Biosensors and Bioelectronics 26 (2010) 92–98

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Nanocrystalline diamond microelectrode arrays fabricated on sapphire technology for high-time resolution of quantal catecholamine secretion from chromaffin cells

V. Carabelli[a,∗], S. Gosso[a], A. Marcantoni[a], Y. Xu[b], E. Colombo[a,b], Z. Gao[b], E. Vittone[c], E. Kohn[b], A. Pasquarelli[b], E. Carbone[a]

[a] Department of Neuroscience, NIS Centre of Excellence University of Torino, Corso Raffaello 30, 10125 Torino, Italy
[b] Institute of Electron Devices and Circuits, Ulm University, 89081 Ulm, Germany
[c] Department of Experimental Physics, NIS Centre of Excellence University of Torino, 10125 Torino, Italy

ARTICLE INFO

Article history:
Received 5 February 2010
Received in revised form 15 April 2010
Accepted 10 May 2010
Available online 19 May 2010

Keywords:
Nanocrystalline diamond
Chromaffin cell
Amperometry
Ca^{2+}-dependent exocytosis
Quantal secretory event

ABSTRACT

The quantal release of oxidizable molecules can be successfully monitored by means of polarized carbon fiber microelectrodes (CFEs) positioned in close proximity to the cell membrane. To partially overcome certain CFE limitations, mainly related to their low spatial resolution and lack of optical transparency, we developed a planar boron-doped nanocrystalline diamond (NCD) prototype, grown on a transparent sapphire wafer. Responsiveness to applied catecholamines as well as the electrochemical and optical properties of the NCD-based device were first characterized by cyclic voltammetry and optical transmittance measurements. By stimulating chromaffin cells positioned on the device with external KCl, well-resolved quantal exocytotic events could be detected either from one NCD microelectrode, or simultaneously from an array of four microelectrodes, indicating that the chip is able to monitor secretory events (amperometric spikes) from a number of isolated chromaffin cells. Spikes detected by the planar NCD device had comparable amplitudes, kinetics and vesicle diameter distributions as those measured by conventional CFEs from the same chromaffin cell.

1. Introduction

In neurons and neuroendocrine cells, neurotransmitters are packed in intracellular vesicles of 50–200 nm diameter and released in the form of "quanta" into the extracellular space after vesicle fusion with the plasma membrane (exocytosis). The quantal nature of neurotransmitter release is at the basis of synaptic functioning and has been investigated since the early 1990s by means of carbon fiber microelectrodes (CFEs) (Wightman et al., 1991). When applied to detect exocytotic events in isolated chromaffin cells, CFEs can provide key information on the formation of the fusion pore and the kinetics of single secretory events with sub-millisecond time resolution (Chow et al., 1992). The technique, however, has some major restrictions. Monitoring quantal secretory events is limited to one cell for each recording and there is no spatial resolution of vesicle distribution below the area of carbon fiber tip.

Following well-established methods used for studying neural networks and acute slices, in this work we adopted a simplified planar microelectrode configuration for recording quantal secretory events. Recently, other planar amperometric devices based on various technologies, e.g. platinum, gold, indium-doped tin oxide (ITO) and nitrogen-doped diamond-like carbon (DLC:N) deposited on glass and silicon layers have been shown to monitor well-resolved quantal amperometric events (Dias et al., 2002; Chen et al., 2003, 2008; Hafez et al., 2005; Parpura, 2005; Cui et al., 2006; Gao et al., 2008). Among these technologies, ITO is the most interesting one for producing transparent electrodes and wires (Guillen and Herrero, 2005). It is more transparent than boron-doped NCD and works nicely in the anodic regime (Sun and Gillis, 2006). However, ITO shows a lower chemical stability, especially at acidic pH and cathodic potentials, and a narrower potential window with respect to the ∼3 V of boron-doped NCD electrodes (Sun and Gillis, 2006; Chen et al., 2008; Sen et al., 2009).

Here we describe a cost-effective long-life device that can potentially be exploited in a wide variety of amperometric measurements, thanks to the superior properties of diamond technology. To this purpose we developed a chip prototype made using nanocrystalline diamond thin-film technology on sapphire, patterned with four boron-doped microelectrodes. Diamond is optically transparent, highly biocompatible, chemically inert and can exhibit a quasi-metallic conductivity when properly doped (Show et al., 2003) or functionalised (Ariano et al., 2009). Moreover, the high corrosion resistance of the NCD electrodes ensures a long-term reliability not easily achievable with other elec-

∗ Corresponding author. Tel.: +39 011 6708488; fax: +39 011 6708174.
E-mail address: valentina.carabelli@unito.it (V. Carabelli).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.05.017