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Graphene-based biosensors for neurodegenerative dementia detection

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A comprehensive study of biosensors for β-amyloid and τ-protein marker detection based on graphene films obtained by thermal decomposition of semi-insulating SiC has shown the possibility of doubling the biosensor response without using graphene functionalization. It has been found that one of the reasons for the low reproducibility of detection results and the decrease in the biosensor response is the aggregation of antibodies and proteins in defective areas of graphene.

Keywords: nanomaterials, graphene, biosensors, Alzheimer's disease.

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Alzheimer's disease is a progressive neurodegenerative disease characterized by cognitive impairment. The accumulation of abnormal β-amyloid and τ-protein in brain tissue plays a central role in its pathogenesis. The number of people suffering from this disease in the world is growing every year, and patients are getting younger. The drugs available to date slow down the progression of the disease, but cannot cure it. In addition, well-proven methods for mass screening for the disease and its diagnosis at an early stage are lacking. Potential solutions to this problem involve the use of graphene-based biosensors [1–3]. However, it has been demonstrated in a recent review [2] that clinical application of such biosensors is hampered by the low reproducibility of graphene parameters in biosensors and the relatively weak dependence of the biosensor signal amplitude (graphene resistance or Dirac potential) on the concentration of detected proteins. These problems occur regardless of the biosensor design (resistance or field-effect transistor). Recent research [2,3] has revealed that graphene functionalization routinely used for antibody binding may also be a source of these problems, since it alters the adsorption properties of graphene. It has been demonstrated for graphene grown by chemical vapor deposition (CVD) [1] that the signal of biosensors may be enhanced by attaching antibodies directly to the graphene surface without functionalization.

In the present study, we determined the efficiency of this novel concept in detection of β-amyloid (beta protein) and τ-protein (tau protein) by biosensors based on graphene obtained by thermal decomposition of silicon carbide. This graphene differs from the CVD one in the nature of organization of the nanomaterial. The influence of aggregation of antibodies to β-amyloids (anti-beta) and τ-proteins (anti-tau) and of beta and tau proteins themselves on the biosensor signal magnitude was also examined.

The studied biosensors are based on a graphene chip with two ohmic contacts. The sensing area (working area of graphene in the chip) was 1×1.5 mm. The biosensor chip type and comprehensive data on the processing of chips and their mounting on holders were provided in [4]. The preparation of graphene films was also discussed in detail in [4]. The high quality of graphene films was verified by Raman spectroscopy and atomic force microscopy (AFM) [4]. Additional washing of the graphene surface with its topography monitored by AFM was also used. This procedure provides an opportunity to restore the root-mean-square (RMS) roughness of the graphene surface to values close to the initial ones (before photolithography) and reduce the spread of resistances of graphene chips by a factor of 2.

Protein detection was carried out in phosphate-buffered saline (PBS) solutions with a constant signal of 40 and 60 mV applied to the chip based on graphene resistance change $(R - R_o)/R_o$ in the biosensor, where R_o is the chip resistance in a pure PBS solution and R is the chip resistance in a PBS solution with tau or beta proteins.

The efficiency of detection of beta and tau proteins was examined for chips produced from a single wafer. In a certain fraction of chips, graphene was not subjected to any functionalization. In the other chips, several types of functionalization (both covalent and by the $\pi-\pi$ mechanism, which is the binding of atoms by lateral

