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Double etched porous silicon nanowire arrays for impedance sensing of influenza viruses

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ABSTRACT

We report new sensing element based on double-etched porous silicon (DEPSi) for sensitive detection of influenza viruses (H1N1). The proposed structure provided efficient penetration of virions into sensitive layer and trapping of them. Adsorption of the viruses led to significant shift of resonant frequency of DEPSi coupled with a coil, measured by impedance spectrometer. The detection limit of virions was lower than 100 TCID50. The results can be used for invention of H1N1 sensor, which provide rapid, label-free and low-cost detection of influenza.

1. Introduction

Influenza virus infection causes more than 9 million hospitalizations and from 0.3 to 0.6 million deaths annually in the world [1,2]. Moreover, prenatal exposure to influenza may lead to adult schizophrenia [3]. Usually, epidemic is seasonal and spreads worldwide within 2–3 weeks [4]. The severity of the disease and rapidness of its expansion require powerful low-cost express tests for early detection of the infection. Several methods including immunofluorescence assay and reverse transcription-polymerase chain reaction (RT-PCR) are widely used [5]. They are known as gold standards and provide high sensitivity and specificity, but rather expensive, long and need a professional laboratory to perform testing. These drawbacks may be crucial during rapid global epidemic, especially in developing countries suffering from low quality health care service. Therefore, new approaches to provide rapid low-cost monitoring of influenza and other viruses are under focus of intensive scientific research.

For instance, silicon chips conjugated with antibodies was used for selective sensing of influenza H3N2 viruses with sensitivity limit down to 30 viruses/μL in sputum [6,7]. Waveguide interferometer also based on silicon chip was proposed herpes simplex virus detection [8]. Although optical sensors provide good sensitivity, they usually require rather powerful light source, sophisticated design of waveguides and CCD-camera, therefore impedance sensors are preferable due to their simplicity. They even can allow continuous monitoring via wearable and flexible sensor element with wireless connection to data analyzer [9].

Porous silicon (PSi) is promising material for sensing applications, because of its high specificity area [10] and tunability of pore morphology [11]. It also allows Si integrated design for both impedance and optical sensors due to formation of PSi layer inside Si monocrystal by etching techniques [11]. It was applied for sensing of various gases [12], but biosensors based on PSi are still under development [13–15]. PSi was previously used for detection of viruses [16–18]. However, in present article its structure has been optimized for more efficient entrapping of virions by using double-etched porous silicon (DEPSi), which has pore sizes similar to virions and enhanced surface roughness desirable for binding with glycoprotein receptors [19]. Previously DEPSi was successfully applied for optical detection of bacteria [13].

2. Methods

For formation of sensing elements, electrochemical etching of silicon wafers (monocrystal with orientation – (100), specific resistivity – 1 … 5 mOhm-cm) was used. The etching was performed in a mixture of
hydrofluoric acid (HF) and ethanol (1:1 by volume) and formed PSi layer. Current density and duration of etching were 50 mA/cm², 2 min, respectively. Metal-assisted chemical etching (MACE) was used to form DEPSi: silver nanoparticles were deposited on the surface of PSi by 30 seconds of submerging of the sample in a mixture (1:1 by volume) of 5 M HF and silver nitrate (AgNO3). Then the sample was moved into a mixture of 5 M HF and H₂O₂ (30%) (1:10 by volume) for 10 min. After that silver nanoparticles were dissolved in nitric acid (HNO₃, 30%, 5 min).

Scanning electron microscopy (SEM) was employed to provide images of DEPSi. Gold top contact was deposited via resistive sputtering in high vacuum on DEPSi layer tilted at 75°. Impedance of the structure was measured by using Anritsu MS2026C vector network analyzer. Obtained dependences were post-processed and fitted by gaussians by using of MagicPlot software.

Influenza virus (strain A/PR/8/1934 (H1N1)) were provided by Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of Russian Academy of Sciences. The initial concentration of viruses in stock solution were about 10⁷ TCID₅₀ (TCID₅₀ indicates 50% tissue culture infective dose in 1 ml). For an adsorption experiment, the stock solution was diluted in 0.9% NaCl to achieve the concentrations of viruses 0.1 TCID₅₀, 100 TCID₅₀ and 1000 TCID₅₀. The prepared 1 ml virus suspension was aerosolized with a nebulizer (LD 212C, Singapore) for 5 minutes into a closed volume of 5 l.

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3. Results

Fig. 1A shows cross-sectional view of DEPSi structure consisted of 4 layers (from top to bottom): deposited gold nanoparticles providing electrical contact (white thin layer), Si nanowire layer (dark), mesoporous silicon layer (light) and Si substrate. The nanowire layer is permeable for viruses, because includes pores with diameter comparable with viruses, unlike underlaying mesoporous silicon. Rough surface of Si nanowires provides strong binding of virions to DEPSi.

Fig. 1B demonstrates adsorption of virions on DEPSi surface. The deposition of gold reduces SEM contrast dramatically, therefore the adsorption of viruses here was made instead of gold deposition. From top both small (5–20 nm) and large (100 nm) pores can be seen. Small pores were formed after first step, i.e. electrochemical etching, and they provide improved trapping of viruses on the surface due to interaction with glycoprotein receptors [19]. Large pores (dark spots) are available for penetration of virions, though SEM allows to see only ones adsorbed on the top surface.

The described DEPSi structure was used as a sensing element, which was inserted into measuring oscillating circuit in parallel to a coil with inductance incabnout 33 μH connected to impedance spectrometer. We used sandwich scheme for connection, i.e. top contact was provided by deposited gold nanolayer, while bottom contact was made by using of silver-based paste. Relatively thick (500 μm) bulk Si layer did not contribute to structure impedance, because of high doping and consequently high conductance. The active conductance of DEPSi layer was negligible, therefore impedance of the whole circuit was governed mostly by capacitance of DEPSi, which was about 1.3 nF.

In conclusion, we described new sensing element ready for detection of influenza viruses. Penetration of virions inside sensitive layer was provided by metal-assisted chemical etching, which formed pores with sizes about 100 nm. Smaller pores formed by electrochemical etching gave efficient trapping of viruses due to strong non-specific interaction with glycoprotein receptors. Variation of capacitance caused by adsorption of viruses led to shift of resonance frequency and allowed detection of viruses with concentration sufficient for tissue infection. Detection limit was 100 TCID₅₀, while the false negative rate was estimated to be 0.1 TCID₅₀. Similar approach can be used of other hazardous respiratory viruses such as coronavirus, respiratory syncytial virus, parainfluenza virus, adenovirus, rhinovirus, etc.

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Fig. 1. SEM images of DEPSi: (A) - cross-sectional view with deposited gold, (B) - top-view with adsorbed H1N1 viruses, inset shows magnification of a virus; (C) - voltage amplitude vs. frequency for DEPSi after adsorption of H1N1 viruses with different concentration (shown on legend).
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