MeV ion beam fabrication of diamond biosensors for action potentials detection

P. Aprà[1,2,3], G. Tomagra[4], A. Battiato[2], S. Ditalia Tchernij[1,2,3], J. Forneris[2],
D. Carlucci[5], L. La Torre[5], A. Marcantoni[4,3]V. Rigato[5], P. Olivero[1,2,3], V. Carabelli[4,3], F. Picollo[1,2,3]

[1] Dipartimento di Fisica, Università di Torino, Torino, Italy. [2] INFN Sezione di Torino, Torino, Italy, [3] Centro inter-dipartimentale "NIS", Università di Torino, Torino, Italy, [4] Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy, [5] INFN, Laboratori Nazionali di Legnaro, Legnaro (Padova), Italy.

INTRODUCTION

Action potential generation is one of the main mechanisms regulating cell excitability and communication. This process consists in a membrane potential variation, induced by the opening and closure of ion channels, which spreads on the plasma membrane. This rapid depolarization is not only a specific communication mechanism between neurons, but it also governs the synaptic transmission, the hormonal release and muscle contraction [1]. In neuroscience, the development of sensors devoted to the detection of these signals is of paramount importance since they would allow performing experiments in which molecular mechanisms or drugs efficacy could be investigated at the cellular level.

Diamond-based devices represent a promising solution to this technological demand due to the actracting properties of this material.

In this paper we present multi electrode array (MEA) cellular sensors based on embedded graphitic micro-channels fabricated by means of MeV ion beam lithography into artificial diamond substrates. The applicability of these sensors on action potentials recording from cardiac tissue is reported.

ION BEAM IMPLANTATION

Artificial polycrystalline diamond substrates produced by chemical vapour deposition by Diamond Materials GmbH (Germany) were employed as a starting material for the sensors fabrication. The crystals are 5×5×0.4 μm^3 in size and are classified as type IIa optical grade, guaranteeing both nitrogen and silicon defect concentration lower than 10 ppm.

Graphitic micro-electrodes were created by means of MeV Ion Beam Lithography. This fabrication technique is based on the introduction of structural defects in the crystal lattice, thus allowing the controlled variation of the properties of the target material. More specifically, promoting the phase transition of diamond into graphite, electrically conductive electrodes for cellular sensing can be created. The conversion is obtained exclusively in regions of the crystal where the ion-beam-induced defect density overcomes a critical threshold, usually referred as graphitization threshold, upon high temperature thermal treatment (2 hours at 950 °C). A collimated 1.3 MeV He^+ beam available at the AN2000 accelerator of the INFN Legnaro National Laboratories was employed for the fabrication of these electrodes. The fluence necessary to overcome the graphitization threshold is 1×10^{17} cm^{-2}. Adopting these parameters, ~250 nm thick regions located at a depth of ~2.2 μm below the sample surface were created, thuse obtaining devices equipped with 60 graphitic independent electrodes. The use, during the implantation, of properly designed metal film masks with variable thickness directly deposited on the diamond crystal, allows the emersion on the surface of the electrodes, thus making them suitable for signals detection.

RAMAN CHARACTERIZATION

To evaluate the effectiveness of the presented fabrication technique, Raman spectral maps were acquired in correspondence of the emerging region of a selected graphitic channel (figure 1). Measurements were performed with a conventional micro-spectrometer (Horiba JobinYvon HR800), with optical excitation given by a continuous 532 nm laser focused with a 100× air objective. The laser power intensity directly incident on the sample was 4.11 mW.

The spectrum collected from the first region (1a, corresponding to purple spot in figure 1c) exhibits the typical first-order Raman peak of diamond at 1332 cm^{-1}. The absence of features related to amorphous or graphite-related features confirms that the areas of the substrate shielded by the mask are indeed consisting of a pristine diamond phase. At lower Raman shift (963 cm^{-1}) a peak addressable to He–related colour centres is evident [2], whose presence is a direct consequence of the He^+ implantation occurred in correspondence of the channel. Differently, the spectrum collected from the endpoints of the sub-surface microchannel (1b, green spot in figure 1d), shows two broad bands at 1361 cm^{-1} and 1582 cm^{-1}, which are respectively referred as the D and G bands, thus providing a direct evidence of the exposure of graphite-like phases at the sample surface [3]. The He-related peak is not observable anymore, since the high fluence reached in this region introduced defects acting as luminescence quencher. In figures 1c and 1e the spatial distributions of the normalized intensities of He-related peak (963 cm^{-1}) and of the diamond peak (blue scale) together with the G band (red scale) are respectively mapped. Concerning the former, the highest intensity is observed all around the outline of the channel structure, where a low fluence (below the

graphitization threshold, but enough to create He-related colour centres) is reached during the implantation, while no traces are assessable far from the channel or above itself. As expected, a clear intensity signal of the G band is observable over the channel, reaching its maximum over the emerging region, while it results to be absent elsewhere. The first-order Raman peak of diamond is prominent outside the implanted area while it is significantly reduced at the endpoint of the electrode. The analysis of the map allows the estimation of the extension of the graphitic region exposed to the surface, indicating an active detection area of ~2×10 μm^2.

