Fabrication of a Diamond-Based Cellular Biosensor with Ion Beam Lithography

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INTRODUCTION

Following our previous studies on the formation of conductive graphitic microchannels in single-crystal diamond by means of direct lithography with a scanning ion microbeam [1-6], we report on the fabrication and preliminary testing of a cellular biosensor with the above-mentioned technique.

The so-called "Lab-on-chip" devices are highly demanded in modern biotechnology to cultivate living cells for long periods while inducing and revealing a broad range of electrical biosignals, such as nerve excitations and synaptic transmission. An integrated diamond-based device can effectively address many open issues in the existing cell-sensing research which to different extents are not met by conventional bio materials (silicon, metals and metals oxides and polymers), such as robustness and reproducibility in performance over repeated biosensing cycles, biocompatibility and long term stability for in vivo measurements and high transparency for optical interfacing.

Here we report on the MeV ion-beam fabrication process of a diamond cellular substrate with graphitic electrodes, and on the results of its preliminary characterization for the detection of exocytosis activity from chromaffin cells.

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 ~5×10^{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 beam line, thus acting effectively as a Faraday cup.

As reported in previous works [1-6], high-fluency MeV ion implantation determines the formation of a sub-surface 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.

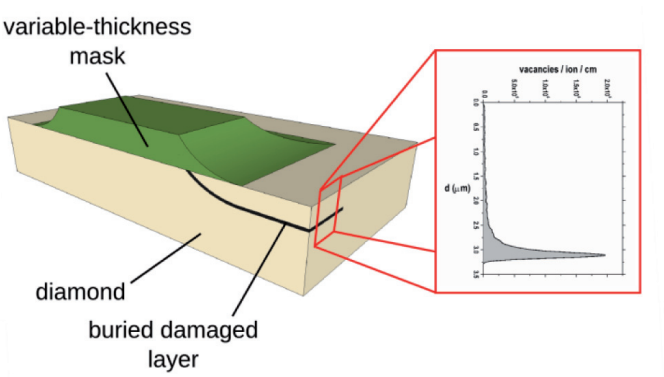

Fig. 1. Schematics of the variable-thickness process allowing the definition of the buried amorphous layer at variable depth.

After ion implantation, the sample was annealed in vacuum at a temperature of 1100 °C for 2 hours. As reported in previous works [1-6], 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. ~3×10^{-3} Ω·cm).

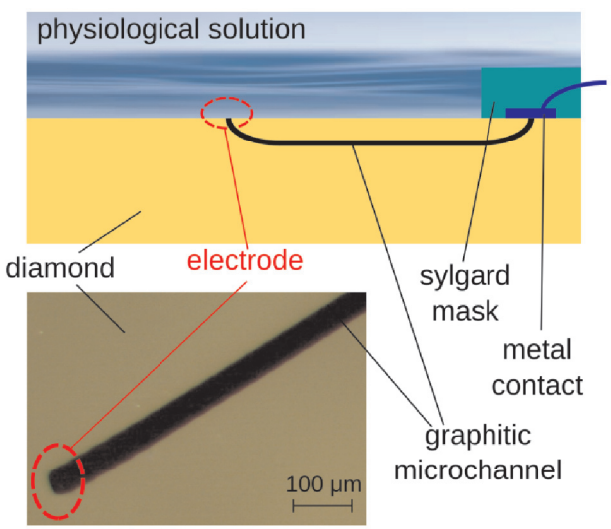

Fig. 2. (Top) Lateral view schematics of the diamond device incorporating the graphitic microchannel; (Bottom) top view micrograph of the microelectrode at the endpoint of the graphitic microchannel.

PRELIMINARY FUNCTIONAL TESTS

The responsiveness of the device towards catecholamines (adrenaline, noradrenaline) was preliminarily tested by means of cyclic voltammetry measurements.

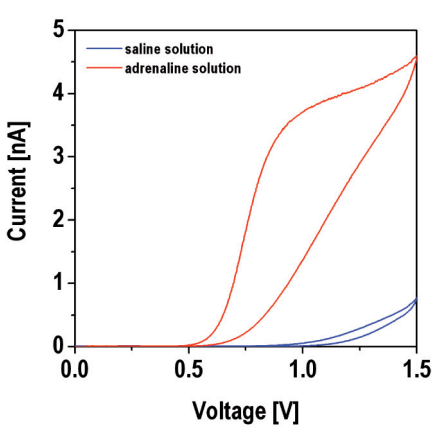

Fig. 3. Cyclic voltammetry tests on saline and adrenaline containing solutions. The adrenaline oxidation feature at V=0.8 V is clearly visible.

As shown in figure 3, when immersed in a saline solution containing 10 mM adrenaline, the device can detect the oxidation of the adrenaline molecules at the electrode when the voltage reaches +0.8 V, as opposed to the case when a standard saline solution (in mM: 128 NaCl, 2 $MgCl_2$, 10 glucose, 10 HEPES, 10 $CaCl_2$, 4 KCl) is employed.

Following these preliminary tests, the device was tested by positioning an isolated chromaffin cell (i.e. a specific type of excitable neuroendocrine cell releasing catecholamines) in correspondence of the active region, while keeping the whole system in physiological solution. The micro-electrode was polarized at the fixed voltage corresponding to the oxidation of the adrenaline (i.e. 0.8 V, as reported in figure 2), in order to detect, via amperometry, the quantal release occurring during exocytosis. As reported in figure 4, the device is able to detect with high signal-to-noise ratio the amperometric spikes of 3-22 pA corresponding to the quantal release of catecholamines.

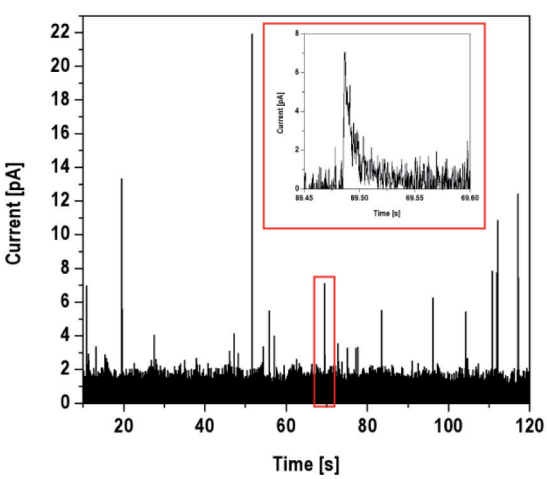

Fig. 4. Time course of amperometric signals detected from a single chromaffin cell located in correspondence of the active region of the device: the spikes (zoom in the inset) correspond to single exocytotic events.

CONCLUSIONS

Ion beam lithography demonstrated to be useful to microfabricate cellular biosensing devices in diamond, which will be further investigated in future works.

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