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Diamond Nanowires

Vertically Aligned Diamond Nanowires for DNA Sensing**

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Nanowires from Si, SiO_2, gold, glassy carbon, SnO_2, and ZnO_2 by bottom-up and top-down techniques have attracted much attention recently.[1] These nanowires have been used for chemical/biochemical sensing applications because of their low weight, as well as the sometimes extraordinary mechanical, electrical, thermal, and multifunctional properties.[2] However, these nanowires do not possess desired chemical stability and reproducibility of biochemical surfaces in electrolyte solutions. Significant improvements in the sensitivity, selectively, parallelism, chemical stability, and biocompatibility towards analytes in solutions are needed.

Diamond is a promising choice for the next generation sensor platforms[3] because of its chemical stability, low background current, and wide potential window. Diamond survives in harsh environments in which other materials such as ZnO_2, SnO_2, and Si fail. Diamond varies from an insulating, to semiconducting, to metal-like conducting materials with increasing doping levels. Surface termination with hydrogen and oxygen also allows the optimization of electronic properties of diamond electrodes. Diamond is also biocompatible towards large biomolecules, such as DNA.[4]

Diamond nanowires were first realized in 1997 by Shiomi,[5] who demonstrated the formation of porous diamond films by reactive ion etching (RIE) using O_2. Later, in 2000, nanostructured diamond honeycomb films were prepared[6] by etching through a porous anodic alumina mask; the work triggered by these results are summarized by Shenderova et al.[7] Growth-induced formation of nanoscale tubular structures by applying a microwave plasma of hydrogen under a bias potential was first reported in 2003.[8] In 2008, Zou et al.[9] reported the fabrication of nanopillar arrays using self-aligned Au nanodots as an etching mask in bias-assisted reactive ion etching with a hydrogen/argon plasma. Although these achievements demonstrate that vertically aligned diamond nanowires can be fabricated by a variety of methods, no applications in electro- or biochemistry have been reported.

Herein, we introduce for the first time the electrochemical application of vertically aligned diamond nanowires for DNA sensing. This new technological pathway marries major advantages of diamond such as chemical stability, biocompatibility, and hardness with geometrically controlled bonding of DNA molecules to realize behavior of the DNA molecules' "like-in-solution" situation. These metal-like nanowires are fabricated from boron-doped single-crystalline CVD diamond (produced by chemical vapor deposition) by use of diamond nanoparticles as a hard mask and by use of RIE in O_2/CF_4 gas mixture for 10 s.[10] These wires are 3–10 nm long and typically spaced 11 nm apart (Figure 1). Nanowires

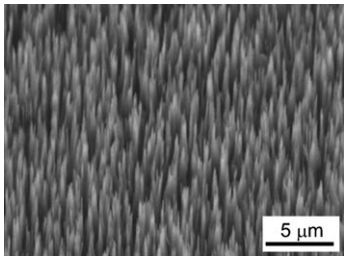

Figure 1. SEM image of vertically aligned diamond nanowires.[10]

separated by approximately 11 nm were selected because anchoring DNA molecules onto these wires will result in a density of DNA of about $10^{12}\,cm^{-2}$, which is promising for DNA sensing with high efficiency. The tips of the nanowires were functionalized electrochemically[11] with phenyl groups.[10] Such functionalized nanowires are used to bond geometrically controlled oligonucleotide molecules to diamond. As DNA self-aligns with the phenyl linker groups, functionalization of the nanowire tips produces a pattern of dispersed DNA bonding governed by the nanowires' structure.

Redox indictors such as $[Fe(CN)_6]^{3-/4-}$ and intercalators such as metal complexes have been widely used for the investigation of DNA sensing on gold and other substrate electrodes. However, no work has been published on diamond-based biosensors that use redox indicators. Herein, we focus of sensing using $[Fe(CN)_6]^{3-/4-}$ as a redox mediator. As shown in Figure 2, the peak currents and peak potential changed remarkably before and after immobilization of probe DNA. The broad peak splitting on unmodified diamond nanowires has been assigned to surface oxidation during fabrication of the diamond nanowires as well as to a relatively low boron doping concentration of the diamond bulk ($7 \times 10^{19}\,cm^{-3}$). Hybridization of target DNA into probe DNA coated diamond nanowires results in a further decrease in peak currents and an enhancement of potential splitting of peak potentials. Figure 2 also shows a cyclic voltammogram (m-cDNA) after exposure to single-base-mismatched DNA. The amplitude is decreased by about 20% relative to single