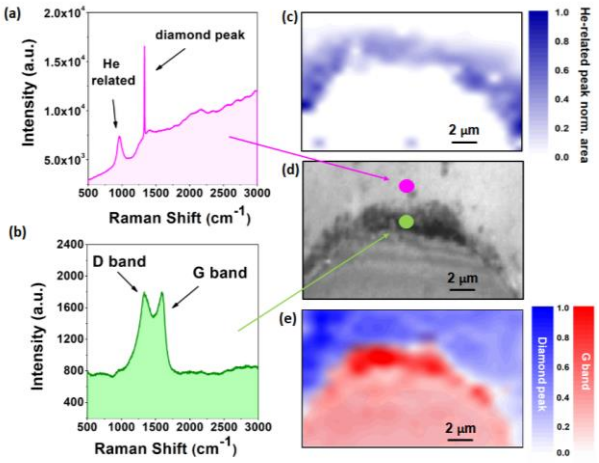

Fig. 1: Raman spectra (a,b) collected from different regions of the diamond which location of these analysis points is indicated in the optical image (d). Spatial map (c) of He-related colour centers distribution around the implanted region. G band (e) intensity maps are presented adopting a colour scale representing the normalized area of the integrated spectral range.

ACTION POTENTIAL DETECTION

The action potential (AP) recordings were performed by employing a commercial "MCS MEA 1060-Inv-BC" amplifier from Multi Channel Systems, since the diamond-based biosensors were developed to be fully compatible with standard acquisition electronics.

These sensors are characterized by a half-band noise of (35 ± 5) μV. These performances are comparable with those of conventional Multi Electrode Array device (60MEA200/300iR-Ti, Multi Channels System), which have a noise level of (22 ± 2) μV. In both cases, the detection of action potentials from cardiac tissues is possible due to the low noise level.

The functional validation of the devices was obtained performing *in vitro* measurements on tissues of the sinoatrial node, which is responsible for the generation of the electrical pulses causing the heart's contraction. This experimental model was chosen since it represents a benchmark biological sample generating intense action potentials (i.e. ~300 μV) across the whole tissue.

The intact sinoatrial node was microsurgically isolated from mice and placed on the sensors recording area while a saline standard solution was perfused.

The potentiometric measurements of the action potentials generated by the sinoatrial node was performed with both the diamond-based biosensors and the conventional MEA devices. Experiments were conducted at room temperature.

Figures 2 show a representative trace of the sinoatrial node spontaneous activity, which fired with a mean basal frequency of (3.5 ± 0.4) Hz and (2.8 ± 0.6) Hz, respectively measured with diamond sensors and conventional MEA.

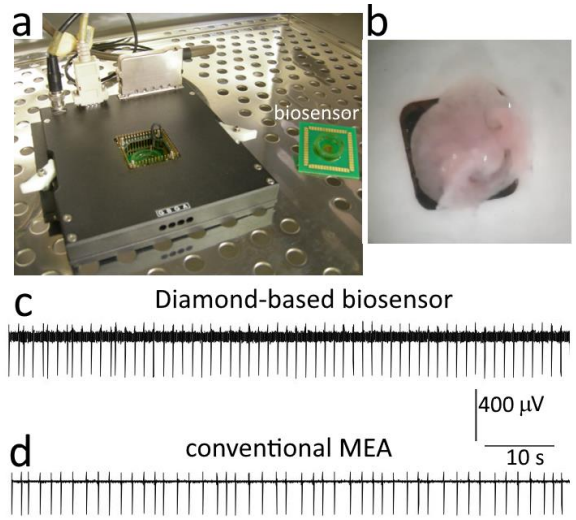

Fig. 2: (a) Picture of the front-end electronic (left side) and of the diamond-based biosensor (right side). (b) Optical image of the tissue placed over the sensor. Potentiometric recording of action potentials from sinoatrial node performed from diamond based-biosensor (c) and conventional MEA (d).

The measured frequencies values are statistically compatible as confirmed by ANOVA test followed by Bonferroni post hoc comparison ($p > 0.05$).

CONCLUSIONS

In the present paper, we report on the fabrication, characterization and *in vitro* functionality validation of diamond-based multi-electrode array biosensors. The technique employed for the fabrication of these devices is the MeV ion beam lithography, which allows the creation of graphitic micro-patterns into the diamond matrix. This approach allows the fabrication of full carbon sensors combining both the properties of diamond (i.e. biocompatibility, chemical inertness) employed as a growth substrate and of graphite (i.e. electrical conduction) working as sensing electrodes for the cellular interfacing.

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