A ION BEAM MICROMACHINED DIAMOND BIOSENSOR FOR DETECTING QUANTAL EXOCYTIC EVENTS FROM CHROMAFFIN CELLS

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Introduction

In the present work we report about a first prototype of single crystal diamond based biosensors for detecting quantal exocytic events from chromaffin cells.

The biosensor was fabricated by using a focused 1.6 MeV He ion beam scanned on a Ib monocrystalline diamond sample (3×3×0,5 mm^3) to define highly damaged regions with a resolution of the order of micrometer.

After the irradiation, the sample was annealed at high temperature (> 900 °C) in order to promote the conversion into a graphitic phase of the region in which the ion-beam process has introduced vacancies density that overcame a critical dose of $1 \cdot 10^{23}$ vacancies cm^{-3} (graphitization threshold) and to recover the sub-threshold-damaged regions to the diamond phase.

This process provided buried highly conductive graphitic channels (resistivity ~mΩ·cm) embedded in a highly insulating and chemically inert diamond matrix, whose end points emerge and are therefore available as surface electrodes.

The microelectrodes show a high electrochemical responsiveness to oxidizable molecules as evaluated by recording the drift redox currents following the addition of consecutive micro-drops of adrenaline in proximity to the emerging channels.

Quantal secretory responses have been measured from stimulated chromaffin cells positioned on the graphitic microelectrodes which were polarized at +800 mV. Sequences of amperometric spikes started after cell stimulation with KCl-enriched solution, with amplitudes well above the background noise within the range of 8-180 pA and comparable with signals obtained by conventional carbon fiber electrodes.

These results demonstrate the potential of the ion beam micromachining technique to fabricate micrometer sized arrays of microelectrodes in monocrystalline diamond with an electrochemical sensitivity suitable for high-resolution measurement of quantal exocytosis.

Experimental: device microfabrication

In the present work an artificial single-crystal diamond sample produced by Sumitomo Electric by means of "high pressure high temperature" (HPHT) technique was employed. Sample size is 3×3×1.5 mm^3 and the crystal is classified as type Ib, i.e. its substitutional nitrogen concentration is comprised between 10 and 100 ppm.

The sample was implanted at the ion microbeam line of the AN2000 accelerator of the Legnaro National Laboratories with a scanning beam of 1.8 MeV He^+ ions at typical fluences of ~$5 \cdot 10^{17}$ cm^{-2}. Ion beam size was 10 μm, while beam currents were comprised between 5 nA and 8 nA, thus ensuring typical implantation times of 50 minutes. The sample was metal-coated to avoid surface charging and fluence was accurately monitored with an electrometer connected to the sample chamber, which is electrically insulated from the rest of the beamline, thus acting effectively as a Faraday cup.

As reported in previous works [1-5], high-fluence MeV ion implantation determines the formation of a sub-superficial amorphized layer in correspondence of the ion end-of-range. In the present work, ion implantation led to the formation of a ~300 nm thick amorphous layer at a depth of 3.2 μm below the sample surface. The employment of variable-thickness masks defined on the sample surface by means of non-contact metal evaporation allowed the gradual modulation of the penetration depth of the MeV ions, thus ensuring the emergence of the buried layers at the sample surface at specific locations, as schematically shown in Figure 1.

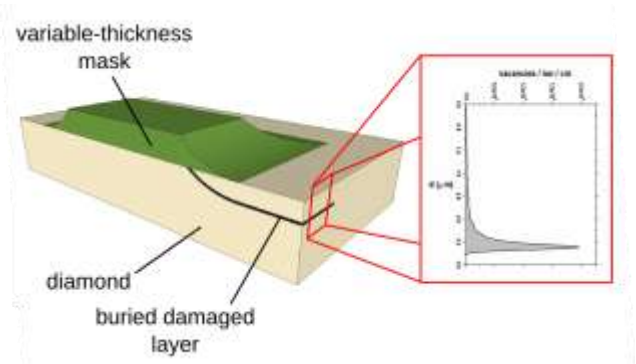

Figure 1. Schematics of the variable-thickness process allowing the definition of the buried amorphous layer at variable depth.

After ion implantation, the sample was annealed in vacuum at a temperature of 1100 °C for 2 hours. As reported in previous works [1-5], the process resulted in the conversion of the amorphized layer to a graphitic phase, while the surrounding regions which were damaged below a critical threshold reconverted to the pristine diamond form. One of the electrically conductive graphitic microchannels was subsequently connected to the acquisition electronic chain by means of a standard metal contact, while the other emerging end defined the location at the sample surface where biochemical sensing was performed, as schematically shown in Figure 2.

It is worth underlying that the micro-electrode is employed to monitor the activity of a living cell, which during the *in vitro* measurements is immersed in a physiological solution: for this reason buried connections embedded in the insulating diamond matrix are demanded, and the metal macro-contacts are shielded with a biocompatible insulating mask in "sylgard" polymer.

Before proceeding with the functional tests reported in the following section, two-points electrical characterization was performed to check that i) the buried electrical channels get in electrical contact with the sample surface at their endpoints and ii) the electrical resistivity of the microchannels is compatible with that of polycrystalline graphite (i.e. ~$3 \cdot 10^{-3}$ Ω cm).