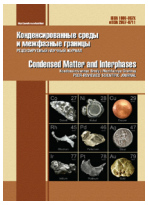

ISSN 1606-867X (Print)
ISSN 2687-0711 (Online)
Condensed Matter and Interphases
Kondensirovannye Sredy i Mezhfaznye Granitsy
https://journals.vsu.ru/kcmf/

Original articles

Research article
https://doi.org/10.17308/kcmf.2021.23/3741

Dps protein localization studies in nanostructured silicon matrix by scanning electron microscopy

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Abstract

The present work is related to the microscopic studies of the morphology of the planar and inner part of silicon nanowires arrays before and after immobilization with a natural nanomaterial, Dps protein of bacterial origin.

Silicon nanowires were formed by metal-assisted wet chemical etching. To obtain the recombinant protein, *Escherichia coli* cells were used as excretion strain and purification were carried out using chromatography. The combination of silicon nanowires with protein molecules was carried out by layering at laboratory conditions followed by drying under air. The resulting hybrid material was studied by high-resolution scanning electron microscopy. Studies of the developed surface of the nanowires array were carried out before and after combining with the bioculture. The initial arrays of silicon wires have a sharp boundaries in the planar part and in the depth of the array, transition layers are not observed. The diameter of the silicon nanowires is about 100 nm, the height is over a micrometer, while the distances between the nanowires are several hundred of nanometers. The pores formed in this way are available for filling with protein during the immobilization of protein.

The effectiveness of using the scanning electron microscopy to study the surface morphology of the hybrid material "silicon wires – bacterial protein Dps" has been demonstrated. It is shown that the pores with an extremely developed surface can be combined with a bio-material by deposition deep into cavities. The protein molecules can easily penetrate through whole porous wires matrix array. The obtained results demonstrate the possibility of efficient immobilization of nanoscaled Dps protein molecules into an accessible and controllably developed surface of silicon nanowires.

Keywords: Silicon wires, Developed surface, Ferritin-like Dps protein, Scanning electron microscopy, Combination

Acknowledgements: the work is supported under scholarship of the President of Russian Federation SP-189.2021.1 for young scientists.

For citation: Parinova E. V., Antipov S. S., Sivakov V., Kakuliia I. S., Trebunskikh S. Yu., Belikov E. A., Turishchev S. Yu. Dps protein localization studies in nanostructured silicon matrix by scanning electron microscopy. *Kondensirovannye sredy i mezhfaznye granitsy = Condensed Matter and Interphases*. 2021;23(4): 644–648. https://doi.org/10.17308/kcmf.2021.23/3741

Для цитирования: Паринова Е. В., Антипов С. С., Sivakov V., Какулия Ю. С., Требунских С. Ю., Беликов Е. А., Турищев С. Ю. Исследования локализации белка Dps в матрице нитевидного кремния методом растровой электронной микроскопии. *Конденсированные среды и межфазные границы*. 2021;23(4): 644–648. https://doi.org/10.17308/kcmf.2021.23/3741

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1. Introduction

Arrays of Silicon Nanowires (Si-NW) are a well-known material, including nanomaterials, whose formation technologies are well developed [1]. Si-NW traditionally attracts the attention of researchers as a material with important properties: visible photoluminescence at room temperature [2] or the possibility of use in hydrogen generation issues [3]. However, the most important property of this material is a simple and economical technology of reproducible formation in combination with an extremely developed surface [4], which is available for activation when combined with numerous functional materials, including nano-biomaterials of natural origin. With this approach to using the available surface of silicon wires arrays of controlled morphology, the issues of the general possibility of combining with the necessary material are the issue of a great interest.

Dps protein (DNA-binding protein of starving cells) of *Escherichia coli* (*E. coli*) bacteria is a representative of bacterial ferritins [5]. The shell size of the bacterial Dps protein is about 9 nm. The protein part includes 12 identical subunits with a homo-dodecamer structure [5, 6]. Dps protein molecules are able to accumulate (deposition) inorganic nanoparticles of the iron-oxygen system [7] inside a hollow part with a diameter of up to 5 nm [7, 8]. Thus, ferritin Dps is a potential container of natural origin, which can be used for the accumulation, storage and targeted delivery of nanomaterials. Previously, synchrotron X-ray absorption near edge fine structure spectroscopy showed a complex composite-like composition of a nanoparticle, which is formed from the oxidation products of Fe^{2+} ions in the ferroxidase centers of natural Dps molecules isolated from *E.coli* bacteria grown aerobically [9]. Moreover, the possibility of forming one-dimensional structures of Dps molecules has been shown by cryo-electron microscopy [5, 8].

