NANO LETTERS

2004
Vol. 4, No. 1
51−54

Direct Ultrasensitive Electrical Detection of DNA and DNA Sequence Variations Using Nanowire Nanosensors

Jong-in Hahm and Charles M. Lieber*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

Received October 2, 2003; Revised Manuscript Received November 10, 2003

ABSTRACT

The development of electrically addressable, label-free detectors for DNA and other biological macromolecules has the potential to impact basic biological research as well as screening in medical and bioterrorism applications. Here we report two-terminal silicon nanowire electronic devices that function as ultrasensitive and selective detectors of DNA. The surfaces of the silicon nanowire devices were modified with peptide nucleic acid receptors designed to recognize wild type versus the ΔF508 mutation site in the cystic fibrosis transmembrane receptor gene. Conductance measurements made while sequentially introducing wild type or mutant DNA samples exhibit a time-dependent conductance increase consistent with the PNA–DNA hybridization and enabled identification of fully complementary versus mismatched DNA samples. Concentration-dependent measurements show that detection can be carried out to at least the tens of femtomolar range. This nanowire-based approach represents a step forward for direct, label-free DNA detection with extreme sensitivity and good selectivity, and could provide a pathway to integrated, high-throughput, multiplexed DNA detection for genetic screening and biothreat detection.

The development of advanced biological sensors could impact significantly the areas of genomics,[1,2] proteomics,[3] biomedical diagnostics,[4] and drug discovery.[5] In this regard, nanoscale sensors based on nanowires (NWs),[6] nanotubes (NTs),[7] and other nanomaterials[8−11] have received considerable recent attention. Nanoparticle labels have been used to enhance the sensitivity in surface plasmon resonance (SPR)-based detection,[9,11] including DNA sensing.[11] Metal submicron wire barcodes also have been used in conjunction with traditional fluorescent assays for DNA detection, where the barcode makes possible multiplexed detection.[10] In contrast to these optical methods, NWs and NTs can be used for label-free, direct real-time electrical detection of biomolecule binding.[6,7] NWs and NTs also have the potential for very high sensitivity detection since the depletion or accumulation of charge carriers, which are caused by binding of a charged biological macromolecule at the surface, can affect the entire cross-sectional conduction pathway of these nanostructures. While both silicon NWs (SiNWs) and NTs have been used previously for detecting biological species,[6,7] we have focused our efforts on SiNWs, since the electrical properties and sensitivity of SiNWs can be tuned reproducibly by controlling dopant concentration and NW diameter.[12−14] The modification of silicon oxide surfaces also has been well studied,[15] and this information can be exploited for tailoring SiNW surfaces with biological or chemical receptors.

Herein, we report the use of SiNWs for real-time, label-free detection of DNA and DNA mismatches. We show that

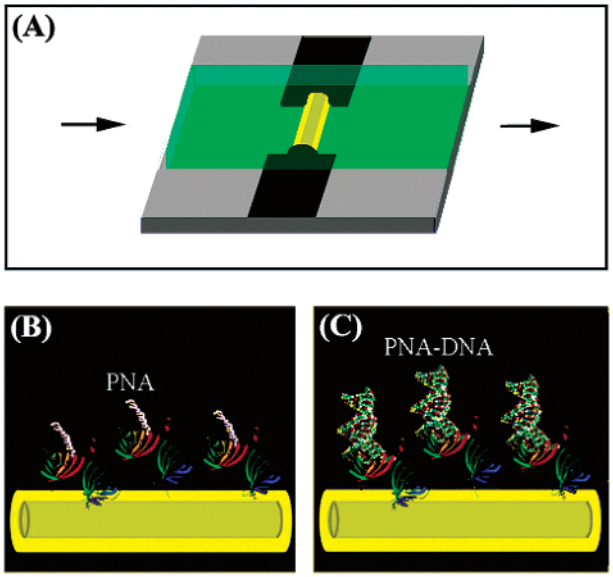

Figure 1. (A) Schematic of a sensor device consisting of a SiNW (yellow) and a microfluidic channel (green), where the arrows indicate the direction of sample flow. (B) The SiNW surface with PNA receptor. (C) PNA−DNA duplex formation.