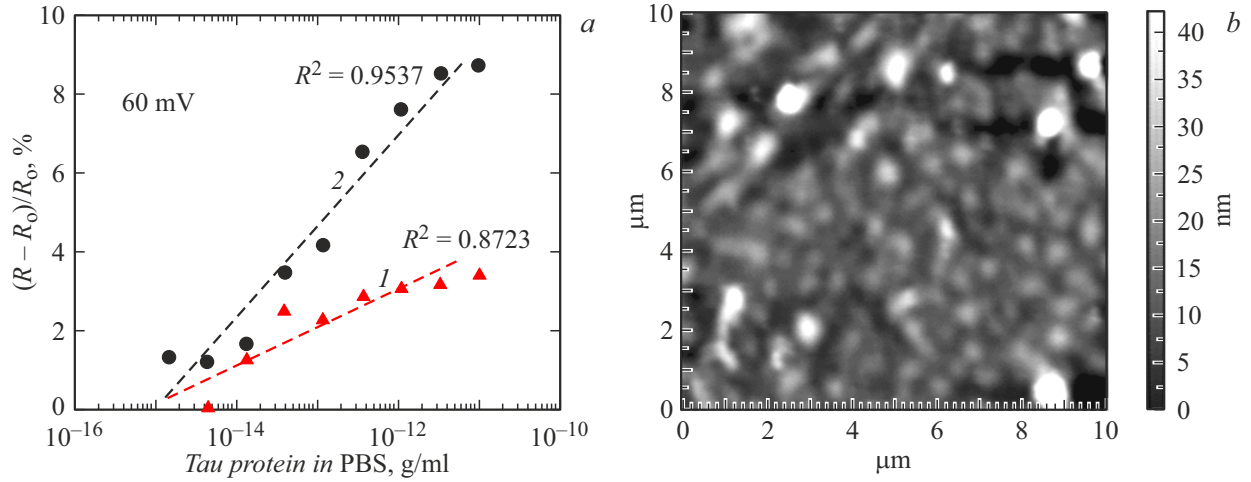

Figure 1. Comparison of parameters of two sensors with different types of graphene surface processing: EG436-E4 — without functionalization; EG436-E2 — with functionalization (electrochemical processing of graphene). *a* — Dependence of biosensor response $(R - R_o)/R_o$ of chips with (*1*) and without functionalization of graphene (*2*) on the concentration of tau protein. The dashed line represents the approximation of results by a logarithmic function. The R^2 parameter value is indicated in the figure. *b* — AFM image of the graphene surface topography within a 10×10 μm field of the EG436-E2 chip after the detection of tau protein.

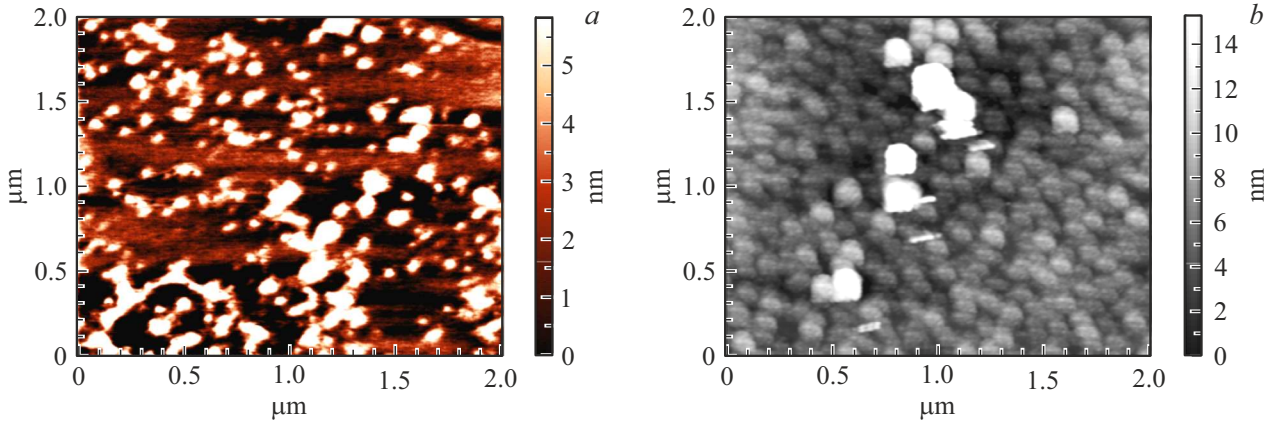

Figure 2. AFM topography of the graphene surface during beta protein aggregation: *a* — in defective regions of the graphene cellular structure that arose during graphene formation; *b* — in defects that arose in the graphene cellular structure during antibody immobilization (anti-tau, dark region) and in the process of formation of aggregates of the antibody–tau protein immune complex in this region (bright formations).

overlap of non-hybridized *p* orbitals) and electrochemical processing of graphene (cathodic reduction and anodic oxidation) in an aqueous solution of $NaClO_4$ (0.1 mol/l) were carried out. The implementation of different types of graphene surface functionalization was detailed in [5,6]. Selective AFM (Ntegra AURA microscope, NT-MDT, Russia) monitoring of the graphene surface state was performed before and after functionalization. In all chips with and without functionalization, immobilization of anti-tau or anti-beta antibodies was carried out under the same conditions from PBS buffer solutions (including diluted ones). Anti-tau and anti-beta antibodies and beta and tau proteins were provided by the Almazov Center. Detection experiments were performed at the Research Institute of Influenza.

