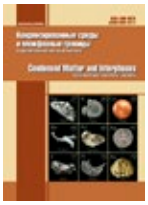

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Localization of the *E. coli* Dps protein molecules in a silicon wires under removal of residual salt

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Abstract

The work is related to the removal of residual salts in hybrid structures formed as a result of silicon wires arrays combining with a nanomaterial of natural origin – bacterial ferritin-like protein Dps. The study of the morphology and composition of the surface and the bulk part of the hybrid structure as a result of combination and subsequent washing in water was carried out.

The method of metal-assisted wet chemical etching was used to obtain silicon wires arrays. To obtain recombinant protein, *Escherichia coli* BL21*(DE3) cells with chromatographic purification were used as producers. The combination of silicon wires with protein molecules was carried out by layering them in laboratory conditions, followed by drying. The residual salt found earlier in the hybrid material was removed by washing in water. The resulting hybrid material was studied by scanning electron microscopy and X-ray photoelectron spectroscopy. A well-proven complementary combination of scanning electron microscopy and X-ray photoelectron spectroscopy together with ion etching was used to study the morphology of the hybrid material "silicon wires – bacterial protein Dps" and the composition with physico-chemical state respectively.

In arrays of silicon wires with a wire diameter of about 100 nm and a distance between them from submicron to nanometer sizes, protein was found as a result of layering and after treatment in water. At the same time, the amount of residual NaCl salt is minimized on the surface of the hybrid structure and in its volume.

The obtained data can be used in the development of coating technology for the silicon wires developed surface available for functionalization with controlled delivery of biohybrid material.

Keywords: Nanostructures, Biomolecules, Hybrid materials, Developed surface, Recombinant ferritin-like protein Dps, Silicon wires, Scanning Electron Microscopy, X-ray Photoelectron Spectroscopy

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1. Introduction

In modern technologies, new functional biohybrid materials are beginning to occupy more and more leading positions, primarily due to the simplicity and efficiency of their formation, properties often dictated by nature itself, and application prospects. Simple methods of formation and convenient, tunable characteristics significantly contribute to the creation of effective devices based on such materials. One of the most relevant and promising directions is the formation, study and further application of hybrid nature-like nanoobjects obtained from various biocultures [1].

A striking representative of the natural functional hybrid nanomaterial is the Dps protein. The Dps protein (DNA-binding protein of starving cells) belongs to the family of bacterial ferritins, whose function is to accumulate iron reserves in the organism. The Dps protein is found in almost all known types of living organisms [2, 3–5]. The Dps protein molecule can be a hybrid nano-biological object consisting of an outer organic shell, the size of which is about 9 nm, and an inorganic core contained in the inner cavity of a molecule up to 5 nm in size [6, 7]. The protein part includes 12 identical subunits with a homododecamer structure [6, 7]. The inorganic core deposited in the inner cavity of the molecule is a nanoparticle of the iron-oxygen system [6]. A hybrid nanomaterial based on Dps protein molecules obtained from *Escherichia coli* cell culture may be of interest as a potential container of natural origin with the wide range of use: from the accumulation and storage of nanomaterials to their targeted delivery.

Earlier, we demonstrated the possibility of forming two-dimensional structures by ferritin Dps molecules [8], and also obtained the understanding of the nuclei morphological features of a biohybrid nanomaterial using the transmission electron microscopy and their composition by X-ray photoelectron spectroscopy (XPS) [9]. An effective combination of the XPS method (with ion profiling) and the scanning electron microscopy (SEM) method has been studied the possibility of filling with Dps protein (free of inorganic nanoparticles) pores of a functional semiconductor material with a developed surface – silicon wires (Si-NW) [10, 11]. However, a significant residual NaCl salt content of buffer solution was found in the formed structures of silicon wires – protein Dps [11]. In this work, the same combination of SEM and XPS was used, providing information on the composition, morphology and physico-chemical state of the object under study, for the silicon wires – protein Dps structures after removal of the NaCl salt residual amount as a result of water washing.

