ÚFE Institute of Photonics and Electronics
The Czech Academy of Sciences

Studijní oddělení
Matematicko-fyzikální fakulta
Univerzita Karlova
Ke Karlovu 2027/3
12116 Praha 2

Doctoral thesis review from the reviewer Michal Cifra

Title of the doctoral thesis:
Electronic effects at the interface between biomolecules, cells and diamond

Author of the doctoral thesis:
Mgr. Marie Krátká

This thesis is focused on characterization of electronic properties of protein-diamond interface by employing a solution-gated field-effect transistor (SGFET) based on hydrogen-terminated diamond surface. The work in this thesis contributes to a research field of nanomaterial-enabled bioelectronics, which is a modern and important science area.

The thesis is built on 4 full chapters and information about the author and her bibliography including list of own publications and poster/oral presentations and attached author´s representative publications.

Introductory chapter concisely covers a selection of current knowledge on diamond as a bio-material, protein-diamond and cell-diamond interactions, and application of the diamond based structures as impedance and transistor-based biosensors. The aims of the thesis are stated here as well.

The next chapter is focused on the brief description of experimental techniques and methods used in the thesis. Preparation of the diamond structures such as the CVD growth of diamond layers, planar SGFET fabrication, direct growth of NCD micro-channels and field effect transistors of diamond layers and diamond SGFETs are first covered. Then the material characterization techniques are described: scanning electron microscopy, Raman spectroscopy and atomic force microscopy. Additionally, setups for measuring transistor characteristics and low-temperature hydrogenation are detailed together with procedures of FBS adsorption and cell cultivation including gamma radiation setup.

The core chapter of the thesis covers results and includes immediate concise discussion of each result.

From the methodical perspective, the techniques and methods used in the thesis both for fabrication and characterization are of high quality and state-of-the-art nature. The techniques and methods are appropriately applied to the research questions in place: CVD and lithography methods for surface gated FET structures fabrication, nanoscale surface characterization (AFM) of FBS protein layers on the diamond surface, scanning electron microscopy for diamond grain size quantification and Raman spectroscopy for diamond

lattice structure probing. Biological methods and approaches are simple but sufficient for demonstration of the working principles.

The thesis presents several novel scientific results. I focus here on the selected results of biophysical interest. At first, the effect of FBS protein (presumably bovine serum albumin) adsorption on a hydrogen-terminated nanocrystalline diamond gate of the transistor is demonstrated. Interestingly, while the albumin possesses a substantial negative charge so one would assume an increase of the drain-source current in hydrogen-terminated (p-doped) channel, the results show the opposite trend. Since rinsing with common solvents does not remove the adsorbed proteins, the low-temperature hydrogenation method for the removal of the adhered protein layer and renewal of SGFETs was also developed. Second group of findings, which I consider very interesting and novel, is related to the interaction of adherent cells with the SGFET. At first, it seems striking to me that the presence of adhered cells increases the leakage (gate-source) current. At second, it is fascinating to observe that the cell seem to respond to the current during the measurement and delaminate from the gate.
The results on experiments of protein and cell layer irradiation are also very interesting in terms of contributing to the elucidation of mechanisms of ionizing radiation with biomolecules using bioelectronics means.
The format of the thesis is adequate. The author uses the visual representation of the data functionally. The photo-documentation is appropriate. The thesis text is succinct, although there are points in the discussion, which could be elaborated further, see comments.

Comments and questions for the author:

1) FBS effect on SGFET characteristics – the author writes on p. 39 "It is attributed to adsorption of a 2–4 nm primary protein layer from FBS which remains on diamond irrespective of rinsing as evidence by AFM [3,32] and which modifies original equilibrium of the surface conductive layer system by replacing ions in the very vicinity of the diamond surface like in the case of DNA molecules or lipid bilayers [86,87]"
   a) Which ions are supposed to be replaced ?
   b) How specifically (what kind of experiments or calculations) would you do to verify if the effects are due to
      i) bovine serum albumin adsorption ?
      ii) ion displacement ?
2) Fig 3.9 - cell delaminating due to the leakage current from the gate. The author writes on p. 43 "We proposed a mechanism of this trigger effect. We attribute it to a close interaction between diamond and cell membrane that releases excess ions to the interface. This interaction becomes negligible when the cells change shape and delaminate" The description of the mechanism in this paragraph and the in the respective article "Osteoblastic cells trigger gate currents on nanocrystalline diamond transistor" is rather vague. It would be very interesting if the author could elaborate on it.
   a) What is the approximate value of the current density (due to leakage current) the cell is exposed to?
   b) What is the approximate value of voltage drop across the cell membrane? Is that sufficient to affect cell membrane voltage-gated ion channels?
3) What is the source of the inherent hysteresis in I-V curves, for example, Figs. 3.5 3.8?

4) What is the reason for the peculiar shape of I-V curves in Fig 3.9. d) ?
5) Fig 3.21 What is the mechanism causing aggregation of proteins under gamma irradiation?

Formal comments

1) p. 2 “For monitoring medical irradiations diamond offers an excellent tissue equivalence due to its atomic number (Z = 6) a boil and boil as you’ve ” atomic number is property of atomic element carbon not a form of carbon
2) inappropriate or missing unit prefixes: p.3 “BSA is a globular protein in dimension of 4 x 4 x 14 mm”, p. 41, Figure 3.9 caption “rectangular squares represent the transistor opening areas with size of 60 m × 60 m”
3) p. 21 - Fig. 2.8: repeated spelling mistake “ELEKTRONS” in the image

The author managed to handle a number of fabrication and experimental methods and showed the ability to interpret the obtained data. The thesis as such fulfills the Aims formulated in section 1.3 and represents an important contribution to the elucidation of electronic effects at the interface between various biomolecular systems and diamond.

Author proved that she is capable of independent creative scientific work. I recommend that the permission for a public defense of the dissertation is granted.

Prague, 17th August, 2018

Ing. Michal Cifra, PhD.

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Signature