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Electrochemical detection of catecholamine release using planar iridium oxide electrodes in nanoliter microfluidic cell culture volumes

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ABSTRACT

Release of neurotransmitters and hormones by calcium regulated exocytosis is a fundamental cellular/molecular process that is disrupted in a variety of psychiatric, neurological, and endocrine disorders. Therefore, this area represents a relevant target for drug and therapeutic development, efforts that will be aided by novel analytical tools and devices that provide mechanistically rich data with increased throughput. Toward this goal, we have electrochemically deposited iridium oxide (IrOx) films onto planar thin film platinum electrodes (20 μm × 300 μm) and utilized these for quantitative detection of catecholamine release from adrenal chromaffin cells trapped in a microfluidic network. The IrOx electrodes show a linear response to norepinephrine in the range of 0–400 μM, with a sensitivity of 23.1 ± 0.5 mA/M mm^2. The sensitivity of the IrOx electrodes does not change in the presence of ascorbic acid, a substance commonly found in biological samples. A replica molded polydimethylsiloxane (PDMS) microfluidic device with nanoliter sensing volumes was aligned and sealed to a glass substrate with the sensing electrodes. Small populations of chromaffin cells were trapped in the microfluidic device and stimulated by rapid perfusion with high potassium (50 mM) containing Tyrode's solution at a flow rate of 1 nL/s. Stimulation of the cells produced a rapid increase in current due to oxidation of the released catecholamines, with an estimated maximum concentration in the cell culture volume of ~52 μM. Thus, we demonstrate the utility of an integrated microfluidic network with IrOx electrodes for real-time quantitative detection of catecholamines released from small populations of chromaffin cells.

1. Introduction

Release of neurotransmitters by Ca^{2+} regulated exocytosis is the fundamental cellular/molecular process that underlies synaptic transmission and neuroendocrine hormone release. The finely tuned mechanisms that regulate neurosecretion are disrupted in a variety of psychiatric, neurological, and endocrine disorders, so are relevant targets for development of novel drugs and therapeutics aimed at improving human health. Hence, there is a need for a thorough, integrated understanding of stimulus–secretion coupling and reliable, high resolution methods to assay transmitter release and determine the neurosecretory phenotype in small populations of cells. Catecholamines including dopamine, norepinephrine, and epinephrine are one important class of neurotransmitter. Various approaches have been used to detect catecholamines including optical (chemical luminescence (Ragab et al., 2000), fluorescence (Nohta et al., 1997), spectrophotometric (Zhu et al., 1997)), electrophoresis (Jin et al., 1999), and liquid chromatography (Hows et al., 2004) methods. The electroanalytical methods (Kita and Wightman, 2008; Troyer and Wightman, 2002) represent one of the most attractive approaches due to the simplicity, high sensitivity, and temporal resolution of the method coupled with the ability to combine thin film electrodes with microfluidic technologies to engineer self contained lab-on-a-chip devices (Berberian et al., 2009; Lu et al., 2004). In recent years, many electrode modifications have been used for improving the determination of catecholamines such as conducting polymers (Liu et al., 2008), carbon nanotubes (Bian et al., 2010; Tsai et al., 2008; Wang et al., 2004), diamond thin films (Dong et al., 2009), and other organic or inorganic compounds

In this manuscript, we report the characteristics of electrochemically deposited IrOx onto thin film platinum electrodes as a sensitive material for the detection of catecholamines. IrOx electrodes are highly stable in cell culture media, show a constant sensitivity over a wide range of concentrations, and are easy to fabricate utilizing electrochemical methods. We also combine these planar sensors with a fluidic network to measure catecholamine secretion from small populations of chemically stimulated adrenal chromaffin cells at in vivo like cell densities.

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2. Materials and reagents

Iridium tetrachloride, oxalic acid dehydrate, hydrogen peroxide (30% solution in water) and anhydrous potassium carbonate were purchased from Aldrich. Platinum (99.95%) rod diameter 2 mm, and titanium (99.95%) rod diameter 2 mm were purchased from Goodfellow Corp. Universal pH buffers from VWR Scientific. The salts used for the Tyrode's solution (potassium chloride, sodium chloride, magnesium chloride, sodium phosphate, sodium bicarbonate) were supplied by Fisher Scientific. Norepinephrine was analytical grade and came from Sigma. Polydimethylsiloxane (PDMS) elastomer composed of prepolymer and curing agent (Sylgard184 kit, Dow Corning, Midland, MI) was purchased from Essex Chemical. All chemicals were used as received.