2. Experimental

The metal-assisted wet chemical etching [12, 13] was used to form Si-NW. Wafers of crystalline silicon of *p*- and *n*-types conductivity (~ 1-5 Ω/cm and < 0.02 Ω/cm, respectively) were used after washing in a solution of hydrofluoric acid HF (2%) for 10 seconds. Next, silver nanoparticles were deposited on the surface of the prepared wafers when immersed in a solution of $AgNO_3$ (0.01 M) and HF (5 M). Immersion times were 15 s for *p*-type substrates and 45 s for *n*-type substrates, followed by three-minute etching in a 30% solution of H_2O_2 and HF (5 M). Finally, silver nanoparticles were removed for 10 minutes by washing in 65% aqueous HNO_3 solution, followed by air drying of the structures. Further, the silicon wires structures were used identical to those studied in [11], as simultaneously processed parts of the same wafers.

Cells of *Escherichia coli* BL21*(DE3) bacteria, hereinafter *E.coli*, transformed by pGEM_dps, were used as producers to obtain the recombinant Dps protein. Detailed information on the recombinant protein preparation is given in [6]. The protein solution had a concentration of 2 mg/ml in a

buffer containing 10 mM NaCl, 50 mM tris-HCl (pH 7.0) and 0.1 mM EDTA. After controlling the size of protein molecules by dynamic light scattering, a single layering of 10 μl of protein molecules solution was performed on the surface of Si-NW arrays. Further, in order to remove the residual NaCl salt [11], a single layering of 10 μl of water was carried out, followed by its removal after 30 seconds using filter paper without contact with the surface of the structure and drying the sample at 37 °C until completely dry.

The morphology of the Si-NW surfaces source array and the hybrid structure based on it with a layered protein after washing was studied by scanning electron microscopy. The Carl Zeiss ULTRA 55 microscope was used in the mode of secondary electrons registration with an accelerating voltage value of 2 kV, which is necessary for working with structures of biological origin. To estimate the areas occupied by the wires array and pores, as well as the degree of the arrays filling with molecular culture after washing, the Image J software package was used.

The XPS research was carried out using the ESCA module electron energy analyzer SPECS Phoibos 150 of the Kurchatov synchrotron (National Research Center Kurchatov Institute, Moscow) NANOFES ultrahigh vacuum experimental station [14]. Monochromatized Al Kα radiation of an X-ray tube (1486.61 eV) was used, the depth of the informative layer was ~ 2–3 nm [15]. Survey spectra were recorded in the range of binding energies 0–850 eV. To normalize and calibrate the data, a standard approach was used based on the independent recording of the pure gold foil (Au 4*f*) signal. To identify the features of the survey spectra, well-known databases were used, from which the most relevant and accurate (monochromatic) spectra were selected [15–17]. A focused source of surface etching with argon ions was used at an accelerating voltage of 3 kV with an etching duration of 20 minutes. The area of the etching area was selected with an excess of the surface area from which the XPS data were recorded.

3. Results and discussion

Fig. 1 shows the data of SEM studies for the initial Si-NW arrays and biohybrid structures. Note that in our previous work [11] we used morphologically identical Si-NW. Simultaneously formed parts of the same plate were used in protein layering [11] and in this work, where washing of the obtained biohybrid structures in water was added to the sequence of formation procedures. For this reason, Fig. 1a, c contains data on the work of [11] Si-NW substrates of *p*- and *n*-type. At the same scale, the SEM images of Fig. 1b, d were obtained for surfaces of biohybrid structures after layering of a molecular protein culture obtained from *E.coli* bacteria and washing. For *p*-type substrates, the formation of more pronounced wires with a uniform distribution of submicron size pores ~ 200-500 nm between the wires has been established. For *n*-type substrates, large pores of similar size are observed together with much smaller ones, about 10–100 nm in size. The formed characteristic upper parts of the wires are indicated by arrow 1, and the pores are indicated by arrow 2 in Fig. 1a, b. It is shown that all the observed Si-NW pores are available for filling as a result of Dps protein molecules layering with a size up to 10 nm [11].

