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Biosensor based on a silicon nanowire field-effect transistor functionalized by gold nanoparticles for the highly sensitive determination of prostate specific antigen

Galina Presnova [a], Denis Presnov [b,c], Vladimir Krupenin [c], Vitaly Grigorenko [a], Artem Trifonov [b,c], Irina Andreeva [a], Olga Ignatenko [a], Alexey Egorov [a], Maya Rubtsova [a,*]

[a] Chemistry Faculty, M.V. Lomonosov Moscow State University, Leninskie gori, Moscow, 119991 Russia
[b] Skobeltsyn Institute of Nuclear Physics, M.V. Lomonosov Moscow State University, Leninskie gori, Moscow, 119991 Russia
[c] Faculty of Physics, M.V. Lomonosov Moscow State University, Leninskie gori, Moscow, 119991 Russia

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ABSTRACT

We have demonstrated label-free and real-time detection of prostate specific antigen (PSA) in human serum using silicon nanowire field effect transistors (NW FETs) with Schottky contacts (Si-Ti). The NW FETs were fabricated from SOI material using high-resolution e-beam lithography, thin film vacuum deposition and reactive-ion etching processes eliminating complicated processes of doping and thermal annealing. This allowed substantial simplifying the transistors manufacturing. A new method for covalent immobilization of half-fragments of antibodies on silicon modified by 3-glycidopropyltrimethoxysilane with thiol groups and 5 nm gold nanoparticles (GNPs) was established. NW FETs functionalized by GNPs revealed extremely high pH sensitivity of 70 mV/pH and enhanced electrical performance in the detection of antigen due to enhanced surface/volume ratio, favorable orientation of antibody active sites and approaching the source of the electric field close to the transistor surface. Si NWFETs were applied for quantitative detection of PSA in a buffer and human serum diluted 1/100. Response time was about 5–10 s, and analysis time per sample was 1 min. The limit of PSA detection was of 23 fg/mL, concentration range of 23 fg/mL–500 ng/mL (7 orders of magnitude). The PSA concentrations determined by the NW FETs in serum were compared with well-established ELISA method. The results matched well with the correlation coefficient of 0.97.

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1. Introduction

During the past decades significant improvements in miniaturization of biomedical systems resulted in the establishment of nanoscale bioelectronic devices (Zhang and Lieber, 2016). Among these devices, semiconductor nanowire field-effect transistors (NW FETs) have been attracted a particular attention due to unique electronic properties, ultra small dimensions and label-free detection in real-time mode (Cui and Lieber, 2001; Zhang et al., 2009; Noor and Krull, 2014). The use of silicon for nanosensors has the advantages provided by its features: biocompatibility, unique electronic, optical, and mechanical properties (Peng et al., 2014). The sensitivity of such biosensors is extremely high, it can reach the level from micromoles (Lin et al., 2010) to amol (Maki et al., 2008) and even allows to achieve the detection of single molecules (Hahm and Lieber, 2004; Wang et al., 2005) or particles (Patolsky et al., 2004). NW based biosensors show the advantages in the registration of proteins at extremely low concentrations which is of great importance for early diagnostics of cancer, acute myocardial infarction, and other disorders (Azmi et al., 2014; Zhang et al., 2012; Kong et al., 2012).

Despite the fact that the use of NWs for biosensors is increasingly widespread, only a few of them were applied for the detection of analytes in real biological samples like blood (A. Kim et al., 2009; Stern et al., 2010). High ionic strength of biological fluids reduces the efficiency of conductivity detection on the surface of NWs. Gao et al. (2015) have recently shown that specific modification of silicon NW by a polymer layer of polyethelene glycol increases the effective screening length of the sensor and enables detection of biomolecules in high ionic strength solutions in real-time. In this paper, we report about a new functionalization technique for silicon by small size gold nanoparticles (GNPs) for the improvement of NW FETs electrical performance as peculiar properties of GNPs such as high surface-to-volume ratio, high surface energy, and conductivity were shown to facilitate an electron transfer between biospecific layer and the electrode

* Corresponding author.
E-mail address: mrubtsova@gmail.com (M. Rubtsova).

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surface (Liu et al., 2003). In our method we used 5 nm GNPs for the covalent immobilization of half-fragments of antibodies on gold through their own thiol groups. New functionalization method was compared with chemical modification by different organosilanes. We have utilized NW FETs for the determination of prostate specific antigen (PSA), which is a molecular marker of prostate cancer. NW FET functionalized by GNPs showed the improved sensing performance and the widest detection range for PSA detection. Application of NW FET biosensors on the serum samples showed good correlation with the standard ELISA while significantly simplifying and reducing the analysis time.

2. Materials and methods

2.1. Materials

Inorganic chemicals, 3-aminopropyltrimethoxysilane (APTMS), ethylendiaminetetraacetic acid (EDTA), 3-glycidopropyltriethoxysilane (GOPS), GOPS with thiol groups (GOPS-SH), 1,4-phenylendiizothiocyanate (PDITC), tetrachloroauric (III) acid, 3,3′5,5′-tetramethylbenzidine (TMB), Tween 20, bovine serum albumin (BSA), casein were purchased from Sigma (St. Louis, USA). PSA and two clones of mouse monoclonal antibodies (mAbs) to PSA were provided by JSC "NVO Immunotek" (Moscow, Russia). MAbs and PSA standard solutions were the same which are used in ELISA kit for total PSA determination (JSC "NVO Immunotek"), registered by the Federal Service of Health Care Control of Russian Federation (Registration Certificate FSR 2008/03082, Technical Specifications TY 9398-341-11361534-2004). Specificity of mAbs towards PSA was confirmed by the manufacturer.

