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ARTICLE *in* NANO LETTERS · SEPTEMBER 2004

Impact Factor: 13.59 · DOI: 10.1021/nl048995x

NANO LETTERS

2004
Vol. 4, No. 9
1713−1716

Electrically Addressable Biomolecular Functionalization of Carbon Nanotube and Carbon Nanofiber Electrodes

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Received June 28, 2004

ABSTRACT

We demonstrate the electrically addressable biomolecular functionalization of single-walled carbon nanotube electrodes and vertically aligned carbon nanofiber electrodes. The method uses an electrochemical reaction in which nitro groups on specific nanostructures are reduced to amino groups and then used to covalently link DNA to only these nanostructures. We demonstrate fabrication of a four-element array of distinct DNA oligonucleotides on carbon nanotube electrodes and the addressable functionalization of submicron bundles of <100 nm diameter vertically aligned carbon nanofibers. DNA hybridization shows that the DNA-modified nanoscale structures have excellent biological selectivity.

One of the major goals of nanotechnology is the fabrication of high-density biosensor arrays composed of nanotubes, nanowires, or nanofibers, each modified with distinct biomolecular recognition elements.[1] Carbon nanotubes and nanofibers are especially attractive candidates for nanoscale biosensing systems because of their exceptional chemical and biochemical stability.[2−4] While methods have been developed for growing single-walled carbon nanotubes (SWNTs)[5] and vertically aligned carbon nanofibers (VACNFs)[6] on surfaces, there has remained an unmet need for methods able to chemically modify closely spaced electrodes. Here, we demonstrate how a combination of chemical and electrochemical reactions can be used to achieve electrically addressable DNA modification of carbon nanotube electrodes and of submicron bundles of VACNFs. Our results provide a general and direct pathway for fabricating high-density arrays of biosensor elements using nanoscale components such as nanotubes and nanofibers tethered to highly specific biomolecular recognition elements such as DNA, proteins, and antibodies.

As schematically illustrated in Figure 1, the heart of this scheme is the functionalization of the SWNTs/VACNFs with nitro groups, followed by the "addressable" electrochemical reduction of the nitro groups to amino groups on SWNTs/VACNFs of interest. The use of an electrochemical reaction to control the functionalization is key, as it provides a way to electrically address individual sensor elements within an array. Since the electron-transfer step in electrochemistry can typically only occur for species within 1 nanometer of an electrode surface,[7] we expect that this process has the potential for extension down to near-atomic length scales.

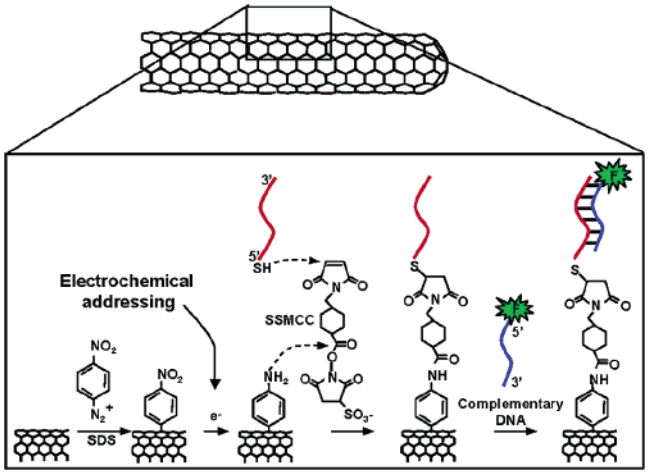

Figure 1. Schematic illustration of addressable biomolecular functionalization of carbon nanotubes. The procedure for carbon nanofibers is identical.

The process we developed is discussed in detail in the Supporting Information. To fabricate an array of distinct biologically modified SWNTs, we started with an oxidized silicon wafer "chip" and used standard lithographic patterning

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10.1021/nl048995x CCC: $27.50
Published on Web 08/10/2004

processes to deposit molybdenum contacts, followed by deposition of an alumina-supported Mo/Fe nanoparticle catalyst for nanotube growth.[5] Single-walled carbon nanotubes (SWNTs) were grown on the electrodes using a commercial thermal chemical vapor deposition system (First Nano). High-resolution SEM images (not shown) and Raman spectroscopy confirm that SWNTs of ~1 nm diameter are covering the Mo electrodes.