According to the results obtained earlier, the surface of Si-NW, which is different in its morphology for *p*-type and *n*-type substrates, was almost completely covered with layered protein, and only the uppermost parts of the wires were fragmentally observed. The large pores dimensions provided a relatively greater penetration of the biomaterial into the silicon wires arrays of the *n*-type substrate. Significantly different morphology of biohybrid structures was observed as a result of washing aiming to remove residual NaCl salt. According to Fig. 1b no observations of micron- and submicron-sized salt microcrystals on the surface of a biohybrid material for a Si-NW *p*-type substrate have been recorded. Moreover, the total amount of protein decreased as a result of washing, since the upper sections of the silicon wires are reproducibly observed, indicated by arrows 1 in Fig. 1b. At the same time, large island formations (Fig. 1b, arrow 2) of micrometer sizes are observed on the surface of the silicon wires arrays, significantly exceeding the dimensions of the pores between wires (pore sizes) and without the faceted shape characteristic of NaCl salt. On the surface of these island formations, in turn, voids with sizes close

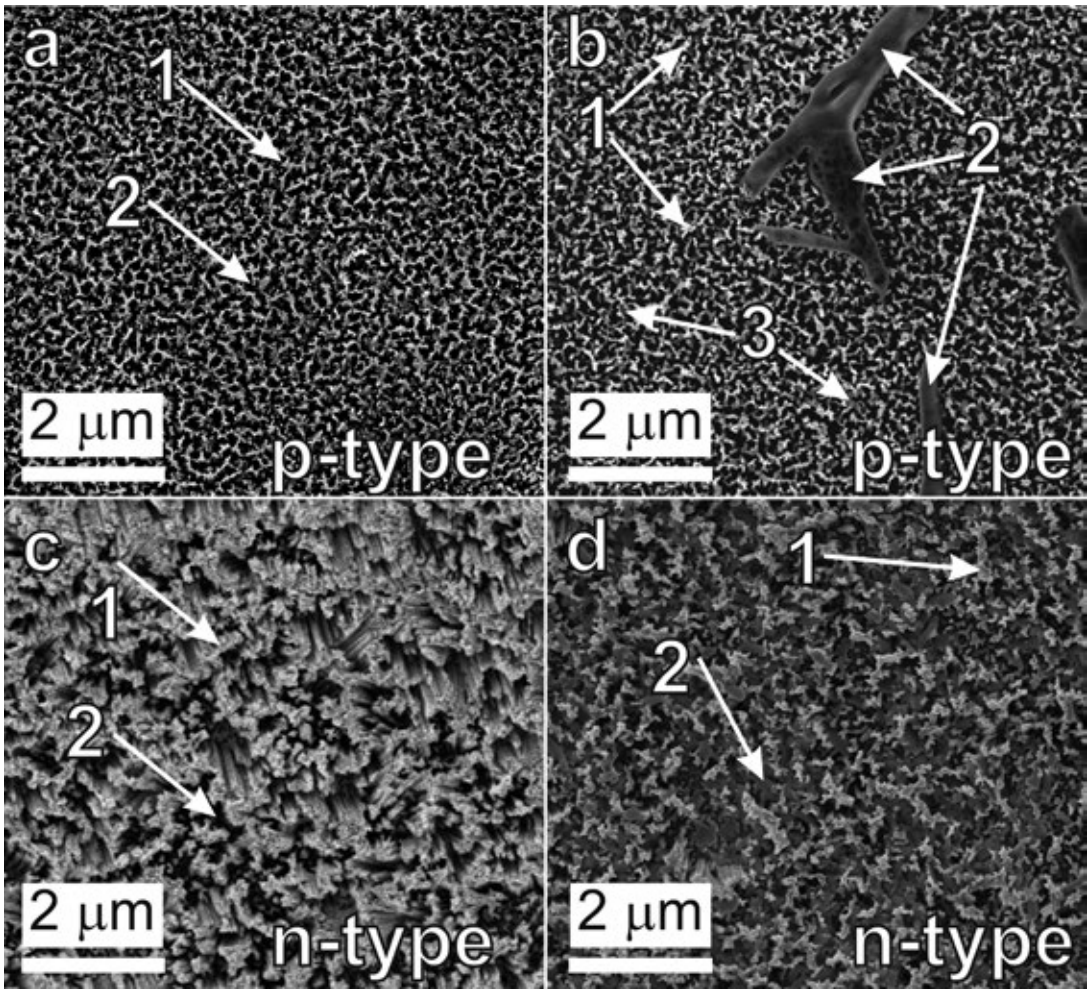

Fig. 1. Scanning electron microscopy of the initial samples of silicon wires arrays surfaces formed from *p*-type (a) and *n*-type (c) substrates [11], as well as after layering of the Dps protein molecular culture and washing in water (b) and (d), respectively. 1 – the tops of the wires (walls of pores), 2 (a and c) – pores between the wires, 2 and 3 (b and d) elements of protein culture

