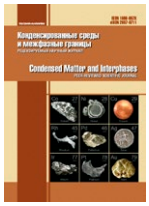

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Localization of the *E. coli* Dps protein molecules in a silicon wires matrix according to scanning electron microscopy and X-ray photoelectron spectroscopy

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Abstract

The work is related to the study of the morphological features of silicon wires arrays combined with a nanomaterial of natural origin, a bacterial ferritin-like protein Dps, and their relationship with the composition of the surface and interior.

A silicon wires array was formed by metal-assisted wet chemical etching. To obtain recombinant protein, *Escherichia coli* BL21*(DE3) cells were used as producers, and purification was carried out by the chromatography method. The combination of silicon wires with protein molecules was carried out by layering under laboratory conditions, followed by drying. The resulting hybrid material was studied by scanning electron microscopy and X-ray photoelectron spectroscopy.

The initial silicon wires array had sharp boundaries on the surface. The diameter of the silicon wires was about 100 nm, while the distances between the wires can vary widely, reaching several hundred nanometres or be less than 100 nanometres, depending on the formation conditions, in the absence of noticeable transition layers. The pores formed in this way are available for filling with protein during deposition.

The effectiveness of using the scanning electron microscopy method to study the morphology of the hybrid material "silicon wires – bacterial protein Dps" as well as X-ray photoelectron spectroscopy method together with ion etching for the investigation of the composition and physico-chemical of the hybrid material was demonstrated. Complementary results have shown that the molecular culture, which is a solution of oligomers of the recombinant Dps protein of *E.coli* bacterial cells, can penetrate deep into the pores of the silicon wires array with an extremely developed surface. The possibility of the control of the filling of silicon wires arrays by varying the pore morphology and other modes of formation of structures and their surface has been demonstrated.

The obtained data can be used to study the possibilities of the functionalization of the developed surface of silicon wires by their driven coating 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

One of the modern and relevant directions of technologies development for the formation of new functional nanomaterials of high scientific and practical interest is the creation and study of structures based on the combination of well-known and well-technologically developed inorganic nanomaterials and biopolymers, including those of natural origin [1, 2]. The well-known silicon wires (Si-NW) remain an attractive material for researchers because of their simple, well-developed and economical production technology, their ability to photoluminescence in the visible spectral region at room temperature [3, 4] and the possibility of using such structures to generate hydrogen [5]. The presence of an extremely developed surface in Si-NW is an important characteristic that enhances the attractiveness of this material. Targeted delivery of nanoparticles, including nano-biohybrid ones, and their controlled distribution during the functionalization of 3D-developed surfaces is a real example of how silicon wires can be applied [6–8].

One of the characteristic examples of a natural functional nano-biohybrid material formation is the accumulation of inorganic nanoparticles inside a natural protein molecule [7, 9]. The Dps protein (DNA-binding protein of starving cells) is a representative of bacterial ferritins [10]. The size of the outer shell of the bacterial protein Dps is about 9 nm. The protein part includes 12 identical subunits with a homododecamer structure [9, 10]. Dps protein molecules are capable of accumulating (depositing) iron ions. The accumulation of iron occurs in the form of inorganic nanoparticles of the iron-oxygen system [9] inside the hollow part of the protein with a diameter of up to 5 nm [9, 10]. Thus, the Dps dodecamer is a potential container of natural origin, which can serve for the accumulation, storage, and targeted delivery of nanomaterials, including into various matrices with a developed 3D surface. Therefore, the issue of studying the result of combining Si-NW arrays with oligomers of recombinant Dps protein obtained from *E. coli* cells is relevant for the development and application of new hybrid materials combining inorganic structures with desired properties with functional nanomaterials of natural origin.

Previously, by scanning electron microscopy (SEM), we showed the potential possibility of filling the developed and available for functionalization surface of silicon wires with *E. coli* Dps protein molecules [6, 8]. In this study, the morphology data are supplemented by the results of the X–ray photoelectron spectroscopy application - XPS method, sensitive to the surface composition and physico-chemical state of the studied object, together with focused ion etching, in order to establish the possibility of filling the space between Si-NW with a recombinant protein molecular culture.