SiNW sensors functionalized with peptide nucleic acid (PNA) receptors can distinguish wild-type from the ΔF508 mutation site in the cystic fibrosis transmembrane receptor (CFTR) gene. Cystic fibrosis is one of the most common fatal genetic diseases among populations of European origin and affects

10.1021/nl034853b CCC: $27.50
Published on Web 12/09/2003

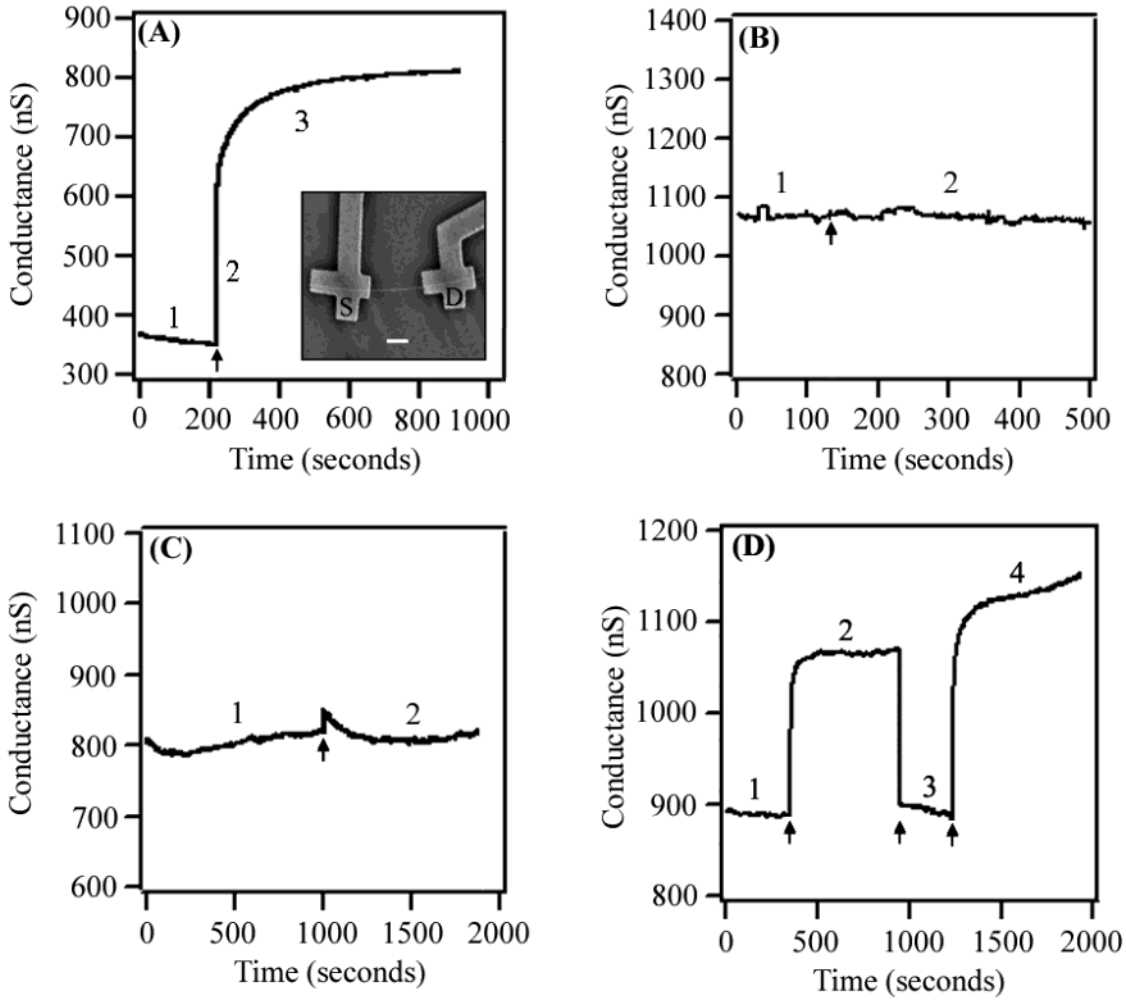

Figure 2. (A) Real-time conductance response from a SiNW device functionalized with PNA receptor. The arrow marks the point in time when the 60 fM WT DNA sample was added. The inset shows a SEM image of a typical SiNW device with source (S) and a drain (D) indicated; scale bar is 1 μm. (B) Time dependent conductance in DNA-free solution; the arrow indicates the point in time when a new solution sample was added. (C) Conductance versus time for SiNW with linked PNA receptor in the presence of 100 fM MU DNA sample (1) and following addition (arrow) of a second 100 fM MU DNA sample. (D) Conductance versus time for PNA-functionalized NW device during flow of DNA-free solution (1), 100 fM MU DNA (2), DNA-free solution (3), and 100 fM WT DNA (4).

approximately one in 3900 live births in the U.S.[16] The ΔF508 mutation is responsible for ~75% of the cases of the disease[17,18] and consists of three missing bases, CTT, which lead to the deletion of the 508th codon.[17] The detection of the presence or absence of the ΔF508 mutation thus serves as an indicator for this disease. More generally, the development of sensors capable of the facile detection of DNA sequence variations could enable genetic risk assessment for disease.

The p-type SiNWs used in these studies were synthesized using a gold nanocluster catalyzed chemical vapor deposition (CVD) approach described previously.[19] SiNWs were assembled into sensor devices consisting of electrically addressable NWs with PNA surface receptors, where the receptors were linked to the NW surface using intervening avidin protein layer, and a microfluidic sample delivery structure (Figure 1).[20] PNA was chosen as a recognition sequence in our studies, since it is known to bind to DNA with much greater affinity and stability than corresponding DNA recognition sequences, and has shown binding with single base specificity.[21,22] A PNA probe with the sequence 5′-ATCATCTTTG-3′, which is fully complementary to the wild type (WT) CFTR sequence, was used as the receptor for detection of WT and mutant (MU) oligonucleotide sequences spanning the ΔF508 region. The WT and MU DNA sequences are 5′-CATAGGAAACACCAAAGATGATATTTTCTTT-3′ and 5′-CATAGGAAACACCAATGATATTTTCTTT-3′, respectively.[23]

