wang and Musameh, 2003; Okuno et al., 2007; Du et al., 2008; Cui et al., 2006, 2009). Recent studies demonstrated that CNTs have a high electrocatalytic effect and a fast electro-transfer rate (Li et al., 2003; Heller et al., 2005; Wang et al., 2003, 2002). CNTs-modified electrodes can accumulate important biomolecules and alleviate surface fouling effects.

In this paper, an ultra-sensitive electrochemical sensor was developed by modifying ITO working electrode with CNTs in a single microfluidic chip. The electrochemical behavior of the sensors was characterized by dopamine (DA) using cyclic voltammetry (CV). With a CNTs modified ITO microelectrode, we have successfully applied this electrochemical sensor in monitoring the release of dopamine from single rat pheochromocytoma (PC 12) cells.

2. Experimental

2.1. Chemicals and materials

CNTs were synthesized through metal organic chemical vapor deposition (MOCVD) and processed through ultrasonic agitation in a mixed sulfuric acid and nitric acid (3:1, v/v) to form carbonyl moi-

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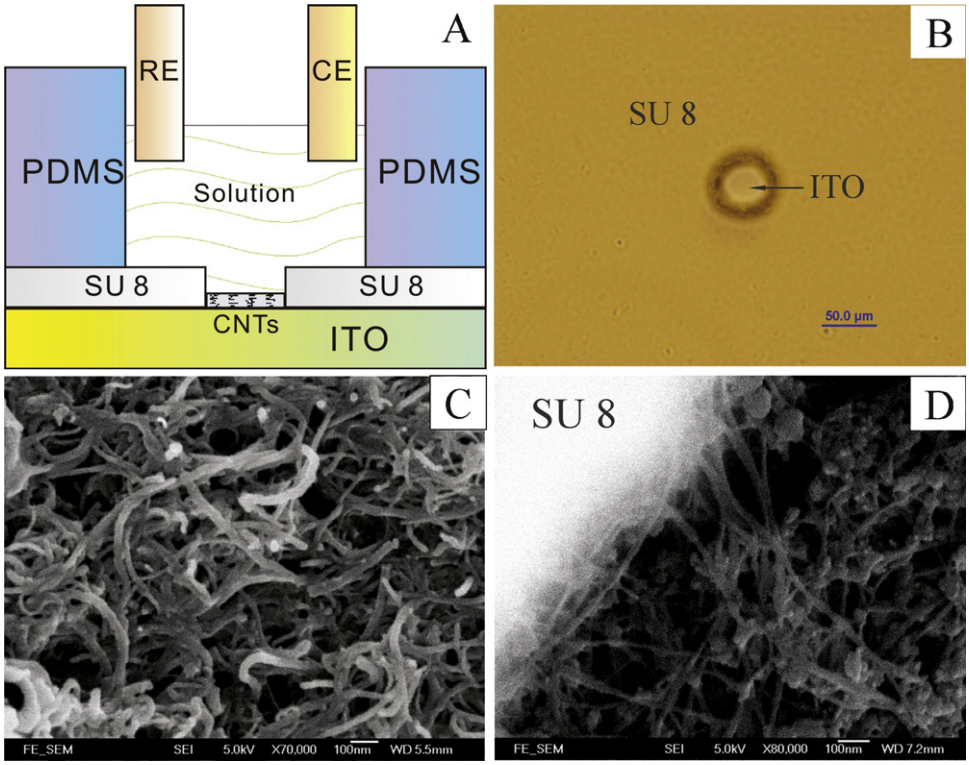

Fig. 1. (a) Cross-sectional schematic of the microfluidic electrochemical sensor with CNTs-ITO microelectrode. Ag/AgCl acted as reference electrode (RE) and Pt wire as counter electrode (CE). (b) Optical photograph of CNTs-ITO microelectrode patterned with SU-8 insulating layer. (c) SEM image of CNTs used for ITO surface modifications. (d) SEM image of the patterned CNTs-ITO microelectrode.

eties on the surfaces of the CNTs and to remove metallic impurities (Cao et al., 2002; Wang et al., 2004; Lin et al., 2008). Dopamine and norepinephrine were obtained from Sigma. Dopamine and norepinephrine samples were prepared by diluting 10 mM stock solutions in 100 mM $HClO_4$. All solutions were prepared with deionized water. The ITO glass (105 ± 20 nm thickness; 15 Ω/sq) purchased from Clover Display Ltd. (Hong Kong, China) was cleaned by sonication for 10 min sequentially in acetone, alcohol, and DI-water before use.

The bath solution contained 140 mM NaCl, 2 mM $CaCl_2$, 4.2 mM KCl, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES with pH = 7.4. The stimulus was high K^+ saline solution (pH = 7.4) containing 50 mM NaCl, 2 mM $CaCl_2$, 80 mM KCl, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES.

2.2. Cell culture

Rat pheochromocytoma (PC 12) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM (Gibco, USA) with an addition of 10% fetal bovine serum (Gibco) and 1% penicillin–streptomycin solution (Gibco). Cells were kept in a 95% humidity atmosphere with 5% CO_2 at 37 °C and the cell medium was refreshed every 2 days.

2.3. Electrochemical measurements and data treatment

Electrochemical measurements were carried out using a CHI electrochemical workstation. CNTs-ITO acted as the working electrode, Ag/AgCl as the reference electrode, and the platinum wire as the counter electrode. Voltammetric measurements were made in phosphate buffer solution (pH = 7.4).

The data were analyzed using home-developed programs. Spike parameters were calculated and analyzed according to the method (Mosharov and Sulzer, 2005). Briefly, spike amplitude, I_{max} (A), is measured between the current at T_{max} (time at spike maximum) and the baseline current under the spike maximum. Spike width, $t_{1/2}$ (s), is evaluated at 50% of I_{max}. The total electrical charge Q (C), is calculated according to the equation, $Q = I_{max} \times t_{1/2}$. I_{max}, and $t_{1/2}$, Q were presented as mean ± SEM (standard error of the mean), respectively.

3. Results and discussion

3.1. Scheme of the microfluidic electrochemical sensor and single cell releasing monitoring procedure

Fig. 1(a) shows the cross-sectional schematic of the microfluidic electrochemical sensor with a CNTs modified ITO (CNTs-ITO) microelectrode. ITO glass was used as the substrate. SU-8 photoresist was patterned on the substrate to limit the dimension of the ITO electrode for final detection (Fig. 1(b)). The SU-8 patterning was completed by conventional spin-coating and photolithographic processes. An exposed ITO microelectrode was modified with CNTs suspension spin-coated on its surface. The CNTs suspension (0.1 mg/mL) was obtained by dispersing purified CNTs (Fig. 1(c)) into an aqueous SDS surfactant solution. Poly(dimethylsiloxane) (PDMS) chamber was formed over the SU-8 pattern as a solution container as well as for reference electrode (RE) and counter electrode (CE) positioning. During the electrochemical characterization, the CNTs-ITO acted as the working electrode, Ag/AgCl as the reference electrode, and the platinum wire as the counter electrode.

The scheme of the single-cell release monitoring with the CNTs modified ITO microelectrode is shown in Fig. 2. As can be seen, an aliquot of PC 12 cells (ca. 1×10^5) was deposited into PDMS chamber positioned over the CNTs-ITO microelectrode. Cells were isolated according to the methods described in Amatore's work (Amatore et al., 2006). When one or two cells were observed to adhere to the CNTs-ITO microelectrode surface, they were allowed