to 100 nm are noted. This indicates the presence of a residual amount of protein that has not entered the pores, but is a source of biomaterial localized in the Si-NW array. Localization of biocomponents in the pores of the studied structures is marked in Fig. 1b by arrows 3. It seems possible to state that the washing of the forming biohybrid structure led to the removal of residual salt crystals from structures surface and the observation of a Si-NW array with a localized molecular culture with incomplete filling of the pores. Protein localization in the spaces between silicon wires is much more noticeable for the Si-NW of the n-type substrate in Fig. 1d. Arrow 1 shows the characteristic upper part of the uncoated silicon wires, and arrow 2 shows a significant amount of protein in the pores of the Si-NW array. The degree of pore filling also decreases as a result of washing, compared with the data [11].

The survey XPS spectra are shown in Fig. 2 for arrays of *p*- and *n*-type silicon wires after layering of the molecular culture of Dps cells of *E. coli* and subsequent washing. All lines corresponding to the biological component of the studied biohybrid structures are marked. For both types of substrates used, the 1*s* oxygen line is the main one. The significant intensity of the 1*s* oxygen line excludes the contribution only from the natural SiO_2 oxide covering the silicon wires. The reason for such a high intensity of the oxygen line, along with the observation of the 1*s* carbon and nitrogen lines (in order of decreasing relative signal intensity), is the Dps molecular culture

localized in the Si-NW array. Also, insignificant in intensity 2*s* and 2*p* silicon lines from wires uncovered by molecular culture are observed. Finally, it should be noted that the set of chlorine (2*s* and 2*p*) and sodium lines (2*p* line and Auger KLL line) is weak in relative intensity. It should be noted that in [11] for biohybrid structures not subjected to removal of the residual NaCl salt, in some cases the signal from the sodium KLL Auger line was the main one in intensity, as was the signal from chlorine atoms. Thus, it can be stated that the applied approach to the dissolution and removal of residual salt leads to the almost complete removal of NaCl from the surface of the studied biohybrid structures Si-NW – molecular culture of Dps cells of *E.coli*. Finally, it should be noted the general similarity in the observed XPS lines in their energy position and relative intensity (Fig. 2, two lower spectra). This indicates an almost identical physico-chemical state of the surface of the studied biohybrid structures after layering of the molecular culture of Dps cells of *E.coli* and subsequent washing, regardless of the type of substrate and its processing mode. That is, the localization of the biomaterial is observed in the pores of Si-NW arrays for both types of surface morphology of the initial array of silicon wires. And the washing process allows removal the residual NaCl salt from the surface of biohybrid structures. Due to the observed identity of the physico-chemical state of the studied structures surface, we removed a significant part of the surface in 20 minutes of etching at a relatively high voltage accelerating argon ions (3 kV) only for a sample of a biohybrid structure formed on an *n*-type substrate. The etching rate estimation using the NANOFES station module along with calibration measurements show the removal rate for silicon atoms ~ 2.5 nm/min. For the residual part of the molecular culture, this rate may differ several times [18]. The removal of more than 50 nm of the surface by an ion beam in this way leads to significant changes. The relative intensity of the O 1*s* line is significantly reduced, which indicates the removal of a considerable part of the Dps molecular culture localized in the pores. At the same time, silicon lines reached almost the same intensity, as a result of a significant