3. Instrumentation

Thin film Ti–Pt electrodes were fabricated on microscope glass slides (24 mm × 76 mm) using e-beam vacuum evaporation (Inotec Corp.) of titanium and platinum from carbon crucible liners (Kurt J. Lesker Company). The deposition rate and the thickness of the films were monitored with a deposition controller MDC-360 (Maxtek, Inc.). A surface profiler (Alphastep200, Tencor Instruments) was used to measure the thickness of the photoresist and IrOx layers. An upright optical microscope (OLYMPUS BX-41) with a CCD camera Micropublisher 3.3 (QImagine, Canada) was used to monitor the surface morphology of platinum, iridium oxide thin films. A stereomicroscope STEMI 2000-C (Carl Zeiss, Germany) was used to align the iridium oxide thin film electrodes relative to the microfluidic network. An inverted microscope (AXIOVERT 25CFL, Carl Zeiss, Germany) in combination with a color CCD camera (KP-D20BU, Hitachi, Japan) was used to image the cells in the microfluidic device. The fluidic flow was controlled and maintained using a microsyringe pump controller MICRO-4 (WPI, Sarasota, FL) for flow rates ranging from 0.1–30 nL/s. Electrochemical experiments were performed with a potentiostat (CHI 660B or CHI 1030) from CH Instruments (Austin, TX). A three-electrode configuration was used for the electrochemical deposition of the IrOx films and the quantification of catecholamine release from chromaffin cells. The counter electrode was either a Pt wire with a diameter of 1 mm for beaker experiments or a Pt film for experiments in a microfluidic device. In all experiments a Dri-Ref-450 from WPI Inc. was used as reference electrode (diameter 0.45 mm).

4. Experimental

In order to characterize the release of the catecholamine from chromaffin cells we utilized a microfluidic device which was fabricated according to the following steps: (i) deposition and patterning of the thin film Pt electrodes; (ii) electrochemical deposition of IrOx onto the Pt electrodes; (iii) replica molding of microfluidic devices with nanoliter cell culture volumes. Fig. 1 depicts a schematic view of the electrode array with micron sized electrodes and a T-type microfluidic channel network. In the next sections, we describe each of these fabrication processes in detail.

4.1. Thin film conducting electrodes

The thin film conductive electrodes were fabricated on 25 mm × 25 mm glass substrates. A Ti layer (~100 Å) is used to mediate the adhesion of the Pt layer (~1000 Å). The main parameters of the deposition process were: substrate temperature ~25 °C; total background pressure during deposition was 5×10^{-6} Torr; the deposition rate for Ti and Pt was ~5 Å/s. The electrode array was patterned using standard photolithography. The exposed part of

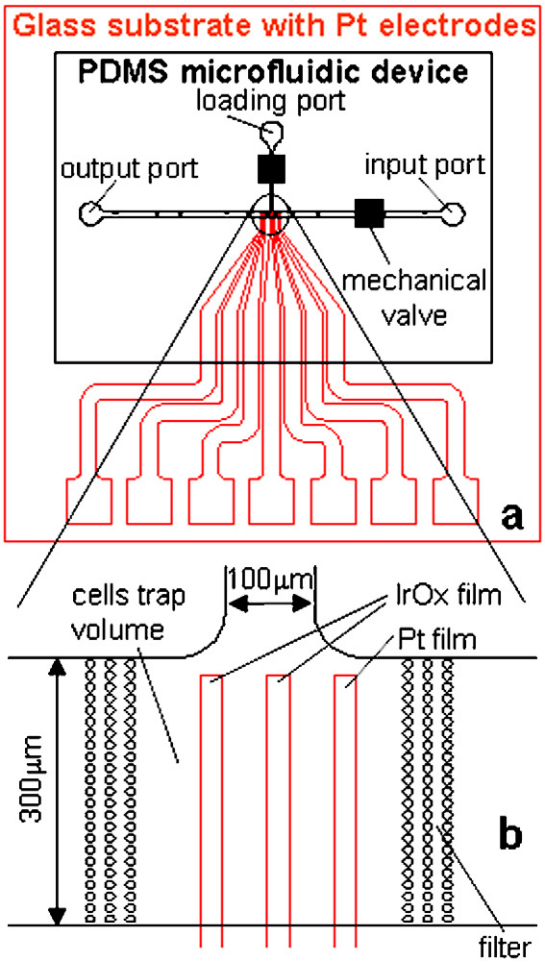

Fig. 1. Schematic overview of the microfluidic device to measure catecholamine release from chromaffin cells in nanoliter volume. (a) black – PDMS microfluidic device with channel network; red – glass substrate with microelectrode array; (b) enlarged view of IrOx and Pt electrodes in the cell culture volume (CCV). The volume of CCV is ~3 nL (300 µm × 300 µm × 30 µm), the size of catecholamine sensitive electrode is 20 µm × 300 µm, the gap in the filter post structure to retain the cells is 3 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the Pt film was removed by ion beam etching. The photoresist was removed with acetone. Finally, the surface of the electrodes was cleaned with isopropyl alcohol, sonicated in DI water and electrochemically pre-treated by cycling the potential between −0.3 and +1.2 V vs Ag/AgCl in 0.5 M H_2SO_4. A schematic drawing of the electrode geometry is shown in Fig. 1a (red lines). The electrode sensing area was 100 µm × 300 µm and 20 µm × 300 µm for experiments in single channels and T-type microfluidic devices, respectively. Three to five central electrodes were used as catecholamine sensitive electrodes and lateral bare Pt electrodes were used as counter electrodes.

4.2. Iridium oxide electrode fabrication

The solution for the electrochemical deposition of iridium oxide films was prepared according to protocols described in the literature (Marzouk et al., 1998; Yamanaka, 1989). In our case we used the following protocol: 150 mg $IrCl_4$ was dissolved in 100 mL of DI water and stirred for 20 min; 1 mL of 30% hydrogen peroxide was added and the resulting solution was stirred for 15 min; 0.5 g of oxalic acid was added and stirred for 5 min; anhydrous potassium carbonate was added to adjust the pH to 10.5. In fresh solution