The addressable biological modification of each specific nanotube electrode is based upon electrochemical reduction of the nitro groups to amino groups. All nanotubes on the entire chip were first modified with aromatic nitro groups by immersing the entire nanotube-modified chip in a 36 mM solution of 4-nitrobenzenediazonium tetrafluoroborate (Aldrich) in 1% sodium dodecyl sulfate (Promega) and shaken using a vortex mixer at room temperature for 24 h,[8−13] following by rinsing in deionized water and acetone. At this stage, the chip consists of molybdenum electrodes coated with SWNTs bearing reactive nitro groups.

This "diazonium chemistry" yields nanotubes modified with nitrophenyl groups as depicted in Figure 1. Functionalization of the nanotubes with nitrophenyl groups can be followed via changes in the characteristic SWNT peak intensities in Raman spectra.[10,14,15] Figure 2a shows spectra of nanotubes grown on patterned Mo electrodes, obtained using a home-built Raman microscope with 514 nm excitation. A comparison of the same sample before and after reaction shows that the nitrophenyl functionalization step increases the intensity of the broad D-band near 1300 cm^{-1} and decreases that of the G-band near 1595 cm^{-1}. These peaks are widely attributed to sp^3-hybridized and sp^2-hybridized carbon, respectively.[14] The changes in Raman intensity therefore show that some of the sp^2-hybridized carbons of the nanotube are converted to an sp^3 carbon due to the formation of the covalent bond between the nanotubes and the phenyl group.[10] Because Raman scattering from nanotubes is resonantly enhanced, the molecules attached to the nanotube are not easily detected above the nanotube signal.[11] Therefore, we attribute the increase in D-band intensity to the sp^3-hybridized carbons of the nanotube due to covalent modification with nitrophenyl groups. The broader peaks near 700 and 900 cm^{-1} are observed on Mo electrodes without nanotubes and are attributed to various molybdenum oxides.

To achieve selective modification of individual nanotube/nanofiber electrodes, we make use of the fact that nitro ($-NO_2$) groups can be easily and selectively reduced to a primary amine ($-NH_2$) by electrochemical reduction.[16] Figure 2b shows several repeated cyclic voltammograms of nanotubes grown on microfabricated molybdenum electrodes, starting at a potential of −0.2 V. As the potential is brought negative, the nitro groups are reduced to amino groups at a potential of ~ −1.0 to −1.4 V, leading to a small reduction (negative-going) peak in the cyclic voltammogram in first reduction sweep (labeled "R1") to −1.8 V. As the potential is brought more positive, no corresponding oxidation peak is observed, demonstrating that the amine groups cannot be easily reoxidized. The second and third cycles are featureless, with no significant peaks characteristic of reduction or oxidation. This demonstrates that the reduction of $-NO_2$ groups to $-NH_2$ groups occurs on the first negative-going (reducing) sweep of the potential.

