NANO LETTERS

2004
Vol. 4, No. 2
245−247

Sequence-Specific Label-Free DNA Sensors Based on Silicon Nanowires

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Received October 30, 2003; Revised Manuscript Received November 23, 2003

ABSTRACT

Highly sensitive and sequence-specific DNA sensors were fabricated based on silicon nanowires (SiNWs) with single stranded (ss) probe DNA molecules covalently immobilized on the nanowire surfaces. Label-free complementary (target) ss-DNA in sample solutions were recognized when the target DNA was hybridized with the probe DNA attached on the SiNW surfaces, producing a change of the conductance of the SiNWs. For a 12-mer oligonucletide probe, 25 pM of target DNA in solution was detected easily (signal/noise ratio > 6), whereas 12-mers with one base mismatch did not produce a signal above the background noise.

One-dimensional nanostructures have been demonstrated as good candidates for ultrasensitive, miniaturized molecule sensors in many applications.[1] Among the variety of systems explored, the sensors based upon semiconductor nanostructures, such as semiconductor single-wall carbon nanotubes,[2−4] silicon nanowires,[5] SnO_2 nanowires,[6] and In_2O_3 nanowires,[7] could be generally understood in terms of change of surface charge of the nanostructures with the presence or absence of molecular species. Because of the high surface-to-volume ratio of the nanostructures, their electronic conductance may be sensitive enough to the surface species that single-molecule detection becomes possible. However, most of the existing studies based on "bottom-up" nanostructures are limited by complex integration, requiring transfer and positioning of an individual nanostructure and making reliable ohmic contacts. Furthermore, the control of doping concentrations in self-assembled semiconducting nanostructures remains a challenge, and the fabrication of high-density sensor arrays is also very difficult. Here we demonstrate the detection of DNA molecules based on their intrinsic charge by using silicon nanowires (SiNWs) fabricated by standard "top-down" semiconductor processes. This method creates a pathway for fabricating high-density, high-quality nanoscale sensors that can be integrated with Si-based signal processing and communication circuits.

Si-based nanoscale sensors with a set of SiNWs 50 nm wide, 60 nm high, and 20 μm long were fabricated using silicon-on-insulator (SOI) wafers. Figure 1 shows a typical microscopic image of a sensor chip and the scanning electron microscope (SEM) images of the SiNW portion. The SOI wafers, which had a 60 nm single-crystal layer on a 200 nm thick SiO_2 insulating layer, were doped by ion implanting with boron or phosphorus to concentrations between 10^{16}/cm^3 and 10^{19}/cm^3, followed by activation at 925 °C in a N_2 ambient for 10 min (the surface was initially covered with 50 nm thermal oxide which was later removed by HF etching after the RIE step).[8] The top Si layer was then patterned by e-beam lithography (for nanowires) and optical lithography (for micron-scale electrical leads). The SiNWs and their micron-scale electrical leads were formed on the top of the SiO_2 insulating layers by reactive ion etching (RIE). To decrease the density of surface dangling bonds on the Si surface and increase the stability of the sensors, a thin SiO_2 layer with a thickness of 3 nm was grown on the Si nanowire surfaces by annealing the wafer at 900 °C in an O_2 ambient for 1 min. An aluminum layer of 100 nm thickness was deposited by e-beam evaporation on top of the Si leads after removing the 3 nm SiO_2 layer (using 1:10 HF solution) in these areas to form electrical contact with the Si and reduce the resistance of the electrical leads. The samples were then annealed in forming gas (3.8% hydrogen in nitrogen) at 450 °C for 30 min to make reliable ohmic contact between the aluminum and Si as well as to remove the interface states between the SiNWs and the thermal oxide. Finally, to eliminate any interference from the electronic leads during sensor tests, the area outside of the SiNW regions was coated with a 100 nm silicon oxide layer.[9] Thus, only the SiNWs were exposed to the environment during sensor testing.

Previously reported methods[10−12] were used to immobilize single-stranded DNA probes on the SiNWs (Figure 2). Before the surface modification, the surface of the SiNWs was treated with a water-vapor plasma. The plasma treatment (1) cleaned the sample surfaces and (2) generated a hydrophilic

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10.1021/nl034958e CCC: $27.50
Published on Web 01/08/2004