2. Experimental

Silicon wires were formed by metal-assisted wet chemical etching [3, 4]. Crystalline silicon substrates of *p*- and *n*- types (specific conductivity ~ 1–5 Ω/cm and < 0.02 Ω/cm, respectively) were washed for 10 seconds in a solution of 2 % hydrofluoric acid (HF). Deposition of silver nanoparticles on the silicon wafers surface was carried out by immersion in a solution of $AgNO_3$ (0.01 M) and HF (5 M) at 15 sec (*p*-type substrates) and 45 sec (*n*-type substrates). Then etching was carried out in a 30 % solution of H_2O_2 and HF (5 M) for 180 sec, followed by removal of silver nanoparticles by washing in a solution of HNO_3

(65 %) in water for 10 minutes. The structures formed in this way were dried in air under laboratory conditions.

Cells of *Escherichia coli* BL21*(DE3) bacteria, hereinafter *E. coli*, transformed by pGEM_*dps*, were used as producers to obtain recombinant Dps protein. Detailed information on the preparation of the recombinant protein, the method of its isolation and purification, removal of inorganic components by stepwise hydrolysis and dialysis are provided in [9]. 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. The sizes of protein molecules were controlled by dynamic light scattering [11]. A single layering of 10 ml protein molecules solution on the surface of Si-NW arrays was performed, followed by drying under laboratory conditions.

The morphology of the initial Si-NW array surfaces and the hybrid structure based on it with a layered protein were studied by Scanning Electron Microscopy. The Carl Zeiss ULTRA 55 microscope was used in the mode of secondary electrons registration with a low accelerating voltage 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 arrays filling with molecular culture, the Image J software package was used.

XPS studies was carried out using the NANOFES beamline ESCA module of the Kurchatov synchrotron ultrahigh vacuum experimental end-station (National Research Center Kurchatov Institute, Moscow), equipped with an electron energy analyzer SPECS Phoibos 150 [12]. Monochromatized Al*K*a radiation of an X-ray tube (1486.61 eV) was used, the depth of the informative layer was ~ 2–3 nm [13]. 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 main features of the survey spectra, well-known databases were used, from which the actual and most accurate (monochromatic) spectra were selected [13–15]. 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 site was selected with an excess of the surface area from which the XPS data were recorded.

3. Results and discussion

The SEM data obtained for the initial arrays of *p*- and *n*-type substrates silicon wires are shown in Fig. 1 (a, c) and, on the same scale, for arrays of silicon wires after layering of a molecular protein culture obtained from *E.coli* bacteria (Fig. 1 (b, d)), respectively. For *p*-type substrates, taking into account their significantly higher specific conductivity (~ 1–5 Ω/cm) compared to *n*-type substrates (< 0.02 Ω/cm), a shorter silver deposition time was chosen according to [3]. This led to the formation of smaller Ag nanoparticles on the surface of *p*-type crystalline silicon and for other equal parameters, to a more pronounced formation of wires during etching. Arrays of *p*-type substrates silicon wires were characterized by a more uniform distribution of submicron size ~ 200–500 nm pores (voids) between the wires, along with generally uniform wall sizes. At the same time, for *n*-type substrates, large pores of similar size were 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. 1 (a, c). All observed pores of silicon wires arrays appear to be available for filling as a result of the layering of Dps protein molecules with a size of up to 10 nm.

The morphology of the surface changed after layering the molecular culture of the recombinant bacterial protein Dps from *E.coli* and subsequent drying. Fig. 1 shows the SEM data for *p*-type (Fig. 1b) and *n*-type substrates (Fig. 1d). These data indicate a clear overflow of pore volumes of the wires array of the *p*-type substrate in the presence of Dps molecules (Fig. 1b). Individual small sections most likely represent the uppermost parts of the wires (arrow 1). There were also quite large submicron-sized formations on the surface, which presumably represent a residual salt from the culture medium, or, more likely, a buffer solution used to maintain the conformation of the Dps dodecamer, in which the molecular culture was directly layered. According to the protocol of experiments this salt is NaCl. However, traces