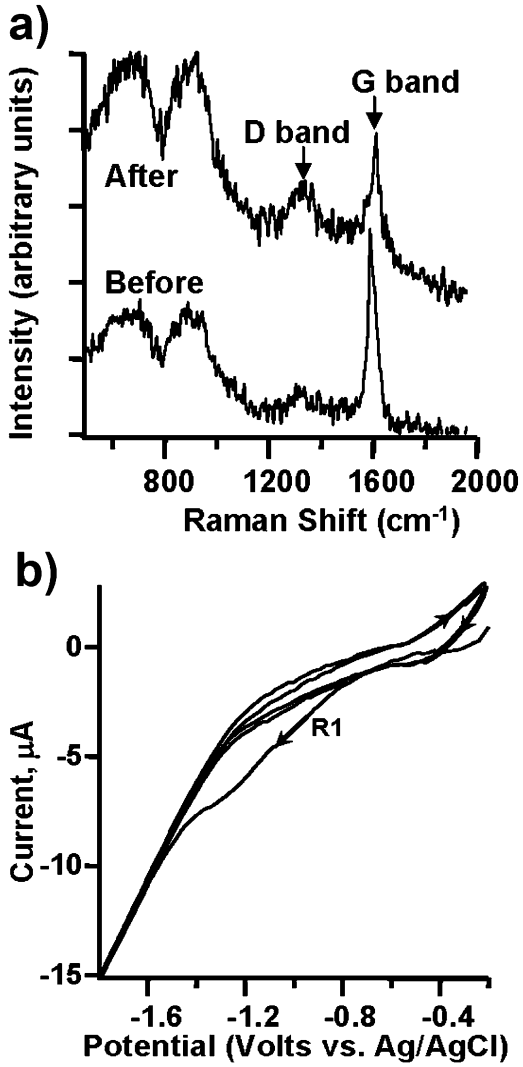

Figure 2. Characterization of nanotube chemical and electrochemical modification. (a) Raman spectra of SWNT-modified Mo electrodes before and after modification with nitrophenyl groups via diazonium chemistry. The characteristic "G" and "D" bands are labeled. The broader peaks near 750 and 950 cm^{-1} are attributed to molybdenum oxides on the underlying electrodes. (b) Cyclic voltammograms of nanotube-modified Mo electrodes after modification with nitrophenyl groups. The reduction of nitro groups to amine groups appears on the first reduction sweep (labeled R1); subsequent oxidizing and reducing sweeps show no subsequent changes.

The electrochemical reduction of $-NO_2$ to $-NH_2$ is the key step in addressability, because the primary amine ($-NH_2$) groups produced by the electrochemical reduction are a ubiquitous starting point for covalently linking biomolecules such as DNA, peptides, and antibodies to surfaces[17,18] and nanostructures.[2] Here, we use a heterobifunctional crosslinker SSMCC to covalently link the amino-modified nanotubes to thio-modified DNA oligonucleotides.[2,17,18] DNA covalent attachment was accomplished by first immersing the entire chip in a 1.5 mM solution of the heterobifunctional

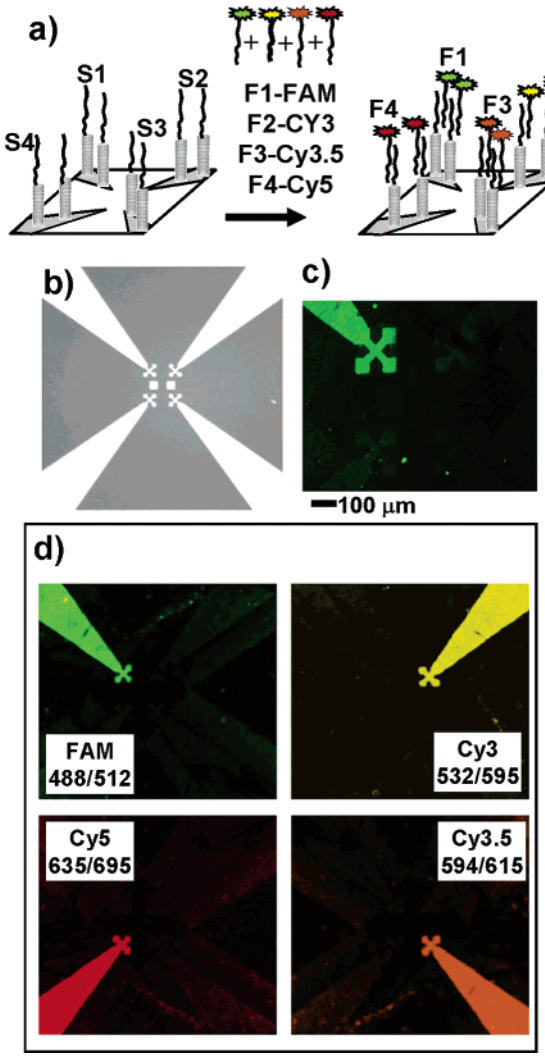

Figure 3. Biomolecular functionalization and hybridization of nanotube-modified electrodes. (a) Schematic of four-element array of nanotube-modified electrodes, modified with sequences S1, S2, S3, and S4, and hybridization with complementary sequences. (b) White-light image of nanotube-modified chip. Nanotube-modified Mo electrodes appear light. (c) Fluorescence image after four-element array was modified with four DNA sequences and exposed only to F1, the fluorescently labeled complement to S1; only one of the four electrodes shows significant fluorescence intensity. (d) Fluorescence images of a single region of a single chip made after exposure to a mixture of four fluorescently labeled complementary oligonucleotides. The specific dyes, excitation wavelengths, and fluorescence wavelengths are indicated.