Finally, it should be noted that the Scanning Electron Microscopy (SEM) method is one of the most popular in the diagnosis of a large variety of objects, including nanoscale structures and biomaterials. The capabilities of the method make it possible to study the morphology features for objects of various origins with high lateral resolution. However, the question of the SEM technique applicability for the precision study of hybrid nano-biomaterials requires experimental verification due to the complex composition and structure of such objects.

Thus, the issue of presented study is related to the possibility of combining Si-NW silicon wires arrays with natural nanomaterial bacterial protein Dps. This is relevant, from the point of view, for the development and application of hybrid materials – combining inorganic structures with desired properties with functional nanomaterials of natural origin. This work is devoted to the application of high-resolution scanning electron microscopy to study structures formed as a result of combining arrays of silicon wires with bacterial protein Dps.

2. Experimental

To obtain Si-NW arrays, the metal-assisted wet chemical etching was used [2, 10]. Standard substrates of n-type crystalline silicon (resistivity of ~ 1–5 Ω/cm were rinsed in a 2% solution of HF for 10 sec. Further, silver nanoparticles were deposited on the surface as a result of immersion of purified Si substrates in $AgNO_3$ (0.01 M) and HF (5M) solution for 30 sec. The next step was etching in 30% H_2O_2 and HF (5M) solution for 180 sec, followed by rinsing in 65% HNO_3 solution (to remove silver nanoparticles) with additional rinsing in water for 10 minutes. The structures formed were dried in the air in ambient conditions.

Recombinant Dps protein was obtained using *Escherichia coli* BL21*(DE3) cells as producers. *E.coli* cells were transformed by the pGEM_dps plasmid. The paper [5] provides detailed information about protein biosynthesis and its subsequent isolation and purification. The solution of protein molecules had a concentration of 2 mg/ml in the initial buffer of 10 mM NaCl, 50 mM tris-HCl (pH 8.0) and 0.1 mM EDTA.

Protein molecules were deposited on the surface of formed and previously studied Si-NW arrays by layering 10 ml solution. After that, the resulting structure was dried in ambient conditions, rinsed in deionized water (by pulling) in order to remove residual salts and dried again under the same conditions.

The surfaces morphology of the initial Si-NW array and the hybrid structure based on

it with layered protein, as well as the sections were studied by SEM. The Carl Zeiss ULTRA 55 microscope was used (secondary electrons registration) with low accelerating voltages of 2, 3 and 5 kV, necessary for working with bio-structures.

The Image J software package was used to estimate the areas occupied by the Si wires, cavities and filling the arrays with molecular culture.

3. Results and discussion

Fig. 1 shows the morphology of the initial surface of Si-NW arrays. The largest wide cavities V1–V6, which were formed as a result of etching, were noted. The width of such pores is up to 737 nm. At the same time, pores with a width of less than 100 nm are observed.

It should be noted that the edges of the wires arrays are sharp, while the arrays themselves have morphological features with sizes less than 50 nm as a result of local etching processes not stimulated by silver nanoparticles [2, 10]. There are no noticeable morphologically transitional layers in the initial array of Si-NW. To estimate the areas in the lateral projection that were taken into account in the calculation are indicated: 1 – the surface of the wires arrays, including the pores side parts available for observation, 2 – cavities (pores). The ratio of the areas of type 1 and type 2 areas is 48 to 52%.

Fig. 2 shows the results of Si-NW arrays combining with Dps protein after drying in laboratory conditions. Before discussing the result of the combination, let us pay attention to the section of the hybrid structure (Fig. 2b) that gives the morphology of individual wires and their height as a whole. Note that the lateral surfaces of the wires are morphologically similar to the surface of the wires arrays. This is especially noticeable for areas of non-etched surface (for example, see the highlighted area 1 in Fig. 1). The height of almost vertical wires is more than a micrometer (Fig. 2b). Consequently, the depth of the cavities (pores) available for filling with protein molecules exceeds a micrometer. At the same time, individual wires, which, on the other hand, we can consider as the walls of cavities (pores) are uniform.