Conductance versus time data recorded with a typical SiNW device prepared in this way (Figure 2A) show an increase in conductance following addition of a 60 femtomolar (fM) WT DNA sample solution. This conductance change includes a rapid (<10 s) increase (part 2, Figure 2A) followed by a longer time scale increase (part 3) occurring over hundreds of seconds. The increase in conductance for the p-type SiNW device is consistent with an increase in negative surface charge density[6] associated with binding of negatively charged oligonucleotides at the surface. Several control experiments have been carried out to assess further the nature of the observed change. First, addition of solution

without either WT or MU DNA exhibited no substantial change in conductance (Figure 2B). Second, no substantial long-term conductance change was observed when a 100 fM solution of MU DNA sample was introduced into a device following initial hybridization with complementary WT sequence (Figure 2C). The small transient conductance increase from the MU sample suggests that there can be nonspecific interaction of negatively charged oligonucleotides with NW sensors, although this nonspecific interaction is reversible and can be quantified (Figure 2D). Specifically, the addition of 100 fM MU DNA solution to a PNA-functionalized NW device that has not been exposed to WT DNA leads to a rapid increase in conductance (Figure 2D, part 2), and then the conductance decreases rapidly to the baseline when DNA-free solution is added (Figure 2D, part 3). Finally, when 100 fM WT DNA solution is added, the conductance increases over a longer time scale, as discussed above, to a level that remains unchanged. These results demonstrate that the longer term increase in conductance is unique to the fully complementary WT DNA samples, and thus that this conductance increase can be used as a measure of WT DNA-to-PNA receptor duplex formation at SiNW surfaces.

The specific conductance changes due to PNA−DNA hybridization were obtained from the time-dependent conductance recorded following introduction of WT and MU DNA samples with the same SiNW device. Direct comparison of these data (Figure 3A) highlights the substantial net conductance change associated with hybridization of the DNA complementary to the PNA receptor. A summary of concentration-dependent data recorded in this way (Figure 3B) demonstrates that the WT DNA can effectively and selectively detect concentrations as low as ca. 10 fM with the devices used in this study without labeling and in real-time. Significantly, this current detection limit demonstrated in our studies is substantially better than that demonstrated by existing real-time measurements, including SPR,[22] nanoparticle-enhanced SPR,[11] and quartz-crystal microbalance[24] for DNA detection.