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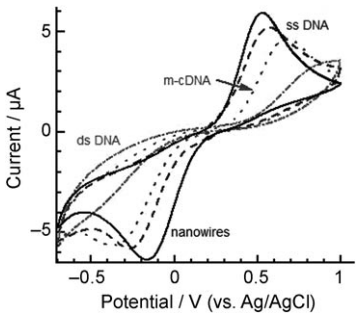

Figure 2. Cyclic voltammograms of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in pH 7.4 phosphate buffer on nanowires before (——) and after functionalization with ss-DNA (-----), single-base-mismatched DNA (m-cDNA) (•••••), and ds-DNA (–•–•). The scan rate was 100 $mV s^{-1}$.

strand DNA (ss-DNA) owing to nonintentional bonding. However, clear discrimination between complementary (ds-DNA) and single-base-mismatched DNA bonding is detected. These results clearly demonstrate that $[Fe(CN)_6]^{3-/4-}$ is a good indicator for DNA bonding to diamond.

Electrochemical detection of DNA hybridization events was then conducted with $[Fe(CN)_6]^{3-/4-}$ as indicator. Figure 3 shows typical examples of cyclic voltammetric (CV) response

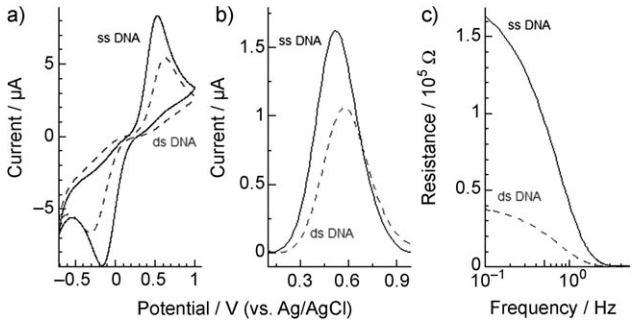

Figure 3. Typical examples of detection of DNA hybridization by cyclic voltammetry (a), differential pulse voltammetry (b), and electrochemical impedance microscopy (c). The concentration of target DNA was 10 nM.

(scan rate: 100 $mV s^{-1}$), differential pulse voltammetric (DPV) signals (scan rate: 100 $mV s^{-1}$, pulse height: 2.5 mV), and electrochemical impedance spectra (Imp) in the regime 0.01 to 10^6 Hz at −0.3 V (vs. Ag/AgCl) in pH 7.4 phosphate buffer as applied on marker DNA (ss-DNA) coated nanowires and after exposure to 10 nM complementary DNA for 1 h (ds-DNA). Hybridization leads to a decrease in resistance of DNA, which agrees with the results on diamond,[12,13] but conflicts with our voltammetric results. The reason for this discrepancy is still unclear and is under investigation in our laboratory. Large amplitude variations in peak current (a, b) and resistance (c) were detected before and after hybridization. The differences before and after DNA hybridization in peak currents from cyclic voltammograms and differential pulse voltammograms and from electrochemical impedance spectra were adopted for the detection of DNA sequence.

Sensitivity curves for DNA hybridization were measured by varying the concentration of complementary target DNA from 1 μM to 10 pM. The overall performance of the diamond nanowire DNA sensor is compared with published data for Au,[14–17] and polycrystalline diamond[13] using comparable DNA structures in Figure 4a. Sensing with diamond nanowires is about 100 to 1000 times better than with smooth

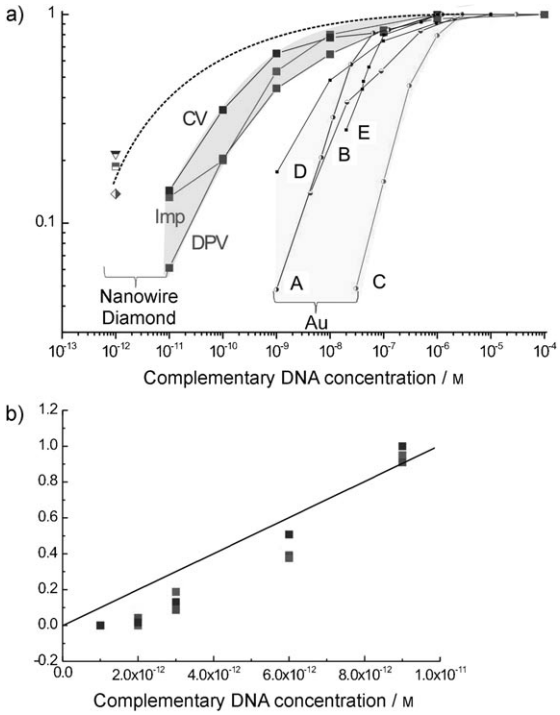

Figure 4. a) Sensitivity curves of diamond nanowires compared with those of gold electrodes (from references [14] (A), [15] (B), [16] (C), and [17] (D)) and diamond[13] (E). b) Detection limit measured on diamond nanowires.

