Neuronal Cells Electrical Activity Recorded by Hydrogen Terminated Diamond Electrode

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Abstract
The paper reports for the first time the use of a hydrogen terminated diamond electrode for the recording of electrical activity from cultured neurons. Diamond is emerging as a most promising substrate for the development of molecular biosensors, but direct evidence that it can be successfully interfaced with living excitable cells is still missing.

1 Introduction

In the field of diamond based biosensors, up to now the emphasis has been on the development of diamond-enzyme interfaces exploiting electron transfer mechanisms [1,2] and of electrodes for electrochemical measurements from cells and tissues [3]. These examples highlight the feasibility of building molecular biosensors: a significant biotechnological breakthrough would be however to develop diamond based cellular sensors to record electrical and optical activity from cultured cells. In a previous paper [4] employing primary neurons, we have shown that it is possible to exploit the optical properties of diamond to record neuronal activity by means of fluorescent probes. Up to now, however, what was lacking was the last and key step, i.e. evidence that conductive hydrogen terminated diamond can be successfully employed in the fabrication of electrodes for recording cellular electrical activity.

To achieve this goal, we used a commercially available (Sumitomo Electric Industries, Japan) high purity (IIa type) 5 μm thick diamond layer epitaxially grown onto a Ib type HPHT (high pressure high temperature) diamond substrate, with (100) orientation and (3x3x0.5) mm^3 dimensions. The homoepitaxial layer showed a surface roughness at the nanoscale (root mean square roughness below 3 nm) as observed by 20x20 μm^2 non contact AFM maps.

2 Materials and Methods

Preparation of the hydrogen terminated diamond electrode

The diamond sample was oxidised in a sulfochromic mixture at 170°C and in a boiling solution of H_2O_2:NH_4OH, in order to remove contaminants and to oxygen terminate the surface; after this treatment the surface was hydrophilic and the sheet resistance, was higher than 10^9 Ohm per square (Ω/sq). The oxidised sample was annealed for 1 hour at 900 °C and 10^{-5} Pa to induce desorption of oxygen and residual adsorbates. The surface hydrogenation was carried out in a hot filament CVD reactor through two Ta hot (2100 °C) filaments and using purified hydrogen gas. The high hydrophobicity of the surface (wetting angle around 90°) and a sheet resistance of 23±1 kΩ/sq confirmed the hydrogen termination of the homoepitaxial layer.

Assuming a hole mobility of 60 $cm^2 \cdot V^{-1} \cdot s^{-1}$, the sheet hole concentration relevant to a sheet resistance of 23 kΩ/sq of the hydrogen terminated surface, is about $5 \cdot 10^{12}$ cm^{-2}, in good agreement with experimental data available in literature [5].

Electrode mounting

The diamond was attached to a high resistance printed circuit board shaped to fit the inverted microscope stage. Two conductive copper pathways were connected respectively to the diamond surface by means of a 20 μm gold wire and a silver paste bond and to a silver chloride reference electrode placed in the extracellular medium. The electrical insulation of conductors, interconnects and bondwires from the electrolyte was ensured by a silicone elastomer (Sylgard 184, Dow Cornig, USA) passivation layer. The fraction of the diamond surface acting as recording area was about 1 mm^2.

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Signal recording

Electrical signals from cells cultured on the diamond surface were fed into a low noise (<5 μV_{RMS}) amplification ($G=10^4$) and filtering (bandwidth: 0.02-24 kHz) custom stage before signal digitalization and recording [6]. Signal acquisition and analysis were performed by means of a Digidata 1440 board and pClamp10 software (Molecular Devices, Toronto, Canada).

Cell cultures

GT1-7 cells represent an appropriate model of differentiated, electrically excitable and autorhytmic neuronal cells, that can be grown at high density [7].

For the recordings, cells were plated on poly-L-lisin (PL) coated hydrogen terminated diamond in the presence of B27 medium supplement (Invitrogen) and cultured for 5-7 days . During the recordings, the medium was replaced with standard Tyrode solution.

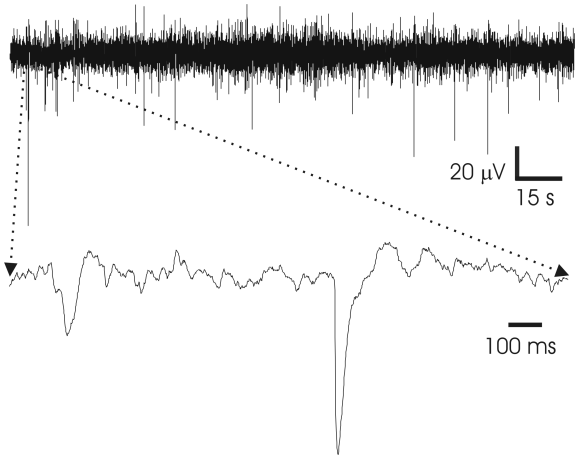

Fig. 1. A typical recording of electrical activity lasting several minutes from GT1-7 cells. The inset shows a stretch from at an expanded time scale.

3 Results and Discussion

In five experiments, electrical activity could be recorded from GT1-7 cells plated at high density on the fraction (1 mm^2) of the HTD surface free of the silicone insulating layer covering interconnections and bond pads. Consistently with previous observations by other groups [7], electrical activity was episodic, with bursts separated by silent intervals. Occasionally, trains of fast (a few ms) spikes could be observed; more often, the recorded activity consisted of slower spikes, lasting some tens of ms and of different amplitude (Fig. 1). While the former could be ascribed to the firing of isolated neurons, the latter were likely the result of the synchronized activity of clusters of cells. Since clusterization is a prerequisite for the emergence of spontaneous electrical activity [7], and since we were recording the collective behaviour of a population of densely packed cells plated on a single macroelectrode, it is quite reasonable that slow signals were the most common.

The uniformity in time courses can be interpreted as evidence that the events are due to similar numbers of synchronously firing cells; the differences in amplitude are likely related to different degrees of adhesion – and therefore of electrical coupling – of different units to the diamond substrate.

Further tests will be carried out with a commercially available multielectrode array (MEA1060, Multichannel System GmbH, Germany).

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