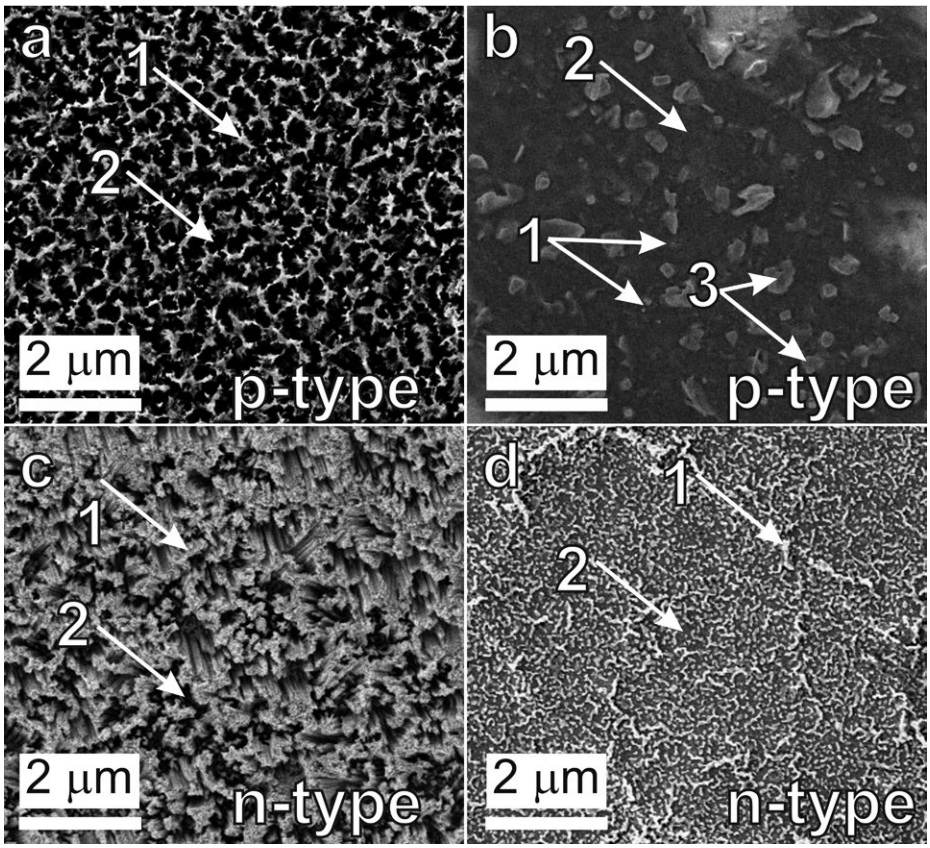

Fig. 1. Scanning electron microscopy of the initial samples of silicon wires array surfaces formed from *p*-type (a) and *n*-type (c) substrates, as well as after layering of the Dps protein molecular culture (b) and (e), respectively. *1* – tops of wires (walls of pores), *2* – pores between wires, *3* – NaCl salt particles after drying

of salt release were not noted for silicon wires formed on *n*-type substrates (Fig. 1d). Here, the filling of pores was also noted (arrow 2), however, a much larger number of uncovered vertices of silicon wires was observed. As the uniform experimental conditions were maintained, we associate this observation with the large volume of formed wires array pores available for filling with molecular culture. The three-fold increase in time of silver deposition on the surface (the size of silver nanoparticles, according to [3] was larger) with a much lower resistivity of *n*-type substrates compared to *p*-type led to a more pronounced "etching" and, as a consequence, large volumes of space between them were available for filling, which is in good agreement with [3, 6, 8]. The absence of NaCl formations on the surface can presumably be explained by the penetration of salt deep into the pores, before the formation of large particles as a result of drying.

The XPS survey spectra are shown in Fig. 2 for *p*-type silicon wire substrates after layering of the molecular culture. It should be noted that we do not provide XPS survey spectra for both types of the initial substrates and arrays of wires before filling with protein due to their almost complete identity. The exception is the intensities redistribution for the 2*s* and 2*p* silicon lines relative to the 1*s* oxygen line intensity which was obviously associated with an increase in the amount of the wires array surfaces available for oxidation. For the wires arrays, after the layering of *E.coli* Dps cells, all lines corresponded to the biological component of the studied sample were noted. First of all, the 1*s* carbon line had most significant in intensity, 1*s* nitrogen and oxygen lines were also present. A weak set of sodium and chlorine lines in combination with the observation of a noticeable Na KLL Auger line indicated some presence of salt on the surface. A comparison with the SEM results may imply the NaCl particles could be observed microscopically with a layer of residual Dps molecular culture covering them. In addition, the presence of barely