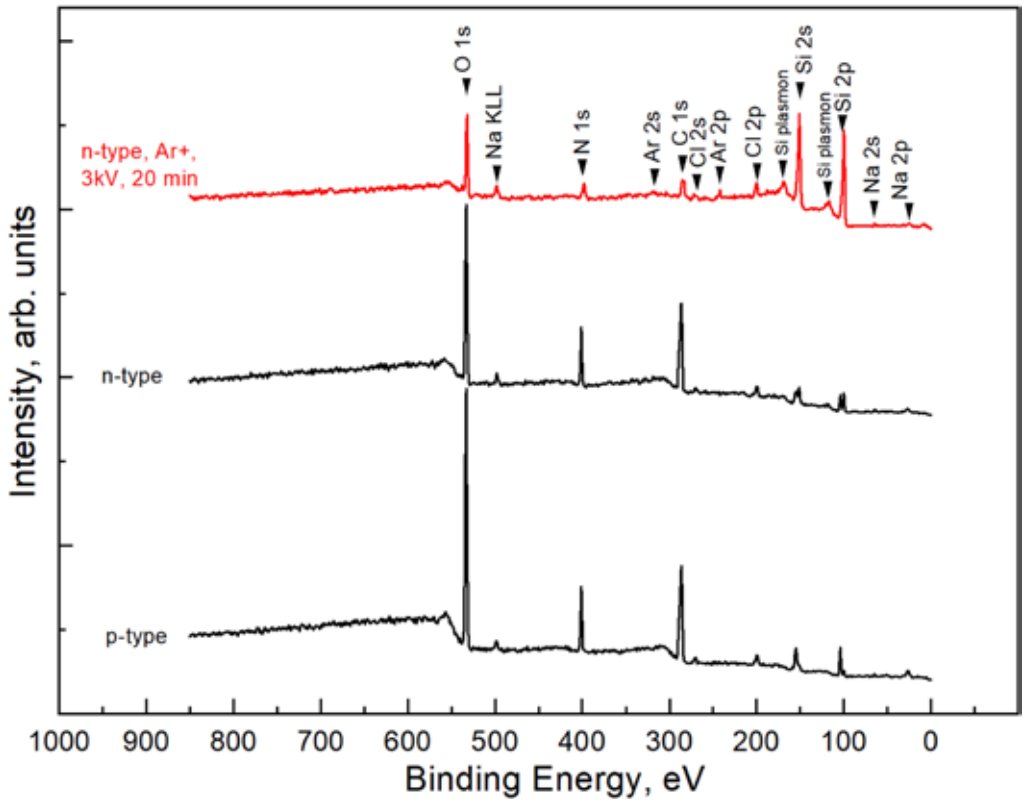

Fig. 2. XPS survey spectra of a silicon wires arrays samples formed on an *n*- and *p*-type substrate before (black) and after (red) ion beam etching (Ar+ 3 kV 20 min). The characteristic elements that make up the studied surface are noted

difference in the rates of ion profiling of the biomaterial and the inorganic silicon "frame". Nevertheless, the nitrogen and carbon lines are still observed, confirming the presence of a Dps molecular culture in the depth of the pores. At the same time, the residual salt lines do not change relative and low intensity. The reason for this observation is the incomplete removal of the NaCl salt, which, nevertheless, is evenly distributed in the volume of the biohybrid structure, probably due to re-precipitation as a result of dissolution during washing with water. Observation of argon lines is associated with ion etching (profiling) of the surface.

4. Conclusions

A combination of X-ray photoelectron spectroscopy and scanning electron microscopy methods demonstrated the possibility of localization of the molecular culture of bacterial ferritin Dps of *E.coli* cells as a result of effective filling of silicon wires arrays pore after washing of the formed biohybrid structures in order to remove the residual NaCl salt.

It is shown that the morphology of the initial silicon wires array does not significantly affect the characteristics of filling pores with a molecular culture of the Dps protein as a result of washing, which leads to the almost complete removal of the residual salt.

Thus, the possibility of silicon wires developed surface functionalization by driven coating with controlled delivery of biohybrid material is confirmed.

Authors comment

During the preparation of this paper, which is a continuation of the research started in [11], it was found that a technical inaccuracy was made. The scale of the image of the initial Si-NW surface formed on a p-type substrate is indicated incorrectly in Fig. 1a of [11], where it is necessary to consider the specified scale equal to 1 μm. However, this scale makes it relatively difficult to compare the morphology with the rest of the SEM images in [11]. For this reason, in this paper we provide an image of the same surface area of the original Si-NW sample from *p*-type substrates with a correctly selected scale of 2 μm. Mistake made does not affect the understanding of the discussed results in the paper [11]. The authors apologize for this technical inaccuracy.

Author contributions

All authors made an equivalent contribution to the preparation of the publication.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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