Applied Surface Science xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Influence of non-adherent yeast cells on electrical characteristics of diamond-based field-effect transistors

Václav Procházka[a,b,*], Michal Cifra[c], Pavel Kulha[a,b], Tibor Ižák[a], Bohuslav Rezek[a,b], Alexander Kromka[b,d]

[a] Faculty of Electrical Engineering, Czech Technical University in Prague, Technická 2, 16627 Prague, Czech Republic
[b] Institute of Physics, The Czech Academy of Sciences, Cukrovarnická 10/112, 162 00 Prague, Czech Republic
[c] Institute of Photonics and Electronics, The Czech Academy of Sciences, Chaberská 57, 182 51 Prague, Czech Republic
[d] Faculty of Civil Engineering, Czech Technical University in Prague, Thákurova 7, 16629 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 30 April 2016
Accepted 1 May 2016
Available online xxx

Keywords:
Nanocrystalline diamond
Yeast cells
Field-effect transistor
Transfer characteristics
pH sensitivity

ABSTRACT

Diamond thin films provide unique features as substrates for cell cultures and as bio-electronic sensors. Here we employ solution-gated field effect transistors (SGFET) based on nanocrystalline diamond thin films with H-terminated surface which exhibits the sub-surface p-type conductive channel. We study an influence of yeast cells (*Saccharomyces cerevisiae*) on electrical characteristics of the diamond SGFETs. Two different cell culture solutions (sucrose and yeast peptone dextrose–YPD) are used, with and without the cells. We have found that transfer characteristics of the SGFETs exhibit a negative shift of the gate voltage by −26 mV and −42 mV for sucrose and YPD with cells in comparison to blank solutions without the cells. This effect is attributed to a local pH change in close vicinity of the H-terminated diamond surface due to metabolic processes of the yeast cells. The pH sensitivity of the diamond-based SGFETs, the role of cell and protein adhesion on the gate surface and the role of negative surface charge of yeast cells on the SGFETs electrical characteristics are discussed as well.

1. Introduction

Diamond is considered as a perspective material in cells and tissue oriented life sciences. In addition to unique mechanical, thermal, and electrical properties, diamond is also biocompatible and exhibits surface conductivity which is highly sensitive to changes in surrounding environment due to surface transfer doping mechanism [1,2]. Thanks to these properties diamond-based transistors do not need any gate dielectric layer.

There are many types of diamond-based bio-electronic devices of new generation. They differ in type of diamond (intrinsic or boron-doped polycrystalline diamond in form of thin films deposited on different substrates (mainly glass), or robust single-crystalline diamond substrates) as well as working principle. The working principle of devices for direct electrical measurement of cells activity and for study the cell-cell or cell-substrate interactions differs for individual device configuration and specific applications such as impedance measurements, field-effect transistor configuration, etc. For example, impedance measurement techniques employ microelectrode arrays (MEAs) [3,4]. MEAs found application in the study of immune system response efficiency [5], direct monitoring of cardiac action potentials of cells [4] or for action potential recording and stimulation of neural networks for both in vitro applications (rigid substrate) as well as for in vivo retinal prostheses (flexible biocompatible substrate) [6]. Interdigital electrodes based on hydrogen-terminated diamond on glass substrates were found prospective as optically transparent devices for real-time monitoring of cellular activity (incubation, cultivation, adhesion, etc.) [7].

In contrast to impedance-based devices, solution-gated field effect transistors (SGFETs) with hydrogen-terminated diamond surface allow study of a single cell. They have found use in research of solution/cell-surface interactions, living microorganisms and tissue cells as biocompatible sensors. The properties of diamond SGFETs, such as influence of the diamond film morphology [8], pH [9], protein adsorption [8] or membrane adsorption and disruption [10] on the electrical characteristics has been well described. In another study enzyme-modified field effect transistors were applied for detection of enzymatic reactions [11] or for measurement of surface potential of living cells [12]. Micro and nanoscopic field SGFETs have a potential for study of electrodynamic cellular

* Corresponding author at: Academy of Science Czech Republic, Institute of Physics, Cukrovarnická 10, Prague, Czech Republic.
E-mail addresses: prochazkav@fzu.cz, prochva4@fel.cvut.cz (V. Procházka).

http://dx.doi.org/10.1016/j.apsusc.2016.05.003
0169-4332/

Table 1
Average conductivity and pH of used solutions.

Measured at 18.9 °C	pH		Conductivity	
	before the addition of cells	30 min. after the addition of cells (measurement start)	Without cells (mS cm^{-1})	C_{CELLS} ~1.10^8 cells ml^{-1} (mS cm^{-1})
YPD	6.4	6.1	3.21 ± 0.04	3.10 ± 0.04
Sucrose (2 wt%)	7.0	6.8	0.43 ± 0.02	0.41 ± 0.02

behavior, which is theoretically predicted [13] yet challenging to detect [14]. The functionalized diamond surface was used in several studies for pattern guided formation of glial and retinal neuron networks [15,16], and it was also demonstrated as perfect solution for photo-sensitive retinal prosthesis and neural interfacing device [17]. However, the studies were focused on the interactions of mammal adherent cells or excitable cells with strong surface potential. There is a lack of studies focused on the interactions of non-adherent, non-excitable cells.