surfaces of Au or diamond. To identify the sensitivity limit exactly, experiments with between 0 and 10 pM of complementary DNA were performed, and the results (Figure 4b) indicate a sensitivity limit of around 2 pM. No degradation of the DNA on the nanowires was detected over 30 cycles of DNA hybridization/denaturation, which is comparable with the chemical stability of optical DNA biosensors from diamond.[4]

In summary, we introduce vertically aligned diamond nanowires for biochemical sensing. Diamond nanowires combine the outstanding electrochemical properties of diamond as a transducer with the advantages of dispersed, controlled binding of linker molecules to realize behavior of DNA molecules' "like-in-aqueous solution" situation. Significant improvements of the sensitivity and chemical stability were obtained, which are required to meet future needs in various fields. Biosensors from diamond nanowires can be recycled to restore the sensitivity by simple chemical cleaning and chemical functionalization without the need for full solid-state sensor production. Such robust devices will perfectly suit demands in high-throughput systems of clinical environments.

Experimental Section

All chemical were analytical grade and used as received. An Autolab 2667 computer-controlled potentiostat (Ecochemie, Utrecht, The Netherlands) was used for electrochemical experiments in a three-electrode configuration with a platinum counterelectrode and an Ag/Ag^+ (0.01 M) reference electrode in organic solvents or an Ag/AgCl (3 M) reference electrode in aqueous solutions.

Electrochemically induced covalent attachment of nitrophenyl molecules to diamond nanowires was performed in 1.0 mM 4-nitrobenzene diazonium in dehydrated acetonitrile ($H_2O < 50$ ppm) containing 0.1 M tetrabutylammonium tetrafluoroborate at −0.05 V (vs. Ag/Ag^+) for 4 s in a N_2-purged glove box. Subsequently, 4-nitrophenyl groups ($-C_6H_5NO_2$) were electrochemically reduced to aminophenyl groups ($-C_6H_5NH_2$) in 0.1 M KCl solution in $EtOH/H_2O$ (1:9).[11] The aminophenyl layer was then treated with 14 nM solution of the heterobifunctional cross-linker sulphosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate in 0.1 M pH 7 triethanolamine (TEA) buffer for 20 min at room temperature in a humid chamber. The ester group in this molecule reacts specifically with the $-NH_2$ groups of the linker molecules to form amide bonds. The maleimide moiety was then treated with 2–4 μL thiol-modified DNA (300 μM thiol DNA in 0.1 M pH 7 TEA buffer) by placing the DNA directly onto the surface in a humid chamber and allowing it to react for 1 h at room temperature. As marker DNA, we used the 23-mer cancer marker cytokeratin 20 (CK20: HS-C_6H_{12}-T_9-CTG TTT TAT GTA GGG TTA GGT CA) and as target the complementary sequence 5′Cy5-TGA CCT AAC CCT ACA TAA AAC AG-3′ (Cy5 indicates the presence of a red fluorescence marker). The sequence of single-base-mismatched DNA (m-cDNA) is TGA CCT AAC C*A*T ACA TAA AAC AG.

For hybridization of DNA, SSPE buffer (saline sodium phosphate EDTA; 5 μL) containing complementary DNA was placed on the sensor surface for 1 h at 20 °C in a humid cell. The density of complementary DNA was varied from 10 μM to 1 pM to investigate sensitivity properties of the sensor. The sensitivity limits were measured in 100 μL of SSPE. After hybridization, the samples were washed in de-ionized water for 1 h at 37 °C to remove nonintentionally bonded DNA molecules. Stability measurements were performed using 1 μM solutions over extended cycles of hybridization/denaturation treatment. Denaturation was performed in 8.3 M urea solution for 30 min at 37 °C, followed by rinsing in de-ionized water.