2.2. Preparation of GNPs

GNPs with an average diameter of 25 nm were obtained by the reduction of tetrachloroauric (III) acid with sodium citrate as described by Frens (1973). GNPs with an average diameter of 5 nm were prepared by the reduction of tetrachloroauric (III) acid with sodium borohydride in the presence of EDTA (Bogatyrev et al., 1994): 0.2 mL of a freshly prepared 1% tetrachloroauric (III) acid and 0.5 mL 0.2 M potassium carbonate were added to 45 mL of 0.3 mM EDTA at 4 °C with stirring. Then, 0.125 mL of 0.5% sodium borohydride was added, and a red-brown sol with an absorption peak of 510 nm was formed.

2.3. Fragmentation of mAbs into symmetric half-fragments

MAbs against PSA in 0.1 M phosphate buffer pH 6.0 containing 0.15 M NaCl and 5 mM EDTA were fragmented into two symmetric half-fragments using 12 mg/mL 2-MEA for 1.5 h at 37 °C and then desalted on a 15-mL Sephadex G-25 column equilibrated with 50 mM Tris pH 8.5 containing 0.15 M NaCl and 5 mM EDTA.

2.4. Conjugation of mAbs half-fragments with GNPs

A solution of second clone mAbs half-fragments (100 μM) was mixed with 1 mL suspension of 25 nm GNPs (pH 7.0 adjusted by freshly prepared Na_2CO_3) and incubated for 2 h at room temperature. Then the suspension was centrifuged at 11,000 rpm for 30 min using a 5810R centrifuge (Eppendorf, Germany). The supernatant was discarded and the pellet was dissolved in 0.01 M K-phosphate buffer with 0.15 M NaCl, pH 7.4 (PBS).

2.5. Functionalization of silicon wafers

The surface of the silicon wafers was purified with oxygen plasma (30 Pa, 25 Wt) for 30 min using a RDE-300 reactive ion-etching instrument (Alcatel, France) and then it was chemically modified by following methods:

2.5.1. Modification with APTMS and PDITC

silicon wafers were heated for 1 h at 100 °C and placed in a vessel containing a 10% APTMS in ethanol. After overnight incubation, the samples were washed three times with ethanol and then with distilled water. The dried samples were incubated in an oven at 100 °C for 10 min and then placed in a solution of 6 mg PDITC in dimethylformamide containing 10% pyridine and stirred for 1 h at room temperature. The resulting samples were washed three times with methanol and deionized water at stirring.

2.5.2. Modification with GOPS

silicon wafers were incubated in 0.2 M solution of GOPS in toluene overnight at 70 °C, then they were washed twice with toluene, methanol, and water at stirring and air dried.

2.5.3. Modification with GOPS-SH and small GNPs

silicon wafers were functionalized by GOPS-SH similarly to the method described above for the GOPS. Then a suspension of 5 nm GNPs was added, incubated overnight at 4 °C, washed twice with PBS.

2.5.4. Immobilization of mAbs

1 μL of mAbs (or their fragments) in PBS (100 μg/mL) was dropped on the modified surface of silicon wafers and incubated for 1 h at 37 °C. Then the samples were washed three times with PBS containing 0.1% Tween 20 (PBST). The surface was blocked with 1% BSA and 1% casein in PBS for 1 h at 37° C.

2.6. Characterization of modified silicon by atomic force microscopy (AFM) and sandwich immunoassay

The samples of silicon were measured using scanning probe microscope AIST-NT SPM (SmartSPM-1000) at ambient conditions. Surface scratching was made in a contact mode of AFM at constant force regime (load 45 nN, velocity 1 μm/s). Topography profiles were obtained in a tapping mode with Pt coated conductive cantilevers (Microscience, model N14/Pt) with a spring constant ranged from 6 to 10 N/m and the cantilever tips (20 nm radius).

To compare the silicon modification methods, the formation of sandwich antigen-antibody complexes was studied. Incubation of mAbs with PSA and then with a conjugate of second clone mAbs with GNPs or peroxidase was performed in PBST for 1 h at 37 °C. Antibodies nonspecific to PSA were used as control. GNPs on the silicon surface were detected by a Supra 40 field emission scanning electron microscope (Carl Zeiss, Germany) with an InLens secondary electron detector integrated in the microscope column. Activity of peroxidase was detected optically with TMB and H_2O_2 as substrates.

2.7. Fabrication of NW FETs

SOI wafers (UNIBOND® wafers, Soitec) with upper silicon layer of 110 nm and buried oxide layer of 200 nm were used for fabrication of NWs, which is described in the Supplementary materials (Fig. S1). Final structure of sensing element with contact pads covered by dielectric is shown in Fig. S2. The NW channel had a length of 3 μm and a width of about 100 nm. Then it was mounted into ceramic chip-carrier and the contacts were connected by 25 μm Al wire using ultrasonic wire bonder (WestBond, USA). Additional insulation of the contacts and connecting wires was performed by silicone sealant. Finally the sensor has the form of a small cap with the open window (about 500 μm in diameter) at

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