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# A New Diamond Biosensor with Integrated Graphitic Microchannels for Detecting Quantal Exocytic Events from Chromaffin Cells

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## Introduction

The quantal release of bioactive molecules from neurons and neuroendocrine cells is a fundamental mechanism that regulates synaptic transmission and hormone release. In particular, chromaffin cells of the adrenal gland, expressing voltage-gated Ca channels functionally coupled to the secretory apparatus, represent an ideal system to study the exocytotic release of catecholamines (adrenaline, noradrenaline) from chromaffin granules, where they are stored at high concentration (1 M) together with ATP, opioids, peptides.[1] Carbon fiber microelectrodes (CFEs) are employed since few decades to detect the exocytotic activity of single excitable cells with amperometric techniques and provide significant information on key mechanisms such as the formation of the fusion pore and the kinetics of single secretory events in real time.[2–5] These classical carbon-based probes possess high, chemical stability and biocompatibility but can be hardly integrated in a miniaturized multi-electrode device. This limits the possibility of using them in multiple single-cell recordings or even to resolve secretory events within microareas of a single secretory cell. Two technical advances that would be beneficial for investigating the molecular basis of synaptic transmission in neuronal networks and the subcellular arrangement of the secretory apparatus.

The accessibility to a graphitic phase in single-crystal diamond through spatially resolved lattice damage by means of energetic ion beams offers several appealing applications in microdevices fabrication. In particular, a technique based on MeV ion-beam lithography through variable-thickness masks was recently developed, which allowed the direct fabrication of buried graphitic microchannels in single-crystal diamond at a variable depth.[6–9]

In this work, we demonstrate that ion-beam lithography can be successfully employed for the fabrication of a monolithic all-carbon miniaturized cellular biosensor (μG-SCD) for recording the exocytotic activity of single chromaffin cells, with detection performances comparable to CFEs.

*Device Microfabrication*: The biosensor was realized with a synthetic single crystal diamond of 3 mm × 3 mm × 1.5 mm. The diamond was produced with high pressure high temperature (HPHT) technique by Sumitomo Electrics and it is classified as type Ib, with a substitutional nitrogen concentration comprised between 10 and 100 ppm. The sample is cut along the (100) crystal direction and it is optically polished on the two opposite large faces. The crystal has good transparency in the visible spectrum, with an absorption band in the blue-violet region.

Sub-superficial conductive micropaths were realized in the diamond matrix by means of a deep ion beam lithography technique based on direct focused ion beam writing through suitable variable-thickness masks, thus allowing for the modulation of the depth at which the channels are formed and therefore their emergence at specific locations of the sample surface. The fabrication method is described in details in previous works.[6–9] The above-mentioned variable-thickness masks were realized on the diamond surface by thermal evaporation of Cu. As shown schematically in **Figure 1**a, this arrangement allowed the deposition of two Cu 3–5 μm thick masking structures with slowly degrading edges. The diamond sample was then irradiated with a 1.8 MeV $He^+$ beam at the ion microbeam line of the AN2000 accelerator of the Legnaro National Laboratories.[10]

The energy transferred by ions to the diamond lattice induces the displacement of carbon ions and hence the creation of defects. Their distribution along the ion track follows a typical depth profile, with a peak at the end of range. In Figure 1a the damage density profile is reported: damage is parametrized in terms of vacancy density and derived from the SRIM-2008.04 Monte Carlo code[11] by taking an atom displacement energy value of 50 eV.[12] The vacancy density is obtained with a simple linear approximation by multiplying the output of SRIM simulations (number of vacancies per ion per unit depth) with the implantation fluence. Such a crude approximation does not take into account complex non-linear damage effects such as defect-defect interaction and self-annealing[13] and therefore leads to a significant over-estimation of the vacancy density

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