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Microelectrode Arrays of Diamond-Insulated Graphitic Channels for Real-Time Detection of Exocytotic Events from Cultured Chromaffin Cells and Slices of Adrenal Glands

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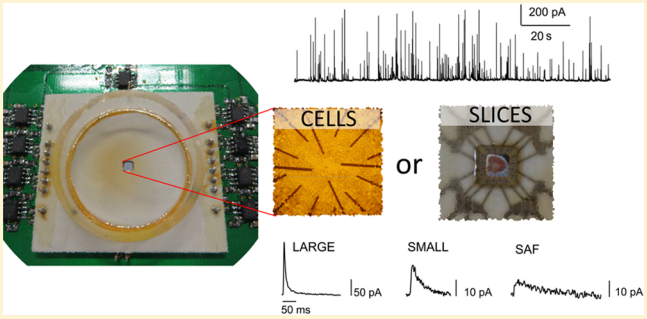
Supporting Information

ABSTRACT: A microstructured graphitic 4 × 4 multielectrode array was embedded in a single-crystal diamond substrate (4 × 4 μG-SCD MEA) for real-time monitoring of exocytotic events from cultured chromaffin cells and adrenal slices. The current approach relies on the development of a parallel ion beam lithographic technique, which assures the time-effective fabrication of extended arrays with reproducible electrode dimensions. The reported device is suitable for performing amperometric and voltammetric recordings with high sensitivity and temporal resolution, by simultaneously acquiring data from 16 rectangularly shaped microelectrodes (20 × 3.5 μm^2) separated by 200 μm gaps. Taking advantage of the array geometry we addressed the following specific issues: (i) detect both the spontaneous and KCl-evoked secretion simultaneously from several chromaffin cells directly cultured on the device surface, (ii) resolve the waveform of different subsets of exocytotic events, and (iii) monitoring quantal secretory events from thin slices of the adrenal gland. The frequency of spontaneous release was low (0.12 and 0.3 Hz, respectively, for adrenal slices and cultured cells) and increased up to 0.9 Hz after stimulation with 30 mM KCl in cultured cells. The spike amplitude as well as rise and decay time were comparable with those measured by carbon fiber microelectrodes and allowed to identify three different subsets of secretory events associated with "full fusion" events, "kiss-and-run" and "kiss-and-stay" exocytosis, confirming that the device has adequate sensitivity and time resolution for real-time recordings. The device offers the significant advantage of shortening the time to collect data by allowing simultaneous recordings from cell populations either in primary cell cultures or in intact tissues.



Single-cell amperometry allows the detection of quantal fusion events with submillisecond time resolution and picoampere sensitivity.[1,2] In the past decade, conventional approaches using carbon fiber electrodes have been combined to a variety of chip-based planar arrays,[3,4] with the aim of increasing the spatial resolution and providing an electrochemical mapping of exocytosis within a single cell[5−7] or collecting events from multiple samples.[8−14] For a recent comprehensive review on the subject, see refs 15 and 16. Focusing on multiple detection of vesicular release, arrays with up to 64 or even 10 × 10 electrodes with subcellular dimensions have been produced,[12,13] thus allowing a multisite detection from chromaffin, PC-12 cells, striatal slices,[17−20] and the coupling of amperometry with fluorescence microscopy.[21,22] An appealing substrate material for these applications is diamond, which offers a wide spectrum of physical-chemical properties which are crucial for the realization of integrated planar sensors, namely, wide optical transparency from infrared (IR) to near-ultraviolet (NUV), high chemical inertness,[23] good biocompatibility,[24] and the possibility of directly writing subsuperficial graphitic microelectrodes by means of MeV ion beam lithography.[25−31]

Taking advantage of the above-mentioned properties, several prototypes of diamond-based electrochemical sensors with integrated graphitic microelectrodes have been developed and tested. A first prototype allowed to detect quantal secretion

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