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Osteoblastic cells trigger gate currents on nanocrystalline diamond transistor

Tibor Izak*, Marie Krátká, Alexander Kromka, Bohuslav Rezek

Institute of Physics ASCR, v.v.i., Cukrovarnická 10, 162 00 Prague 6, Czech Republic

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ABSTRACT

We show the influence of osteoblastic SAOS-2 cells on the transfer characteristics of nanocrystalline diamond solution-gated field-effect transistors (SGFET) prepared on glass substrates. Channels of these fully transparent SGFETs are realized by hydrogen termination of undoped diamond film. After cell cultivation, the transistors exhibit about 100× increased leakage currents (up to 10 nA). During and after the cell delamination, the transistors return to original gate currents. We propose a mechanism where this triggering effect is attributed to ions released from adhered cells, which depends on the cell adhesion morphology, and could be used for cell culture monitoring.

1. Introduction

Diamond is an attractive material for bio-electronic systems due to its biocompatibility, chemical stability and favorable combination of optical, mechanical and electrical properties. In spite of diamond's stability, its surface can be chemically modified in order to control its properties such as electrical conductivity, electron affinity, and surface wettability. This creates suitable conditions for cell adhesion [1] and differentiation [2], as well as for electrical detection of biological effects [3–5]. For example, oxygen terminated (O-terminated) surfaces are hydrophilic and highly resistive, while hydrogen terminated (H-terminated) surfaces are hydrophobic and they induce p-type surface conductivity even on intrinsic diamond. Hence, in comparison to conventional silicon FETs, the H-terminated diamond-based SGFETs operate without a gate oxide layer, which allows direct contact between biomolecules and the surface of the FET channel [6]. Moreover, diamond-based devices are also fully transparent. This enables both top- and back-side monitoring of cellular morphology and activity in real-time monitoring [7,8].

At present, mostly monocrystalline and polycrystalline diamond (MCD) films are used for electrical measurements of cells [5,9] because of their higher conductivity in comparison to nanocrystalline diamond (NCD) [10]. On the other hand, NCD films are more likely to be widely applicable in bioelectronics as they are inexpensive, they can be easily fabricated on arbitrary substrates (including glass and plastics) and on large areas at low temperatures [11]. However, from the electronic point of view, NCD is a complicated system due to the presence of sp^2 graphitic phase and grain boundaries [10]. In our previous study we discussed the influence of grain boundaries and sp^2 phase on the NCD interface toward aqueous solutions, bio-molecules, and on the SGFET function [12]. SGFETs based on NCD with grain sizes and thicknesses from 500 nm down to below 100 nm were operational and their function did not depend on the grain size [12].

Here we show that the diamond SGFET function is significantly influenced by the adhesion and morphology of osteoblastic cells. We discuss a possible mechanism and introduce a schematic model of this effect.

2. Material and methods

Fused silica glass substrates were used for the deposition of nanocrystalline diamond (NCD) films by microwave plasma chemical vapor deposition (CVD) in an Aixtron reactor. Different deposition durations resulted in different grain sizes of the NCD films: ~80 nm after 1 h and ~250 nm after 4.3 h (see corresponding SEM morphology Fig. 1a). The detailed surface morphology of both diamond films together with their Raman spectra can be found in ref. Krátká et al. [12]. After the NCD growth, the surfaces of diamond films were hydrogenated in the same reactor at 600 °C for 10 min. Several photolithographic steps were applied to define: (i) 20 μm wide H-terminated transistor channel surrounded by oxygen-terminated areas, (ii) source and drain contacts

* Corresponding author. Tel.: +420 220318511; fax: +420 233343184.
E-mail address: izak@fzu.cz (T. Izak).

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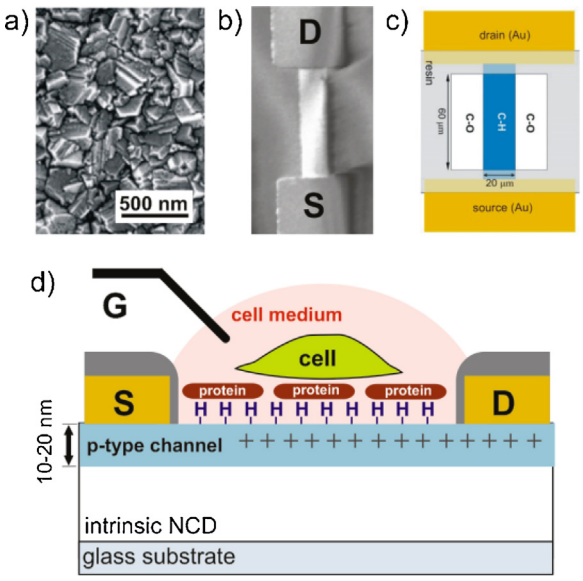

Fig. 1. SEM images of (a) nanocrystalline diamond surface morphology (4.3 h deposition) and (b) detail of the NCD SGFET transistor channel with source-drain Au contacts. (c) Top-view scheme of the NCD SGFET with active H-terminated diamond gate area of 20 μm × 60 μm defined by the opening in the insulating resin. (d) Cross-section scheme of the NCD SGFET transistor with protein layer and cells.

(10 nm of Ti and 50 nm of Au), (iii) the sensing gate area opening of 60 μm × 60 μm in insulating resin layer (Fig. 1b and c). More details about photolithographic steps as well as NCD deposition parameters can be found in reference Krátká et al. [12].