Thus in presented work we focused on yeast cells (*Saccharomyces cerevisiae*), which are widely used as basic model of eukaryotic cells in molecular biology and genetics [18]. Yeast cells enable a wide variety of genetic engineering techniques and the strict functional analyzes of proteins. They are non-polar, non-excitable and non-adherent cells. Their inner and surface structures are well defined and understood. They have a stable negative surface charge with very weak pH dependency, which determines their behavior in the solution [19,20]. In general, yeast cells are known as non-adherent cells, although some yeasts such as *S. cerevisiae*, *Candida albicans* and *Candida glabrata* have the ability to adhere to plastic surfaces [21,22]. The yeast cell adhesion to plastic substrates is believed to depend on hydrophobic interaction [23]. Novel methods for attachment and cultivation of specifically positioned single yeast cells on a microelectrode surface employ a weak electrical potential (−0.2 and −0.4 V) [24]. It was reported that the yeast cells attached to the negative potential-applied ITO electrodes showed normal cell proliferation.

We study the influence of different solutions (sucrose and yeast peptone dextrose without or with yeast cells) on the H-diamond SGFET electrical characteristics. We discuss interactions of the yeast cells and cell culture solutions with H-terminated diamond surface on the transistor gate. We suggest that metabolic activity of yeast cells can be detected by the H-diamond SGFET.

2. Experimental

2.1. Fabrication of the sensor

Fused silica glass substrates (in size of 10 mm × 10 mm × 1 mm) were ultrasonically cleaned in isopropyl alcohol and deionized water (DI) and subsequently were immersed for 10 min into an ultrasonic bath with a colloidal suspension of diamond nanopowder with nominal particle size of ~5 nm. This process leads to the formation of a 5 ÷ 25 nm thin layer of diamond powder necessary to initiate the diamond growth in a thin film.

Nanocrystalline diamond (NCD) thin films were grown in a microwave ellipsoidal cavity reactor by chemical vapor deposition (CVD) process for 4.5 h, at following conditions: gas pressure 30 mbar, gas mixture 1% CH_4 in H_2 and microwave power 1000 W. The deposition temperature was in the range of 550 ÷ 600 °C. These deposition parameters led to the growth of ~450 nm thick diamond film with grain sizes ~250 nm [3]. Diamond films were further hydrogenated in the same microwave plasma reactor at 600 °C in hydrogen plasma for 10 min to induce the surface conductivity.

Photolithographic masks were applied on H-terminated NCD films using a positive MA-P1215 photoresist to define FET channels and contacts. The NCD films were treated in oxygen radio-frequency plasma (300 W, 3 min exposition time) to generate insulating O-terminated areas, which surround the H-terminated channels (5 μm wide and 60 μm long stripes) connecting source and drain contacts. The source and drain contacts were prepared by thermal evaporation (10 nm of Ti and ~50 nm of Au) followed by the lift-off technique. The samples were cleaned in acetone and photoresist stripper (mr-REM 660). The area between contacts was covered with a positive photoresist ma-P 1240 (thickness 4 μm). The final photolithographic step created openings of 60 μm × 60 μm to define the active gate area (Fig. 3) [25].

2.2. Experimental procedure

Yeast cells *Saccharomyces cerevisiae* (Euroscarf collection; genetic background BY4741, *MAT*a) were used in experiments. Sucrose medium was prepared by dissolving it in DI water to achieve 2% weight concentration. YPD medium was autoclave-sterilized contained: 1%(w/v) yeast extract (Chemos CZ, s.r.o.), 2%(w/v) peptone (Chemos CZ, s.r.o), 2%(w/v) D-glucose (Penta, s.r.o.) in DI water. Glucose was filtration sterilized (200 nm diameter filter pores) and added to medium after sterilization. Cells for experiments were cultivated as follows. Glass Erlenmeyer flask (100 ml) was filled with autoclave sterilized 10 ml of YPD medium. The medium was inoculated with yeast cells from YPD agar plate and the culture was cultivated at 30 °C on an orbital shaker at 180 rpm for 16 h. Then, the cell culture was centrifuged at 3000 RPM for 5 min, supernatant discarded and cells transferred to either sucrose or YPD medium to achieve final cells concentration C_{Cells} = 108cells ml^{-1}. Blank media (without cells) were used as references. Electrical conductivities of the employed media were measured by the conductivity meter calibrated after each measurement with 10 repetitions for accuracy. The resulting values are shown in Table 1.

For electrical measurements, two high precision source-meter units were used (Keithley 327). The first unit was connected between the channel electrodes of the sensor (i.e. source and drain), the second between the AgCl electrode and source electrode. The AgCl electrode was immersed into a drop of solution on the sensor gate area. Image of the setup is shown in Fig. 1. The drain and source electrodes are completely commutable due to sensor symmetry, thus there is no physical difference between them. For measurements of transistor transfer characteristics the U_{DS} was kept constant (U_{DS} = −0.6 V). First set of measurements was done with blank solutions (sucrose or YPD) by covering of the SGFETs active opening area with a 10 μl droplet. After each measurement, the SGFETs were rinsed by ethanol followed by deionized water. Second set of measurements was performed with cells contained in the medium (sucrose or YPD). The cells were let to sediment on the surface for 10 min prior to the measurements.

3. Results and discussion

Functionality and properties of diamond-based transistors were at first characterized by electrical measurements in standard phosphate buffer saline solutions (PBS) at different pH. Fig. 2a shows the gate voltage dependence of the diamond-based SGFET measured at constant current I_{DS} = 5 nA for pH varied from 9 to 4

Please cite this article in press as: V. Procházka, et al., Influence of non-adherent yeast cells on electrical characteristics of diamond-based field-effect transistors, Appl. Surf. Sci. (2016), http://dx.doi.org/10.1016/j.apsusc.2016.05.003