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Carbon nanotube multi-electrode array chips for noninvasive real-time measurement of dopamine, action potentials, and postsynaptic potentials

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ABSTRACT

Multi-electrode arrays (MEAs) can be used for noninvasive, real-time, and long-term recording of electrophysiological activity and changes in the extracellular chemical microenvironment. Neural network organization, neuronal excitability, synaptic and phenotypic plasticity, and drug responses may be monitored by MEAs, but it is still difficult to measure presynaptic activity, such as neurotransmitter release, from the presynaptic bouton. In this study, we describe the development of planar carbon nanotube (CNT)-MEA chips that can measure both the release of the neurotransmitter dopamine as well as electrophysiological responses such as field postsynaptic potentials (fPSPs) and action potentials (APs). These CNT-MEA chips were fabricated by electroplating the indium–tin oxide (ITO) microelectrode surfaces. The CNT-plated ITO electrode exhibited electrochemical response, having much higher current density compared with the bare ITO electrode. Chronoamperometric measurements using these CNT-MEA chips detected dopamine at nanomolar concentrations. By placing mouse striatal brain slices on the CNT-MEA chip, we successfully measured synaptic dopamine release from spontaneous firings with a high *S*/*N* ratio of 62. Furthermore, APs and fPSPs were measured from cultured hippocampal neurons and slices with high temporal resolution and a 100-fold greater *S*/*N* ratio. Our CNT-MEA chips made it possible to measure neurotransmitter dopamine (presynaptic activities), postsynaptic potentials, and action potentials, which have a central role in information processing in the neuronal network. CNT-MEA chips could prove useful for in vitro studies of stem cell differentiation, drug screening and toxicity, synaptic plasticity, and pathogenic processes involved in epilepsy, stroke, and neurodegenerative diseases.

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1. Introduction

Multi-electrode biochip technologies allow analysis of neural function at both the single cell and network levels and permit stable long-term recordings from cultures and isolated tissues. Current applications include monitoring changes in the transmitter or electrophysiological phenotype in culture, drug screening and toxicity assays, and studies of long-lasting synaptic plasticity. For example, planar multipatch clamp techniques have been widely used to assess the effects of drugs on ion channel gating (Kiss et al., 2003; Py et al., 2011). Among the most clinically important potential applications is analysis of optimal conditions for expansion and lineage guidance of human induced pluripotent stem (iPS) cells (Takahashi et al., 2007; Saporta et al., 2011; Braun et al., 2012). In addition, the development of MEA biochips that can analyze electrophysiological responses and changes in the extracellular chemical microenvironment, including transmitter release, would be a significant leap for studies of neural network properties and neuropathogenesis.

Several biochips have been developed with planner MEAs to measure APs and field postsynaptic potentials (fPSPs) from cultured neuronal networks and brain slices (Gross, 1979; Pine, 1980; Jimbo et al., 1999; Suzuki et al., 2005; Yi et al., 2009). The construction of MEAs requires precise microfabrication techniques to print integrated microelectrodes on glass, and the basic design has been widely used to obtain long-term multisite recordings with high temporal resolution (Jimbo et al., 2003; Mielke et al., 2007; Zhang et al., 2009; Pimashkin et al., 2011). Although PSPs and APs can be measured by several types of MEAs, it is still difficult to measure presynaptic activity such as neurotransmitter release from the presynaptic bouton. The pathogenesis and symptoms of many neurological diseases stem from dysregulation of neurotransmitters; hence, the simultaneous measurement of transmitter release and postsynaptic response could provide

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further insight into these diseases. For example, abnormal release of neurotransmitters such as dopamine and glutamate contribute to progressive and as yet incurable diseases such as Parkinson's disease and schizophrenia.

The traditional technology for measuring many electrochemically active neurotransmitters involves oxidation–reduction reactions at the electrode surface (Hutson and Curzon, 1983; Ge et al., 2011; Ozel et al., 2011). Dopamine has been measured using carbon fibers, glassy carbon, or carbon nanotube (CNT) electrodes (Zhao et al., 2001; Wightman, 2006; Keithley et al., 2011; Shi et al., 2011; Li et al., 2012). It has been reported that CNTs show particularly high sensitivity to electrochemically active species like dopamine (Liu et al., 2007; Rivas et al., 2007; Saleh Ahammad et al., 2009; Sansuk et al., 2013). Indeed, modified CNTs on the surface of the electrode have improved both the sensitivity and temporal resolution of these measurements (Haghighi and Bozorgzadeh, 2011; Jacobs et al., 2011; Palanisamy et al., 2012; Goran et al., 2013).

The most widely used methods for modifying CNTs on the electrode surface are immersion drying (Gabriel et al., 2009; Haghighi and Bozorgzadeh, 2011; Jacobs et al., 2011; Li et al., 2012; Palanisamy et al., 2012; Goran et al., 2013) and direct chemical vapor deposition (CVD) (Bareket-Keren and Hanein, 2012; Heim et al., 2012; Viet et al., 2012). However, immersion drying yields CNT coatings with limited strength and durability, while direct CVD requires expensive technology. Therefore, we have focused on developing simple electroplating techniques to coat CNTs on the surface of indium–tin oxide (ITO) microelectrode chips. Although CNT electroplating has been described (Yang et al., 2010; Hu et al., 2012), electroplating onto microelectrodes for both electrophysiology and highly sensitive detection of dopamine has not been achieved.