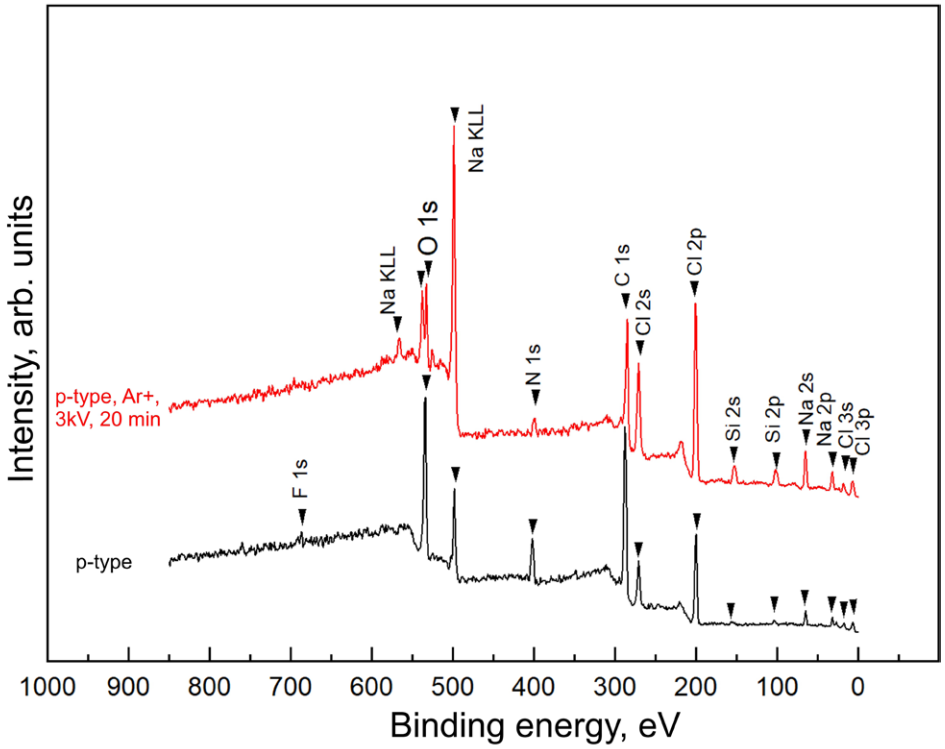

Fig. 2. XPS survey spectra of a silicon wires sample formed on a *p*-type substrate before (black) and after (red) ion beam etching (Ar+ 3 kV 20 min). The characteristic elements that make up the studied sample surface are noted

noticeable 2*s* and 2*p* silicon lines should be noted, confirming the assumption that wire arrays formed on a *p*-type substrate overflow, according to the SEM data.

Unlike the studies published earlier, where "soft" modes were used for etching [16, 7], in the present study we used a relatively high accelerating voltage of argon ions (3 kV) for the removal of the significant part of the surface in 20 minutes of etching. The estimation of the etching rate used at the NANOFES station module, along with calibration measurements, showed the removal rate for silicon atoms of ~ 2.5 nm/min. For the residual part of the molecular culture, this rate may differ by several times [17]. The removal of more than 50 nm of the surface by an ion beam led to significant changes. NaCl lines became the most intense, confirming the assumption made above about the salt particles coverage by the residual protein. The intensities of the silicon lines also became more pronounced, which also implies the removal of a significant amount of protein from the part of the surface subjected to ion etching.

Finally, it should be noted that after the removal of the part of the surface, the intensity of the oxygen 1*s* line practically did not change, but the peak became two-component. The preservation of intensity may be associated with the preservation of pores filling with molecular culture. The appearance of the second component of the oxygen peak may be due to the contribution of natural silicon oxide from the wires array "opened" after ion etching. The preservation of the position and relative intensity of the carbon line, obviously the main for the molecular culture, confirms the penetration and filling of pores with Dps protein under the selected method and modes of formation.

XPS spectra survey for *n*-type silicon wire substrates after layering of molecular culture are shown in Fig. 3. It should be noted that the silicon lines were among the most intense, confirming the observations of SEM, indicating the not so pronounced filling of pores in comparison with *p*-type substrates. The two-component oxygen line had the highest intensity here. This result was undoubtedly obtained by the cumulative contribution of oxygen atoms of naturally oxidized silicon wires and protein in the pores of the wires matrix.

