H1N1 influenza virus interaction with a porous layer of silicon nanowires

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Abstract

Here, the non-specific interaction of the H1N1 influenza virus with a porous layer of silicon nanowires (PSi NWs) was studied by transmission and scanning electron microscopy (TEM, SEM, respectively) and optical spectroscopy. PSi NW layer with a thickness of about 200 nm was fabricated by metal-assisted chemical etching of p-type highly doped crystalline silicon wafers, and consist of porous nanowires with a diameter of 50–200 nm, and a distance between the nanowires of 100–200 nm. It was shown that during the adsorption of viruses, viral particles with a diameter of about 100 nm bind to the porous surface of the nanowires. This interaction was revealed using TEM, SEM, and causes wavelength shifts in the Fabry–Perot fringes in the reflection spectrum of visible light from the PSi NW layer. The results show that thin layers of PSi NWs are a promising nanomaterial for creating filters and sensors for binding and detection of viruses.

1. Introduction

Due to the increased mobility of the population, a large number of viruses are spreading rapidly around the globe, causing the simultaneous circulation of several SARS pathogens at the same time [1]. Among other pathogens of acute respiratory viral infections, the influenza virus occupies a special place. In addition to the usual seasonal epidemics, pandemics of a completely new in pathogenicity of which cannot be predicted [2]. Thus, the development of new methods for the early diagnosis of viral diseases is an urgent task.

To create sensors for virus detection, nanotechnological approaches are actively studied, in which gold nanoparticles [3, 4], carbon nanotubes [5, 6] and silicon nanowires (Si NWs) [7] etc are proposed as sensitive elements. For example, it was shown that a Si NW-based field effect transistor, whose surface was modified by antibodies, can selectively detect even individual influenza viruses in biological fluids and air [8–10]. However, the manufacture of such transistors, in which single nanowires with a diameter of 50 nm and a length of about a micrometer are used as a sensitive element, requires expensive high-precision equipment. Typically, such Si NWs are produced by CVD (chemical vapor deposition), which is also quite expensive and time consuming. Also Si NWs, obtained by CVD, is often contaminated with gold [11], which can affect their electronic properties.

The metal-assisted chemical etching (MACE) is another simple and cheap method of obtaining Si NWs [10, 11]. It was shown that if a highly doped silicon substrate is used for etching, the resulting arrays consist of porous in volume nanowires (PSi NWs) with a diameter of 100 nm, pore size of 5–10 nm, and the distance...
between the wires of 100–300 nm \([12]\). The surface of the nanowires is coated with a thin oxide layer, which is formed during their preparation. This oxide layer protects the nanowires from further oxidation due to contact with environmental molecules, including atmospheric gases and water under normal environmental conditions \([13, 14]\). The properties of light interference in the layers of Si NWs shown can be used to detect protein A and sucrose \([15]\). In addition, the binding of various viruses to porous silicon nanoparticles has been shown, which can be caused by Van der Waals interactions arising between the huge porous surface of nanoparticles and virions \([16]\).

In this paper, we investigate H1N1 influenza virus interaction with layers of PSi NWs. TEM and SEM images revealed the effective binding of virions to the porous surface of NWs. At the same time, the presented changes in the spectra of white light reflection from the PSi NW array after virus adsorption indicate the possibility of creating an optical interferometric sensor based on it for the detection of influenza viruses.

2. Methods

PSi NWs were fabricated by two step MACE \([17]\) of p-type crystalline silicon (c-Si) wafers with crystallographic orientation (100) and resistivity of 1–5 m\(\Omega\) \(\cdot\) cm. In a first step of MACE, c-Si substrate was covered with silver nanoparticles by immersing it in solution of 0.02 M AgNO\(_3\):5 M HF (1:1) for 15 s. In the second step of MACE, PSi NWs were fabricated throw the etching process under silver nanoparticles in solution of H\(_2\)O\(_2\) (5%):5 M HF = 1:10 for 10 min. After the etching process, PSi NWs were washed in a 30% HNO\(_3\) solution for 15 min to remove silver nanoparticles from the PSi NWs/c-Si interface. After this, the samples were additionally washed with Millipore water and air dried.

Influenza virus (strain A/TW/3355/1997(H1N1)) were provided by Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of Russian Academy of Sciences. Virus stocks were propagated in the allantoic cavity of 10-day-old embryonated SPF chicken eggs (CE). CE were incubated at 37°C, cooled at 4°C 48 h post infection and harvested 16 h latter. The study design was approved by the Ethics Committee of the Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products (Approval #4 from 2 December 2014).