Strong and irreproducible changes in the graphene surface topography after functionalization were revealed in the studies of graphene in biosensor chips before and after functionalization. For example, the initial RMS roughness values determined by AFM increased from 0.23 to 2–10 nm in a scanning field of $10 \times 10\,\mu$m, and these changes could be accompanied both by a several-fold increase in the resistance of graphene in biosensor chips and by its reduction. With strong changes in RMS roughness after functionalization, the spectral density of low-frequency noise (S_U) of graphene chips increased by 1–2 orders of magnitude compared to the initial values, which is indicative of generation of a large number of defects in graphene.

Antibodies (anti-tau) were applied under the same conditions to such graphene chips and to chips with

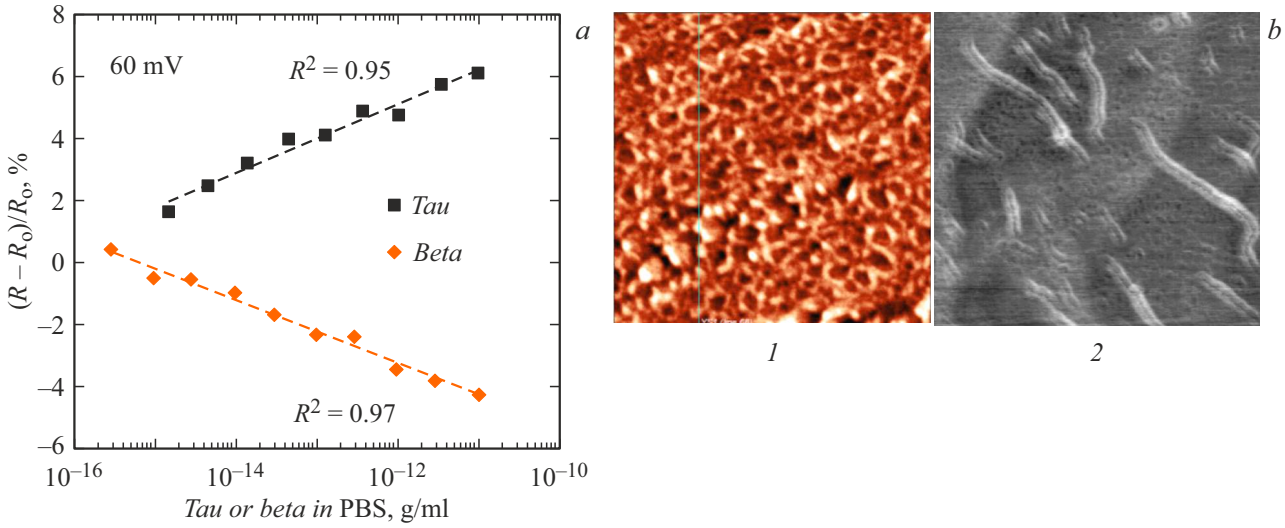

Figure 3. *a* — Concentration dependences of biosensor signals. Diamonds and squares represent the dependences of the beta protein biosensor (EG427-B4) and the tau protein biosensor (EG436-B6) signals. *b* — AFM images of the graphene surface in the same biosensors as in panel *a*. Scans $2 \times 2\,\mu$m in size are shown; the change in contrast reflects the variation of height from 0 to 5 nm. Fragments of the topography of graphene surfaces with tau (*1*) and beta amyloide (*2*) proteins are presented. Beta protein aggregates form between graphene steps, anchoring themselves in small defects of the cellular structure of the surface. Tau protein forms doughnut-shaped aggregates around small defects of the cellular structure.

graphene without functionalization, and an immune reaction of antibodies to tau protein was carried out. The sensitivity of biosensors based on these two types of graphene chips was evaluated by the change in resistance of the biosensors observed as the concentration of tau protein diluted in a PBS solution increased gradually from 10^{-15} to 10^{-10} g/ml. The concentration dependences of biosensor response $(R - R_o)/R_o$ with (triangles) and without functionalization (circles) are shown in Fig. 1, *a*. The initial detectable concentration of tau protein is fairly low for both types of biosensors (Fig. 1, *a*): it is several orders of magnitude lower than the detection limit of these proteins by modern diagnostic methods, such as enzyme immunoassay. However, the response of the biosensor without graphene functionalization reaches a level of 8.7%, which is almost 2 times higher than the one corresponding to the biosensor with electrochemical processing of graphene. Other types of functionalization also induced a noticeable suppression of chip response compared to the response of the chip without functionalization. It should be noted that the obtained results for graphene formed by thermal decomposition of silicon carbide are on par with the data presented in [1] for CVD graphene with and without functionalization. In practical applications, a larger response magnitude and a more pronounced concentration dependence for the chip without graphene functionalization (curve *2* in Fig. 1, *a*) should contribute to an enhancement of resolution and accuracy in determining intermediate protein concentration values.