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[1] a) T. Strother, W. Cai, X. S. Zhao, R. J. Hamers, L. M. Smith, *J. Am. Chem. Soc.* **2000**, *122*, 1205 – 1209; b) K. Hashimoto, K. Ito, Y. Ishimori, *Anal. Chem.* **1994**, *66*, 3830 – 3833; c) K. M. Millan, A. J. Spurmanis, S. R. Mikkelsen, *Electroanalysis* **1992**, *4*, 929 – 932; d) F. J. Yusta, M. L. Hitchman, S. H. Shamlian, *J. Mater. Chem.* **1997**, *7*, 1421 – 1427; e) S. S. A. Elrehim, S. M. A. Elwahab, E. E. Fouad, H. H. Hassan, *Mater. Corros.* **1995**, *46*, 633 – 638.

[2] a) G. Zheng, F. Patolsky, Y. Cui, W. U. Wang, C. M. Lieber, *Nat. Biotechnol.* **2005**, *23*, 1294 – 1301; b) Y. Cui, Q. W. Wei, H. Park, C. M. Lieber, *Science* **2001**, *293*, 1289 – 1292.

[3] a) J. C. Angus, Y. V. Pleskov, S. C. Eaton in *Thin Film Diamond II, Semiconductors and Semimetals*, *Vol. 77* (Eds.: C. E. Nebel, J. Ristein), Elsevier, Tokyo, **2004**, p. 97; b) G. M. Swain in *Thin Film Diamond II, Semiconductors and Semimetals*, *Vol. 77* (Eds.: C. E. Nebel, J. Ristein), Elsevier, Tokyo, **2004**, p. 121; c) C. E. Nebel, B. Rezek, D. Shin, H. Uetsuka, N. Yang, *J. Phys. D* **2007**, *40*, 6443 – 6466.

[4] W. Yang, O. Auciello, J. E. Butler, W. Cai, J. A. Carlisle, J. E. Gerbi, D. M. Gruen, T. Knickerbocker, T. L. Lasseter, J. N. Russell Jr, L. M. Smith, R. J. Hamers, *Nat. Mater.* **2002**, *1*, 253 – 257.

[5] H. Shiomi, *Jpn. J. Appl. Phys.* **1997**, *36*, 7745 – 7748.

[6] H. Masuda, M. Watanabe, K. Yasui, D. Tryk, T. Rao, A. Fujishima, *Adv. Mater.* **2000**, *12*, 444 – 447.

[7] O. A. Shenderova, C. W. Padgett, Z. Hu, D. W. Brenner, *J. Vac. Sci. Technol. B* **2005**, *23*, 2457 – 2464.

[8] K. Kobashi, T. Tachibana, Y. Yokota, N. Kawakami, K. Hayashi, K. Yamamoto, Y. Koga, S. Fujiwara, Y. Gotoh, H. Nakahara, H. Tsuji, J. Ishikawa, F. A. Köck, R. J. Nemanich, *J. Mater. Res.* **2003**, *18*, 305 – 326.

[9] Y. S. Zou, T. Yang, W. J. Zhang,, Y. M. Chong, B. He, I. Bello, S. T. Lee, *Appl. Phys. Lett.* **2008**, *92*, 053105.

[10] N. Yang, H. Uetsuka, E. Osawa, C. E. Nebel, *Nano Lett.*, submitted.

[11] a) H. Uetsuka, D. Shin, N. Tokuda, K. Saeki, C. E. Nebel, *Langmuir* **2007**, *23*, 3466 – 3472; b) D. Shin, N. Tokuda, B. Rezek, C. E. Nebel, *Electrochem. Commun.* **2006**, *8*, 844 – 850.

[12] a) V. Vermeeren, N. Bijnens, S. Wenmackers, M. Daenen, K. Haenen, O. A. Williams, M. Ameloot, M. vandeVen, P. Wagner, L. Michiels, *Langmuir* **2007**, *23*, 13193 – 13202; b) W. Yang, J. E. Butler, J. N. Russell, R. J. Harmers, *Langmuir* **2004**, *20*, 6778 – 6787.

[13] H. Gu, X.-d. Su, K.-P. Loh, *J. Phys. Chem. B* **2005**, *109*, 13611 – 13618.

[14] G. Carpini, F. Lucarelli, G. Marrazza, M. Mascini, *Biosens. Bioelectron.* **2004**, *20*, 167 – 175.

[15] O. Paenke, A. Kirbs, F. Lisdat, *Biosens. Bioelectron.* **2007**, *22*, 2656 – 2662.

[16] J. Kafka, O. Paenke, B. Abendroth, F. Lisdat, *Electrochimica Acta* **2008**, DOI: 10.1016/j.electacta.2008.01.031.

[17] H. Aoki, H. Tao, *Analyst* **2005**, *130*, 1478 – 1482.