In addition, there are several other features of the nanowire DNA sensors that deserve further comment. First, the 10 fM detection limit determined for these studies is not an absolute limit since it should be possible to improve electrical sensitivity of SiNWs through variations in effective dopant concentration and improvements in the contacts.[25] The detection limit in a equilibrium binding assay will be limited ultimately by the binding constant of the recognition sequence with the DNA sample being tested, although this does not represent a fundamental detection limit, since nanowires with sufficient sensitivity could be operated in a stochasitic or single-molecule detection mode. Second, the plots of the conductance change as a function of WT DNA concentration for two independent devices (Figure 3C) show a very similar systematic increase in net conductance with increasing DNA concentrations. This device-to-device reproducibility is an important validation of the potential of the SiNWs for development as integrated sensors in the future, and differs from results reported for NT sensors where

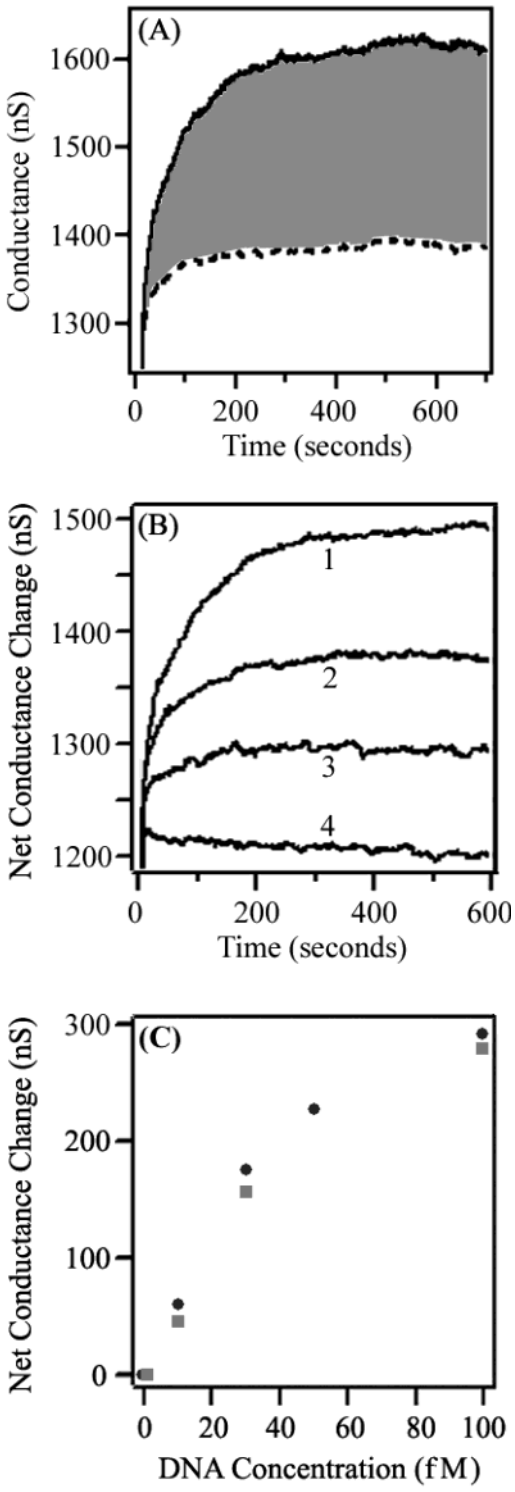

Figure 3. (A) Conductance graphs obtained from 100 fM of WT (solid line) and MU (dashed line) fragments on the same subject NW. (B) Net conductance changes vs time for 100 fM (1), 30 fM (2), 10 fM (3) and 1 fM (4) DNA samples. (C) Conductance vs DNA concentration where data points shown as squares and circles were obtained from two independent SiNW devices.

mixture of semiconducting and metallic material is believed to affect individual device behavior.[7]

In summary, we have demonstrated that p-type SiNWs functionalized with PNA receptors function as ultrasensitive and selective real-time DNA sensors at concentrations down

to tens of femtomolar range. We have shown that the SiNW devices could distinguish the WT versus MU DNA sequences associated with the ΔF508 mutation site in the cystic fibrosis transmembrane receptor gene, and that this specific response was reproducible for different devices. More generally, it should be possible to extend this approach to the detection of other genetic markers of diseases, and, by exploiting recent advances in large-scale assembly,[26] we believe it will be possible to develop and study highly integrated NW sensor arrays that could enable high-throughput, highly sensitive DNA detection for basic biology research and genetic screening.