The study of AFM topography of the graphene surface after the application of antibodies and the immune reaction on the graphene surface made it possible to identify the influence of aggregation of antibodies and proteins on the biosensor signal magnitude. The AFM topography mages of biosensor chips with a weaker concentration dependence (curve *1* in Fig. 1, *a*) normally feature rather large aggregates (presumably of the immune complex of tau proteins; light regions in Fig. 1, *b*), which were lacking on the surface of graphene in the chip before the experiments on protein detection. Aggregation of the immune complex of tau or beta proteins was also observed on graphene in chips with a stronger concentration dependence, but their dimensions (lateral size and height) were 2–5 times smaller in most cases. The process of aggregation of antibodies and proteins is affected both by their high concentration and the morphological features of initial graphene (e.g., large defects in its cellular structure; dark regions on the steps in Fig. 2, *a*). Similar defects may emerge in the process of application (immobilization) of anti-tau or anti-beta antibodies to the graphene surface from a PBS solution with their high concentration (10^{-5} g/ml; see Fig. 2, *b*).

A comparison of the concentration dependences of signals of beta and tau protein biosensors (diamonds and squares in Fig. 3, *a*) revealed the opposite nature of variations of graphene resistance with an increase in concentration of the corresponding proteins; their topography is also illustrative of the differences in nature of interaction of these proteins with graphene. Fragments *1* and *2* of topography images of these two surfaces are shown in Fig. 3, *b*. Different patterns of aggregate formation, which are made clear at different concentrations of detected proteins (10^{-11}–10^{-9} g/ml) or antibodies applied to graphene (10^{-5} g/ml), are seen. The observed features agree well with known facts revealed in the studies of affected brain

cells: τ-proteins are positively charged, while β-amyloids are negatively charged [2]. The identified features of interaction of antibodies and proteins with graphene are the reasons behind the irreproducibility of detection results and the suppression of biosensor response and require further in-depth study.

The efficiency of application of graphene obtained by thermal decomposition of silicon carbide in detection of tau and beta proteins without any special functionalization of its surface for antibody binding was demonstrated. Aggregation of antibodies and protein complexes in large defects of the cellular structure of graphene, which form both in the process of growth of graphene and during its interaction with a high concentration of antibodies, was detected. This has a negative effect on the reproducibility of graphene parameters and the sensitivity of biosensors.

It appears that a detailed study of the processes and mechanisms of interaction of beta and tau proteins with graphene should provide an opportunity to develop Alzheimer's disease markers suitable for mass clinical application.

Conflict of interest

The authors declare no conflict of interest.

References

[1] S.S. Kwon, D. Kim, M. Yun, J.G. Son, S.H. Lee, Biosens. Bioelectron., **192**, 113519 (2021). DOI: 10.1016/j.bios.2021.113519
[2] M. Sun, C. Zhang, S. Lu, S. Mahmood, J. Wang, C. Sun, J. Pang, L. Han, H. Liu, Adv. Funct. Mater., **34**, 2405471 (2024). DOI: 10.1002/adfm.202405471
[3] J. Zhang, J. Zhu, F. Guo, J. Jiang, M. Xie, L. Hao, J. Chao, Chem. Commun., **59**, 6869 (2023). DOI: 10.1039/d3cc01830j
[4] A.A. Lebedev, S.Yu. Davydov, I.A. Eliseyev, A.D. Roenkov, O.V. Avdeev, S.P. Lebedev, Yu. Makarov, M. Puzyk, S. Klotchenko, A.S. Usikov, Materials, **14**, 590 (2021). DOI: 10.3390/ma14030590
[5] V. Georgakilas, M. Otyepka, A.B. Bourlinos, V. Chandra, N. Kim, K.C. Kemp, P. Hobza, R. Zboril, K.S. Kim, Chem. Rev., **112**, 6156 (2012). DOI: 10.1021/cr3000412
[6] A.Yu. Plekhanov, M.V. Puzyk, A.S. Usikov, A.D. Roenkov, A.A. Lebedev, S.P. Lebedev, S.A. Klotchenko, A.V. Vasin, Yu.N. Makarov, Opt. Spectrosc., **130** (9), 1141 (2022). DOI: 10.21883/EOS.2022.09.54835.3628-22.

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