We modified ITO-MEAs by CNT electroplating to create a novel biochip (CNT-MEA) that can measure all three principal outputs of functional neuronal networks, i.e., neurotransmitter release, APs, and PSPs. We succeeded in detecting nanomolar concentrations of dopamine in solution and dopamine release from rat striatal slices. Furthermore, we measured APs and PSPs in cultured hippocampal neurons and hippocampal slices with microsecond temporal resolution and signal-to-noise ratio $(S/N) > 100$.

2. Materials and methods

2.1. Reagents

Bamboo-structured multi-wall carbon (diameter 20–40 nm, length 1–5 μm) powders were purchased from NanoLab (USA). Double-stranded DNA samples were kindly gifted by Maruha Nichiro Holdings Inc. (Japan). Dimethylformamide was purchased from Wako Pure Chemical Industries (Japan). Potassium ferricyanide and dopamine were purchased from Sigma-Aldrich (USA).

2.2. Apparatus

All electrochemical experiments and electroplating were performed with an ALS 1140A electrochemical analyzer (ALS, Co., Ltd., Japan). Electrophysiological recordings for APs and PSPs were performed using CNT-MEA chips connected to the MED planar MEA recording system (Alpha MED Sciences Co., Ltd., Japan).

Surface characterization of the electroplated CNTs was conducted by high-resolution scanning electron microscopy (JSM-6060LV, JEOL). Sample chips were sputter-coated with a thin layer of Pt using a Pt coater (JFC-1600, JEOL), and SEM images were obtained using an acceleration voltage of 5 kV and 50,000 × magnification.

2.3. Fabrication of CNT-plated ITO MEA chips

Planar ITO MEAs were produced on glass slides using photolithography. Arrays contained either 64 electrodes, 50×50 μm, spaced 300 μm apart and arranged in $4 \times 4 \times 4$ grids or 16 electrodes, 200×200 μm (Alpha MED Sciences Co., Ltd., Japan), the latter used for detection of dopamine. To insulate each electrode, acrylic imide insulating film was coated on the entire chip surface and the recording terminals were exposed by chemical etching. MWCNTs were electroplated onto the surface of the ITO electrode. Briefly, MWCNTs and double-stranded DNA were sonicated at 2:1 (w/w) in 1:1 dimethylformamide plus deionized water for 10 min. The dispersed MWCNT solution was dripped onto ITO planar MEA chips and electroplated using an electrochemical analyzer in a two-electrode mode using a Pt reference electrode. Applied voltage was 2.4 V/60 s for 50×50 μm electrode chips and 4.3 V/60 s for 200×200 μm chips.

2.4. Electrochemical measurements of dopamine

Electrochemical measurements of dopamine were performed using CNT-MEA chip with an Ag/AgCl reference electrode and a Pt counter electrode. Ag/AgCl and Pt electrodes were inserted into culture rings on the CNT-MEA chip and fixed at the ring cap [Fig. S1A (Electronic Supplementary Information; ESI)]. All potentials in this study are reported with respect to the Ag/AgCl electrode. The amperometric response of dopamine was recorded at +0.3 V. For in vitro measurements, dopamine was dissolved in phosphate buffered saline (PBS) at pH 7.4. All electrochemical detection experiments were performed at room temperature.

2.5. Brain slice preparation and neuronal culture

Striatal and hippocampal slices were prepared from 4-weeks-old male mouse (ICR, Charles River Laboratory Japan, Inc.). In brief, mice were rapidly decapitated under anesthesia. The brains were quickly removed, and in some cases the hippocampal tissue was carefully isolated. Striatal slices of 300 μm thickness were prepared in both the coronal and sagittal planes and hippocampal slices in the transverse plane using a microslicer (Dosaka DTK-1000, Japan). The slices were maintained at room temperature (22–25 °C) in a holding chamber containing oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid [ACSF (mM): 120.0 NaCl, 25 $NaHCO_3$, 3.3 KCl, 1.23 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgSO_4$, and 10.0 D-glucose, at pH 7.4] and allowed to equilibrate for at least 1 h prior to physiological measurements.

For neuronal cultures, hippocampi from E18 rat were removed and trypsinized (0.25%) at 37 °C for 15 min and dissociated by trituration in Hank's balanced salt solution (HBSS, Invitrogen). The cell suspension was briefly centrifuged and the pellet resuspended in 5 ml neurobasal medium containing 2% B27 serum-free supplement and 1% penicillin–streptomycin (Invitrogen). A droplet (50 μl) of cell suspension was loaded onto poly-d-lysine-coated CNT-MEA chips at 130 cells/mm^2 and maintained in culture under a 5% CO_2 atmosphere at 37 °C.

2.6. Real-time measurement of dopamine release from striatal slices

For real-time measurement of synaptic dopamine release, the striatal region of coronal or saggital brain slices was placed over a 200×200 μm CNT-plated ITO-MEA chip that formed the bottom of a superfusion chamber. The chips were connected to a 30 °C heating plate and the chamber was continuously perfused with oxygenated ACSF at a rate of 2 ml/min at 29–30 °C. An Ag/AgCl wire and a Pt wire in the superfusion chamber acted as reference and counter electrodes, respectively [Fig. S1B, ESI]. To detect

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