Acknowledgment. We acknowledge Y. Cui, D. Keen, and L. Lauhon for helpful discussions and thank Y. Cui for synthesizing the silicon nanowires, and D. Keen and D. Housman for providing PCR amplified CFTR samples used in preliminary experiments. C.M.L. acknowledges support of this work by the Defense Advanced Research Projects Agency, National Cancer Institute and Ellison Medical Foundation.

References

(1) Ramsay, G. *Nature Biotech.* **1998**, *16*, 40.
(2) Shoemaker, D. D.; Schadt, E. E.; Armour, C. D.; He, Y. D.; Garrett-Engele, P.; McDonagh, P. D.; Loerch, P. M.; Leonardson, A.; Lum, P. Y.; Cavet, G.; Wu, L. F.; Altschuler, S. J.; Edwards, S.; King, J.; Tsang, J. S.; Schimmack, G.; Schelter, J. M.; Koch, J.; Ziman, M.; Marton, M. J.; Li, B.; Cundiff, P.; Ward, T.; Castle, J.; Krolewski, M.; Meyer, M. R.; Mao, M.; Burchard, J.; Kidd, M. J.; Dai, H.; Phillips, J. W.; Linsley, P. S.; Stoughton, R.; Scherer, S.; Boguski, M. S. *Nature* **2001**, *409*, 922.
(3) MacBeath, G.; Schreiber, S. L. *Science* **2000**, *289*, 1760.
(4) Alizadeh, A. A.; Eisen, M. B.; Davis R. E.; Ma, C.; Lossos, I. S.; Rosenwald, A.; Boldrick, J. C.; Sabet, H.; Tran, T.; Yu, X.; Powell, J. I.; Yang, L.; Marti, G. E.; Moore, T.; Hudson, J., Jr.; Lu, L.; Lewis, D. B.; Tibshirani, R.; Sherlock, G.; Chan, W. C.; Greiner, T. C.; Weisenburger, D. D.; Armitage, J. O.; Warnke, R.; Levy, R.; Wilson, W.; Grever, M. R.; Byrd, J. C.; Botstein D.; Brown, P. O.; Staudt, L. M. *Nature* **2000**, *403*, 503.
(5) Scherf, U.; Ross, D. T.; Waltham, M.; Smith, L. H.; Lee, J. K.; Tanabe, L.; Kohn, K. W.; Reinhold W. C.; Myers, T. G.; Andrews, D. T.; Scudiero, D. A.; Eisen, M. B.; Sausville, E. A.; Pommier, Y.; Botstein, D.; Brown, P. O.; Weinstein, J. N. *Nature Genet.* **2000**, *24*, 236.
(6) Cui, Y.; Wei, Q.; Park, H.; Lieber, C. M. *Science* **2001**, *293*, 1289.
(7) Chen, R. J.; Bangsaruntip, S.; Drouvalakis, K. A.; Kam, N. W. S.; Shim, M.; Li, Y.; Kim, W.; Utz, P. J.; Dai, H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4984. Star, A.; Gabriel, J.-C. P.; Bradley, K.; Gruner, G. *Nano Lett.* **2003**, *3*, 459.
(8) Park, S.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1703.
(9) McFarland, A. D.; Van Duyne, R. P. *Nano Lett.* **2003**, *3*, 1057.
(10) Nicewarner-Pena, S. R.; Griffth-Freeman, R.; Reiss, B. D.; He, L.; Pena, D. J.; Walton, I. D.; Cromer, R.; Keating, C. D.; Natan, M. J. *Science* **2001**, *294*, 137.
(11) He, L.; Musick, M. D.; Nicewarner, S. R.; Salinas, F. G.; Benkovic, S. J.; Natan, M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 9071.
(12) Cui, Y.; Duan, X.; Hu, J.; Lieber, C. M. *J. Phys. Chem. B* **2000**, *104*, 5213.
(13) Cui, Y.; Lieber, C. M. *Science* **2001**, *291*, 851.
(14) Cui, Y.; Lauhon, L. J.; Gudiksen, M. S.; Wang, J.; Lieber, C. M. *Appl. Phys. Lett.* **2001**, *78*, 2214.