Gating of the SGFET was realized by immersing the H-terminated channel into an electrolyte solution which was in contact with Ag/AgCl gate electrode (Fig. 1d). We used McCoy's 5A medium with 15% FBS (fetal bovine serum) and 15 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer as the working solution. The sensing gate area of SGFET was covered by a 5 μl droplet of this medium, and the transistor characteristics were measured using Keithley K327 source-measure units (note: this behavior is labeled further as "after FBS absorption"). Then the sensors were washed with deionized water, dried with compressed air and were used for cell incubation.

SAOS-2 cells (sarcoma osteogenic, human osteoblast-like cell line; DSMZ GmbH) were grown in McCoy's 5A medium (Bio-Concept) supplemented with heat inactivated 15% FBS (Biowest), penicillin (20 U/ml) and streptomycin (20 μg/ml). HEPES buffer was added to the medium because of the absence of CO_2 atmosphere during measurement. Sterilization of SGFETs prior to cell plating was performed by UV-C for 10 min. We used a standard germicidal low-pressure mercury lamp (TUV 15W/G15 T8, Philips), which emits UV-C radiation with the peak at 253.7 nm by power of 15W. Cells were plated in the densities of 10,000 cells/cm^2 using a droplet technique; i.e. the transistor sensing gate area was covered by 10 μl droplet of cell suspension in the medium [12]. After 1 h incubation, 1.4 ml of the medium was added and cells were cultivated for 2 days in 5% CO_2 at 37 °C. Stable 5% CO_2 concentration in the atmosphere during the cell cultivation helps the bicarbonate buffer in cultivation media to sustain the optimal pH (7.1–7.4). After 2 days of cell cultivation, the SGFETs were taken out from the cell medium, and again the sensing gate area was covered by a 5 μl droplet of HEPES buffer to prepare for electrical measurements. After experiments, rinsing of cells was performed by phosphate buffered saline (PBS) solution, and the transistor characteristics were measured again.

3. Results and discussion

Fig. 2 shows the transfer characteristics and leakage currents measured at different stages. As reference initial stage, SGFET with absorbed FBS protein was used (Fig. 2a and b). In our previous studies we showed that the SGFET transfer characteristics after FBS adsorption is shifted by −50 mV, which is permanent even after rinsing using deionized water and phosphate buffered saline [12,13]. This shift after the FBS adsorption is caused by replacement of ions by proteins in the very vicinity of the diamond surface [14].

Additional shift of transfer characteristics was observed after the cell growth and delamination (−115 ± 10 mV at I_{ds} = −2 nA) (Fig. 2f and g). The shift direction (sign) as well as the value is in agreement with previous reports [12]. However, just after the cell growth (i.e. cell incubation), the transistors reproducibly exhibited

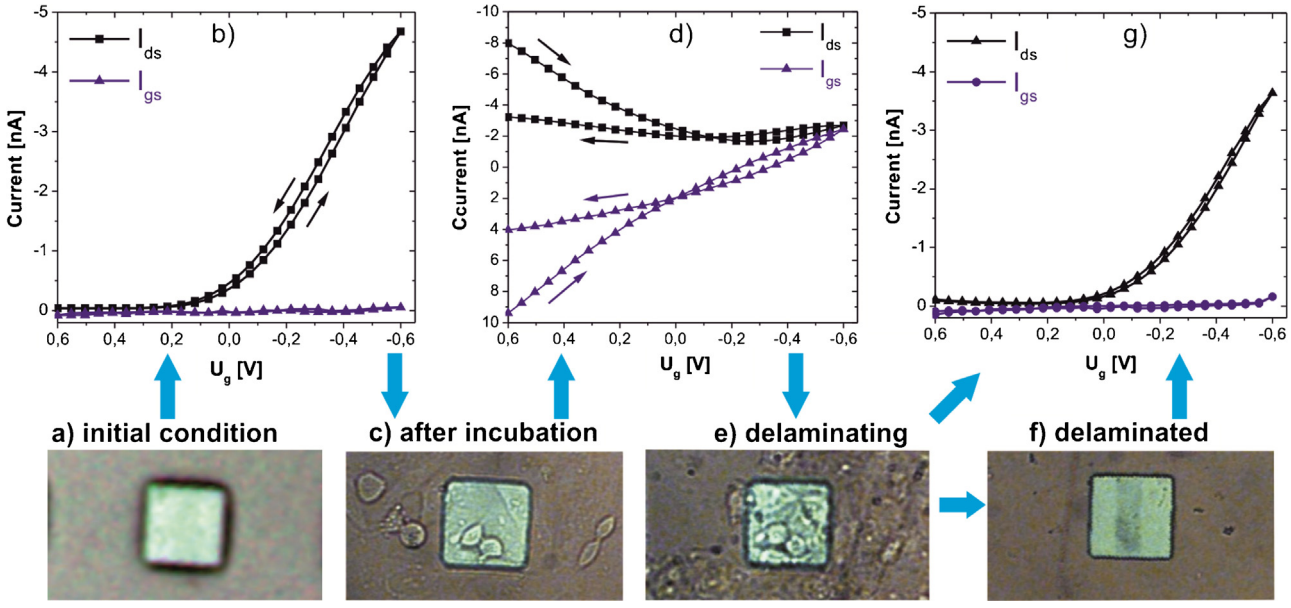

Fig. 2. Transfer characteristics and leakage currents at U_{ds} = −0.6 V of NCD SGFETs with large grain size measured: (b) after FBS protein absorption (labeled as "initial"), (d) after cell incubation and (g) during or after full delamination of cells. Fig. (a), (c), (e), (f) are optical photos of transistor openings at the specific stage of the measurement (rectangular squares represent the transistor opening areas with size of 60 μm × 60 μm).