The structure properties of PSi NWs before and after influenza virus adsorption were studied by a scanning electron microscope (SEM) of Carl Zeiss SUPRA 40 FE-SEM, Germany, and transmission electron microscope (TEM) JEM1210, Japan. For TEM measurements the samples were placed on microscopic carbon grids and stained with 0.5% phosphotungstic acid at pH 7.0.

For the adsorption experiment, the virus containing liquids were diluted in Millipore water to achieve a final concentration of 18.6 \(\mu\)g ml\(^{-1}\). The prepared virus suspension was aerosolized with a nebulizer (Little doctor LD 212 C, Singapore) for 5 min into a closed volume of 3 liters in which the samples were placed. Then the samples were taken out and dried in air for 30 min – 1 h.

Interferometric reflectance spectra of PSi NWs were investigated by Perkin Elmer spectrometer Lambda 950, US, equipped with an integrating sphere at the spectral range from 450 to 800 nm.

3. Experimental results and discussion

SEM microphotographs of the obtained PSi NWs are shown in the figure 1. From the SEM images it can be seen that the samples have a homogeneous porous structure and consist of porous nanowires with a diameter of 50–200 nm and a distance between the nanowires of 100–200 nm. The thickness of the nanowires (\(d\)) is 200 nm. At the bottom of the samples there is a thin porous layer with the thickness of 100–200 nm. Such porous layer is often present in the MACE of highly doped c-Si \([18, 19]\). The porosity of the samples was calculated using the Bruggeman effective medium approximation \([20]\) and amounted to 42%.

Figures 2(a), (b) show SEM microphotographs of the surface of PSi NWs with different magnification after influenza H1N1 virus adsorption. Viruses are visible on the surface in the form of spheroidal white nanoparticles with a diameter of 80–100 nm. According to the obtained images, viruses bind to the porous surface of PSi NWs, and also partially penetrate into the pores between the nanowires.

TEM microphotograph of negatively stained influenza virions is shown in figure 2(c). Here, the virions look like spheroidal nanoparticles with a diameter of about 100 nm. It is clearly seen that surface of the virion is sheathed in a lipid bilayer, which is studded with the membrane glycoproteins receptors. Figure 2(d) shows TEM image of virions after interaction with PSi NWs, which were detached from the c-Si substrate. As in the SEM image, it can be seen that the virions are bind to the porous surface of the nanowires. The cause of this non-specific interaction can be the forces of Van der Waals, as well as the fact that the glycoprotein receptors of viruses can cling to the pores of the nanowires and get stuck there \([16]\).
Figure 3(a) shows reflectance spectra of PSi NWs before and after adsorption of influenza H1N1 virus. To test the effect of the suspension in which the virus was diluted, the reflectance spectra of PSi NWs were measured before and after adsorption of Millipore water (see figure 3(b)).

The spectra in figure 3(a) display a series of interference fringes. These fringes result from Fabry–Perot interference and be explained by the reflection of white light at the top and bottom interfaces of the PSi SiNW layer and is related to the effective optical thickness (EOT) of the porous layer by equation (1) [21]:

$$2dn_{eff} = m\lambda,$$

where $m$ is the spectral order, $\lambda$ is the wavelength of light, $d$ is the thickness and $n_{eff}$ is the effective refractive index of PSi NW layer (nanowire diameter and distance between the nanowires are less than the light wavelength).

From the presented spectra it can be seen the shift of the interference peaks for PSi NWs before and after virus adsorption. This can be explained by a change in the $n_{eff}$ of the samples when the virus fills the pores of PSi NWs.

Figure 1. SEM microphotographs of PSi NWs: (a) cross-sectional and (b) planar view.

Figure 2. (a), (b) SEM microphotographs of PSi NWs after virus adsorption with different magnifications; (c) TEM microphotographs of influenza H1N1 virions; (d) TEM microphotographs of PSi NWs, which were detached from the c-Si substrate, after their interaction with H1N1 virions.
neff was calculated by the distance between interference peaks ($\Delta \lambda$) and the thickness of the PSi NW layer, $d$.
Thus, initial $n_{\text{eff}(\text{ini})} = 2.1$ before the adsorption of the virus and changed to $n_{\text{eff}} = 2.3$ after the adsorption of the virus.