(15) Iler, R. K. *The Chemistry of Silica*; Wiley: New York, 1979.
(16) Cystic Fibrosis Foundation, http://www.cff.org.
(17) Rommens, J. M.; Iannuzzi, M. C.; Kerem, B.; Drumm, M. L.; Melmer, G.; Dean, M.; Rozmahel, R.; Cole, J. L.; Kennedy, D.; Hidaka, N.; Zsiga, M.; Buchwald, M.; Riordan, J. R.; Tsui, L.; Collins, F. S. *Science* **1989**, *245*, 1059.
(18) Cystic Fibrosis Mutations Database, http://www.genet.sickkids.on.ca/cftr/.
(19) SiNWs used in the current work were boron-doped using a SiH_4/B_2H_6 ratio of 4000:1 during growth. SiNWs with an average diameter of 20 nm were suspended in ethanol and deposited on oxidized Si substrates. Electrical contacts were made to NW ends using electron-beam lithography, followed by Ti/Au (50/50 nm) metallization. Linear current (*I*) versus voltage (*V*) behavior was observed for all devices, and these devices had conductance values between 200 and 1700 nS.
(20) SiNW devices were cleaned using an oxygen plasma etcher (20 W for 30 s at 0.5 Torr with the O_2 flow rate of 250 sccm, Anatech Ltd., SP100, Springfield, VA). A PDMS microfluidic channel was fabricated using a standard photolithography with channel dimensions of 500 μm × 3 mm. Biotinyl *p*-nitrophenyl ester, pyridine (99.9%), and 4-(dimethylamino)pyridine (DMAP) were purchased from Aldrich (St. Louis, MO). Immediately after the plasma cleaning, SiNWs were incubated overnight at room temperature in 10 mL pyridine solution containing 10 mg of biotinyl *p*-nitrophenyl ester and 5 mg of DMAP. SiNWs were subsequently rinsed three times with pyridine and the microfluidic channel was placed on the device for further NW surface modification and sample introduction. A 30 μg/mL avidin solution in PBS, pH 7, was introduced for 30 min to terminate the NW surface with avidin. The avidin-modified NW was further linked with biotinylated PNA probes by adding 100 μmol of PNA capture probes for 30 min.
(21) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.
(22) Jensen, K. K.; Orum, H.; Nielsen, P. E.; Norden, B. *Biochem.* **1997**, *36*, 5072.
(23) PNA probes with WT base sequence of ΔF508 in the CFTR gene, 5′-ATCATCTTTG-3′, were purchased from PE Applied Biosystems (Branchburg, NJ) and resuspended in distilled water. The capture PNA probes were biotinylated at the 5′ end. Single-stranded DNA pieces containing WT and MU ΔF508 in the CFTR gene, 5′-CATAGGAAACACCAAAGATGATATTTTCTTT-3′ and 5′-CATAGGAAACACCAATGATATTTTCTTT-3′ respectively, were obtained from Operon Technologies Inc. (Qiagen, San Francisco, CA). The oligonucleotides were reconstituted in nuclease-free water (Promega, Milwaukee, WI) at pH 7.
(24) Hook, F.; Ray, A.; Norden, B.; Kasemo, B. *Langmuir* **2001**, *17*, 8305.
(25) Cui, Y.; Zhong, Z.; Wang, D.; Wang, W. U.; Lieber, C. M. *Nano Lett.* **2003**, *3*, 149.
(26) Whang, D.; Jin, S.; Wu, Y.; Lieber, C. M. *Nano Lett.* **2003**, *3*, 1255.

NL034853B