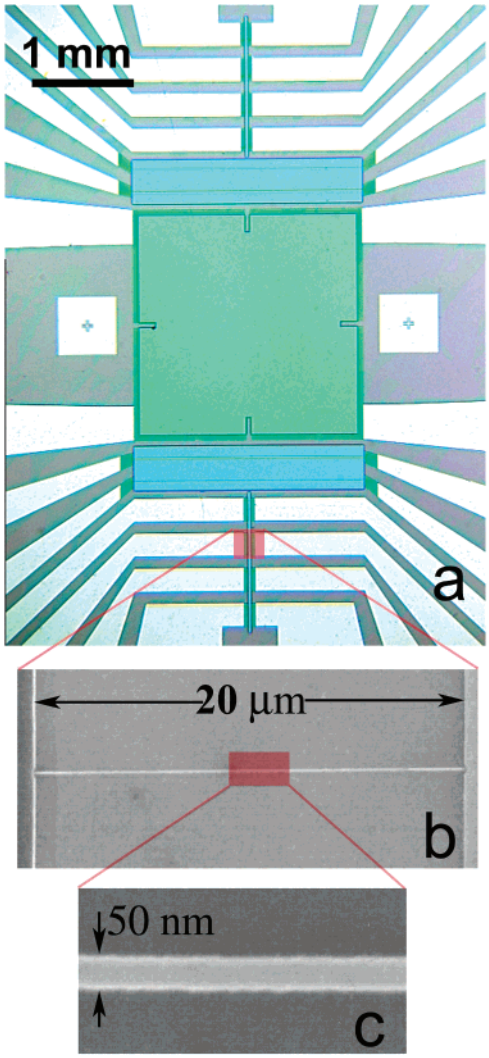

Figure 1. (a) The optical image of the central region of a sensor test chip used for the DNA sensing study. (b) and (c) Representative SEM images showing the SiNW bridging two contact leads.

surface by hydroxy-terminating the silicon oxide surfaces. A self-assembled monolayer with free thiols on the surface was then prepared using a gas-phase method.[13] The samples were exposed to the vapor of 3-mercaptopropyltrimethoxysilane (MPTMS, Aldrich, Milwaukee, WI) in argon for 4 h, followed by rinsing with absolute ethyl alcohol, and blown-dry with nitrogen. The immobilization of ss-DNA probes was achieved by exposing the MPTMS-covered samples to a 5 μM solution of the oligonucletides modified with acrylic phosphoramidite functional groups at the 5′ position (Integrated DNA Technologies, Inc., Coralville, IA) for 12 h. The immobilization of DNA probes was confirmed by attenuated total reflection infrared spectroscopy (ATR−IR) on a silicon ATR crystal (Harrick Scientific Corporation, NY). Compared with noncovalent attachment methods,[14] the covalent anchoring of oligonucleotides on the SiNW surface provided better stability and less nonspecific hybridization for DNA sensing.

The immobilization of the oligonucleotides on semiconducting silicon surfaces was independently studied by the surface photovoltage (SPV) technique.[15] A prime grade p-type Si(111) wafer (SEH brand, boron-doped, resistivity

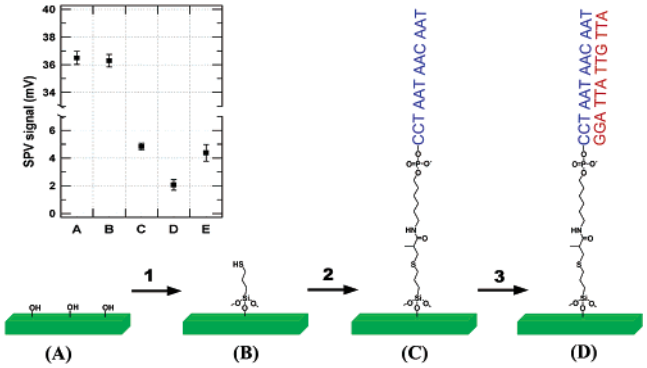

Figure 2. Modification scheme of the SiNW surface for the DNA detector: (1) self-assembly of 3-mercaptopropyltrimethoxysilane (MPTMS) by gas-phase reaction in Ar for 4 h; (2) covalent immobilization of DNA probes by exposing the previous surface to 5 μM solution of oligonucleotide CCT AAT AAC AAT modified with acrylic phosphoramidite at the 5′-end for 12 h; (3) DNA detection based on hybridization between label-free complementary DNA target GGA TTA TTG TTA and the immobilized DNA probes on the SiNW surfaces. The inset is the SPV signal on a p-type Si surface at different stages of the modification; A, B, and C correspond to the schematic diagrams, D is with 25 pM solution of complementary DNA target exposed to the surface C, and E is with 25 pM solution of noncomplementary DNA (GGA TCA TTG TTA) exposed to the surface C.

3−6 Ω cm) was cleaved into ∼1 in^2 pieces and then subjected to the surface modification steps described in Figure 2. The SPV signal was measured at different stages of the surface modification procedure as shown in the inset of Figure 2. The SPV signal of the clean Si wafer barely changed after forming the 3-mercaptopropyltrimethoxysilane (MPTMS) monolayer on the surface, whereas the SPV signal significantly decreased (∼31.5 mV) after the attachment of the DNA probes. According to Fritz et al.,[16] a change in surface potential of ∼3 mV corresponds to the binding of 3 × 10^4 12-mer oligonucleotides per μm^2. Therefore, ∼31.5 mV change should correspond to a coverage of ∼3 × 10^5 12-mer oligonucleotides per μm^2, i.e., ∼1 probe DNA per 3.3 nm^2, which is in good agreement with the footprint of a 12-mer oligonucleotide. The hybridization of the DNA probes on the surface with the complimentary DNA target further reduced the SPV signal by an additional ∼3 mV. Assuming the same effective charge on both the probe and target DNA, ∼3 mV of potential change implies a 10% efficiency of hybridization between the surface probe DNA and target DNA, which is reasonable considering the electrostatic repulsion between negatively charged oligonucleotides. For the control sample with a one base mismatch DNA, on the other hand, the SPV signal remained constant within the measurement error, indicating negligible nonspecific binding between mismatching 12-mer oligonucleotides.