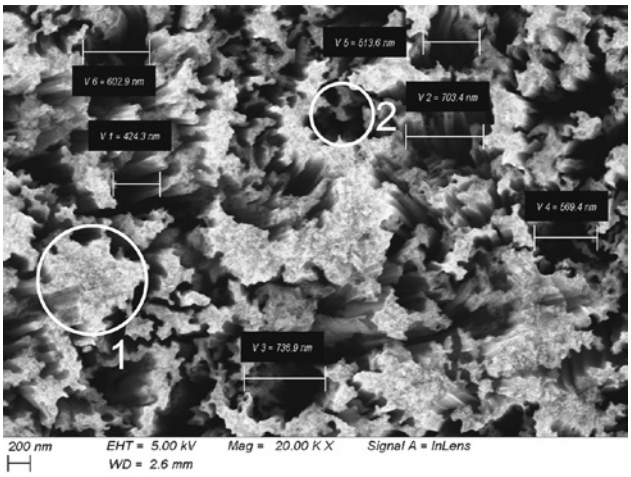

Fig. 1. Morphology of the silicon wires array initial surface. The sizes of cavities (pores) of the largest width are underlined

The small accelerating voltages used for registration (shown in the figures) were sufficient to form images (Fig. 2) of the morphology of hybrid bio-structures of sufficient sharpness and degree of detailing.

In Fig. 2 it is clearly visible that the cavities between the wires of the Si-NW array are filled with protein. Areas 1 (as in Fig. 1) mark the surface of non-etched areas, including those not covered with deposited molecular culture. At the same time, areas 2 clearly contain morphologically pronounced areas corresponding to the residual volumes of the molecular culture, which are located in the cavities after drying the hybrid structures in the laboratory and vacuuming in the microscope working chamber. In fact, the material that fills the pores is morphologically more homogeneous than the surface (walls) of Si-NW and is located precisely in the cavities available for deposition (combining). In Fig. 2b area 3 is additionally marked confirming the fact that the entire volume of the pore is filled with the molecular culture. The ratio of unfilled surface areas (1) and protein coating (2) is estimated as 30% and 70% of the total area of the microscopic surface image. This fact confirms the success of the first time combination of the nano-biomaterial of the molecular culture of the Dps protein with the available developed surface of the silicon wires array.

4. Conclusions

For the first time, a combination of a nano-biomaterial as the Dps protein molecular

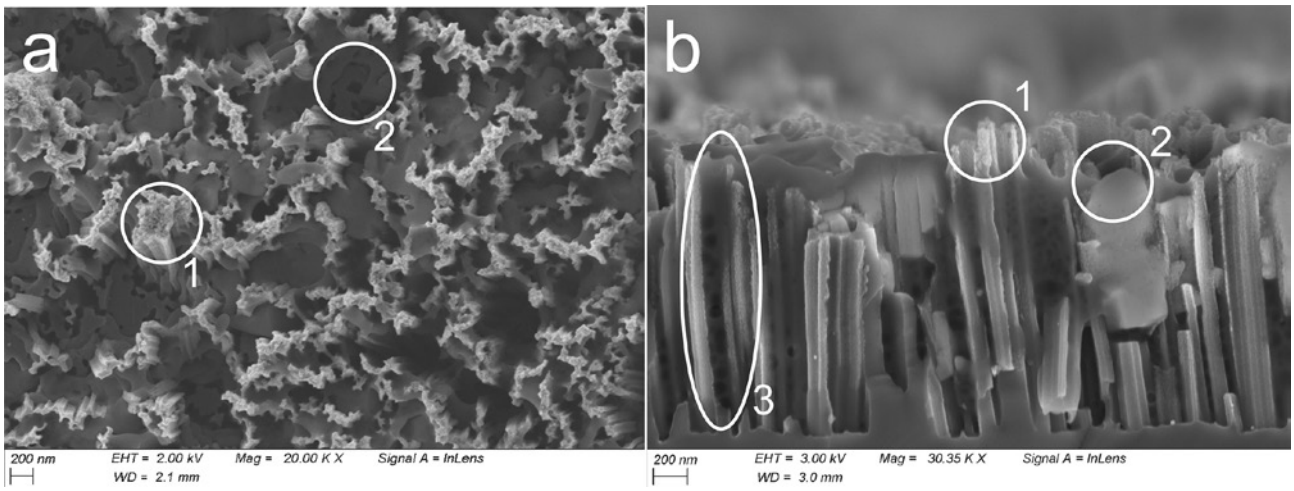

Fig. 2. Morphology of (a) – the surface and (b) – the inner part (section) of the silicon wires array after combining with the Dps protein

culture with an accessible developed surface of a silicon wires array was performed. It has been shown by high-resolution scanning electron microscopy that ferritin Dps molecules can successfully penetrate into the submicron size pores, successfully filling them and covering the silicon wires array highly developed surface as a whole. Suggested approach can be used for the functionalization of the Si wires arrays surface by using ferritin molecules as nanometer-sized containers for targeted delivery of materials tasks and the formation of functional hybrid nanobiomaterials in general.

Conflict of interests

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

Author contributions

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

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Received September 9, 2021; approved after reviewing October 15, 2021; accepted for publication November 15, 2021; published online December 25, 2021.

Translated by Sergey Turishchev