According to the Bruggeman effective medium approximation, after the adsorption on PSi NW layer, the degree of filling with the virus reached 25%. So, equation (2) describes PSi NWs without virus, and equation (3) describes PSi NWs after virus adsorption:

\[
\begin{bmatrix}
  f_1 \cdot \frac{\varepsilon_0 - \varepsilon_1}{\varepsilon_0 + L \cdot (\varepsilon_1 - \varepsilon_0)} + f_2 \cdot \frac{\varepsilon_0 - \varepsilon_2}{\varepsilon_0 + L \cdot (\varepsilon_2 - \varepsilon_0)} \\
  f_1 \cdot \frac{\varepsilon_0 - \varepsilon_1}{\varepsilon_0 + L \cdot (\varepsilon_1 - \varepsilon_0)} + f_2 \cdot \frac{\varepsilon_0 - \varepsilon_2}{\varepsilon_0 + L \cdot (\varepsilon_2 - \varepsilon_0)} + f_3 \cdot \frac{\varepsilon_0 - \varepsilon_3}{\varepsilon_0 + L \cdot (\varepsilon_3 - \varepsilon_0)}
\end{bmatrix} = 0
\]

(2)

\[
\begin{bmatrix}
  f_1 \cdot \frac{\varepsilon_0 - \varepsilon_1}{\varepsilon_0 + L \cdot (\varepsilon_1 - \varepsilon_0)} + f_2 \cdot \frac{\varepsilon_0 - \varepsilon_2}{\varepsilon_0 + L \cdot (\varepsilon_2 - \varepsilon_0)} + f_3 \cdot \frac{\varepsilon_0 - \varepsilon_3}{\varepsilon_0 + L \cdot (\varepsilon_3 - \varepsilon_0)}
\end{bmatrix} = 0
\]

(3)

where $\varepsilon_1 = 1$ is the permittivity of the air, $\varepsilon_2 = 11.8$ is the permittivity of the c-Si, $\varepsilon_3 = 2.2$ is the permittivity of the virus [22], $\varepsilon_0$ is an effective permittivity which is calculated from the figure 3; $L = 0.5$ is the depolarization factor for cylinder, $f_1$ is the fill factor of the air, $f_2$ is the fill factor of the c-Si, $f_3$ is the fill factor of the virus.

From equation (2), the fill factor of the air calculated, which amounted to $f_1 = 0.42$, and the fill factor of the c-Si $f_2 = 0.58$, for PSi NWs without virus ($n_{\text{eff}} = 2.1$ and $\varepsilon_0 = 4.41$). After the adsorption of the virus, $f_2$ remains the same, and from equation (3) the fill factor $f_1 = 0.17$ and the fill factor of the virus $f_3 = 0.25$ ($n_{\text{eff}} = 2.3$ and $\varepsilon_0 = 5.29$).

It is clearly seen that there were no differences in the spectra in figure 3(b) before and after adsorption of Millipore water, in which then the virus was diluted. This also confirms the fact that the change in $n_{\text{eff}}$ is associated with the binding of the virus.
The presented changes in the spectra of white light reflection from the PSi NW array after virus adsorption indicate the possibility of creating an optical interferometric sensor based on it for detecting influenza viruses. Figure 4 shows schematic of the PSi NW-based optical interferometric sensor for virus detection.

4. Conclusion

PSi NWs were fabricated by metal-assisted chemical etching of p-type highly doped crystalline silicon wafers. The obtained thin films with a layer thickness of about 200 nm have a porous structure and consist of porous nanowires with a diameter of 50–200 nm, with a distance between the nanowires of 100–200 nm. According to images obtained using a scanning and transmission electron microscope, during adsorption, viruses bind to the porous surface of PSi NWs, and also penetrate into the pores between the nanowires. The cause of this non-specific interaction can be the forces of Van der Waals, as well as the fact that the glycoprotein receptors of viruses can cling to the pores of the nanowires and get stuck there.

The reflectance spectra of the samples display a series of interference fringes, which result from Fabry–Perot interference and be explained by the reflection of white light at the top and bottom interfaces of the PSi NW layer. It is shown that the distance between the interference peaks is different for a thin PSi NW layer before and after the adsorption of the virus. The results show that thin layers of porous nanowires are a very promising nanomaterial for virus filtration as well as for creating an optical sensor for virus detection.

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