For the sensitized SiNWs, sequence-specific DNA detection was achieved by monitoring the conductance of the SiNWs, while the target DNA solution was injected into a purpose-built sensor chip testing apparatus. We used only high purity water (>18 MΩ, Nanopure, Barnstead, IA) as the solvent in this study because the charge-based detection is most sensitive when counterion screening of the negatively charged DNA molecules is minimized. Figure 3 shows the

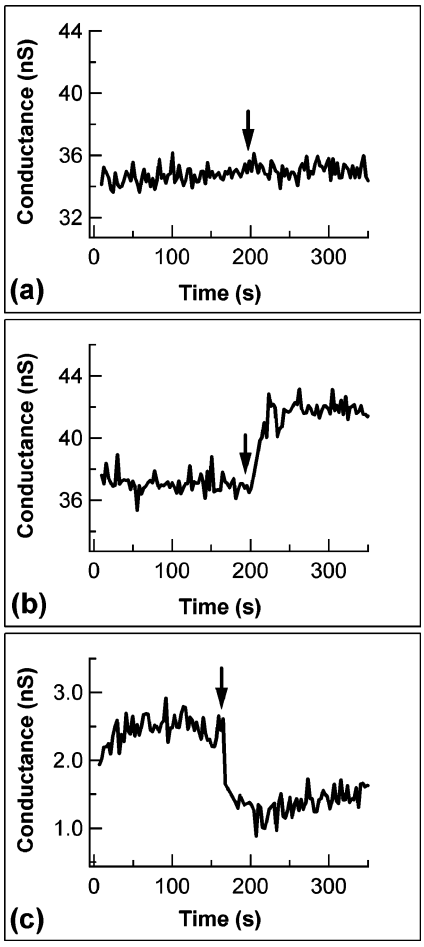

Figure 3. Real-time detection of DNA. (a) Conductance of a p-type SiNW modified with DNA probes (CCT AAT AAC AAT) versus time, where the arrow indicates the addition of 25 pM noncomplementary DNA (GGA TCA TTG TTA) solution. (b) Conductance of the same p-type SiNW shown in (a), where the arrow indicates the addition of 25 pM complementary DNA (GGA TTA TTG TTA) solution. (c) Conductance of an n-type SiNW modified with the same DNA probes as in (a) and (b), where the arrow indicates the addition of the 25 pM complementary DNA solution.

conductance traces of two representative SiNWs: (a, b) a boron-doped, p-type wire with nominal doping density of 10^{19} cm^{-3}, and (c) a phosphorus-doped, n-type sample with nominal doping density of 10^{18} cm^{-3}. Control experiments with noncomplementary DNA with a single-base mismatch did not change the conductance of the SiNWs above the noise level (as shown in Figure 3a). On the other hand, the real-time increase of the conductance of the p-type SiNW upon the addition of complementary ss-DNA was observed (as shown in Figure 3b). A similar change of the magnitude of the conductance was observed in n-type samples, but in the opposite direction (Figure 3c). When the target DNA attached to its complementary DNA on the SiNW surfaces, the increase of negative charges introduced by the DNA enhanced (or reduced) the carrier concentrations in the p-type (or n-type) SiNWs, resulting in the observed changes of the SiNW conductance. The responses of the sensors for n-type and p-type nanowires were 12% and 46%, respectively, and signal/noise ratios were 8 and 6, respectively. (Response is defined as $(I_{DNA}-I_0)/I_0$, and S/N ratio is calculated by dividing the change of the conductance by the standard deviation of the baseline.)

Compared to label-dependent DNA detecting methods, where fluorescent, chemiluminescent, redox, or radioactive labels are required for signal readout, the label-free DNA detecting method illustrated here offers the advantages of eliminating expensive labeling steps and simplifying the signal readout. Furthermore, the nanoscale features of the silicon wires fabricated by "top-down" semiconductor processing makes it possible to fabricate sensor arrays with extremely high density and direct integration with Si-based circuits.

In summary, highly sensitive and sequence-specific DNA sensors were demonstrated on silicon nanowires (SiNWs) with single stranded (ss) DNA probes covalently immobilized on their surfaces. For a 12-mer oligonucletide probe, a 25 pM solution of target DNA could be detected with excellent discrimination against single-base mismatches.

Acknowledgment. We thank Henrietta Gamino and Margie Flores for assistance with the sample processing. This work is partially supported by DARPA.

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NL034958E