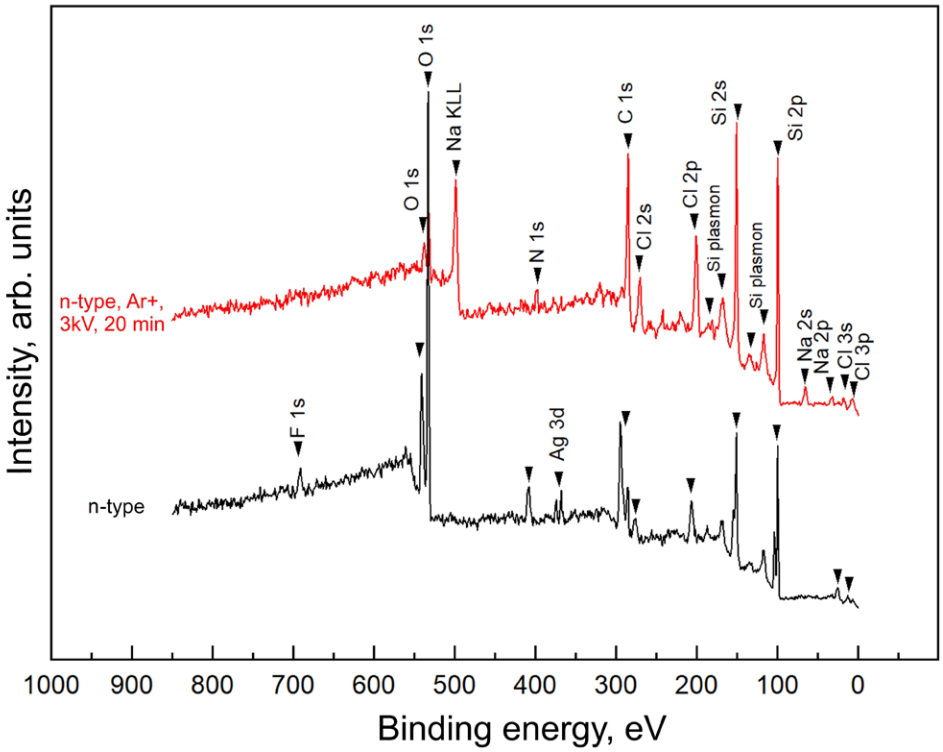

Fig. 3. XPS survey spectra of a silicon wires sample formed on a *p*-type substrate before (black) and after (red) ion beam etching (Ar+ 3 kV 20 min). The characteristic elements that make up the studied sample surface are noted

The intense carbon line as well as nitrogen line, also indicated the presence of a significant amount of protein in the matrix pores of wires. The weak contribution of chlorine lines to the survey spectrum, together with the absence of sodium lines, may indicate insignificant, residual traces of salt in the most superficial layers (~ 3 nm). An interesting observation was the presence of an Ag 3*d* doublet line. This finding is explained by the three times longer deposition time of silver on the silicon surface for *n*-type wafers compared to *p*-type with identical washing time after the formation of a wires array.

As in the previous case, ion etching led to a change in the physicochemical state of the studied structure. The silicon line became one-component after etching. Together with a significant decrease in the intensity of the two-component 1*s* oxygen line, this observation confirmed the noticeable removal of natural silicon oxide from the developed (including SEM data, Fig 1) surface of the wires array. The 1*s* oxygen line remained two-component indicating that the Dps protein molecules were also preserved in the pores. The increase in the intensity of 1*s* carbon line also confirmed this observation. The appearance of all the lines characteristic of NaCl observed for structures on *p*-type substrates confirmed the assumption made during the analysis of SEM data about the presence of residual culture medium salts or buffer solutions after drying in the depth of pores for wire arrays of *n*-type substrates.

Finally, the presence of fluorine atoms on the studied structures surface after protein layering and the decreased intensity of the nitrogen line after ion etching of the structures formed on the both types of substrates can be considered as the subject of separate studies. Additionally the issue of the removal of residual salts of buffer solutions and the culture medium of *E.coli* cells producers of Dps deserves separate consideration.

4. Conclusions

The possibility of the effective filling of silicon wires array pores with bacterial ferritin Dps molecules of *E.coli* cells has been demonstrated

for the first time using X-ray photoelectron spectroscopy and scanning electron microscopy.

It was shown that the morphology of the initial silicon wires array has a significant effect on the characteristics of pores filling with a molecular culture of the Dps protein. The possibility of the controlled filling of silicon wires arrays by varying the morphology of pores and other modes of structure formation was established: the resistivity of the initial crystalline silicon wafers, etching time, layering characteristics, and salt concentrations of working solutions.

The obtained self-complementary data of the SEM and XPS methods can be used to study the possibilities of the silicon wires developed surface functionalization by driven coating under the controlled delivery of biohybrid material.

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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