cross-linker sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexan-1-carboxylate (SSMCC, Pierce) in 0.1 M triethanolamine buffer (Aldrich), pH 7.0 for 20 min to expose reactive maleimide groups, and then by applying the thio-modified DNA oligonucleotides (50 μM in triethanolamine buffer, pH 7) to the surface in a humid chamber for 2 h, followed by rinsing.

Figure 3 demonstrates the use of electrochemical addressing to produce and characterize an array of four SWNT electrodes modified with distinct DNA olignonucleotides.[19] In this experiment, a silicon chip with four independent electrodes was modified with carbon nanotubes and then reacted such that all four electrodes were modified with nitrophenyl groups. Figure 3b shows a white-light image of the nanotube-modified chip, showing the light-colored SWNT-modified electrodes and the darker regions of oxidized silicon. The electrochemical potential was applied to one of the four electrodes and was swept from −1 down to −2.0 V to reduce the nitro groups to amine groups. A thiol-modified DNA oligonucleotide (sequence S1)[19] was linked to the amino groups via its terminal 5′-thiol group using SSMCC as described above. After rinsing, the electrochemical reduction step was applied to electrode 2, and the entire chip was then exposed to SSMCC and a different thiol-modified DNA oligonucleotide with sequence S2.[19] Electrodes 3 and 4 were similarly electrochemically addressed and functionalized with the DNA sequences S3 and S4. In each of the four cycles, the entire chip was exposed to all reactants and DNA oligonucleotides; no mechanical barriers or specialized fluid handling was needed, as the modification chemistry is entirely controlled by the ability to selectively address one specific electrode at a time for electrochemical modification.

We tested the specificity of the DNA-modified nanotube electrodes by exposing the entire chip to a mixture of four complementary DNA molecules, each of which was labeled at the 5′ end with a different fluorescent dye[20] with a unique spectral signature of optimal absorption and emission wavelengths. Figure 3d shows four fluorescence images obtained after the entire chip was immersed in an equimolar mixture of the four different complementary sequences for 20 min.[20] Each image was obtained at the excitation and emission wavelengths specific to the four dyes used to label the DNA oligonucleotides and then represented in false color approximating the color of the fluorescence. The images show that the complementary DNA molecules are able to recognize their appropriate complementary sequence with a high degree of selectivity, each sequence in solution hybridizing only with the complementary sequence bonded to the carbon nanotubes. Thus, the electrically addressable modification process leads to biologically modified electrodes exhibiting a high degree of biological specificity.

To test the ability to achieve high spatial resolution, we conducted experiments on ∼500 nm-diameter bundles of vertically aligned carbon nanofibers (VACNFs). In these experiments, Mo electrodes were fabricated on silicon wafers covered with 300 nm silicon nitride insulator. Using electron-beam lithography, a thin film of 20 nm titanium followed by 20 nm nickel was selectively deposited in the patterned regions. VACNFs with typical diameters of of 50−100 nm each were grown on the catalyst-patterned substrate using 4 Torr of a mixture of acetylene (16 sccm flow rate) and ammonia (80 sccm) in a home-built DC plasma-enhanced chemical vapor deposition reactor.[6] Nanofibers shown here were grown using using 330 W of power applied to the sample for 12 min; the plasma heated the sample to approximately 700 °C.

Figure 4a shows a scanning electron microscope image of a ∼500 nm diameter bundle consisting of approximately ∼10 nanofibers, grown on a 3-micron-wide Mo electrode. Figure 4b shows a white-light image of this same sample,

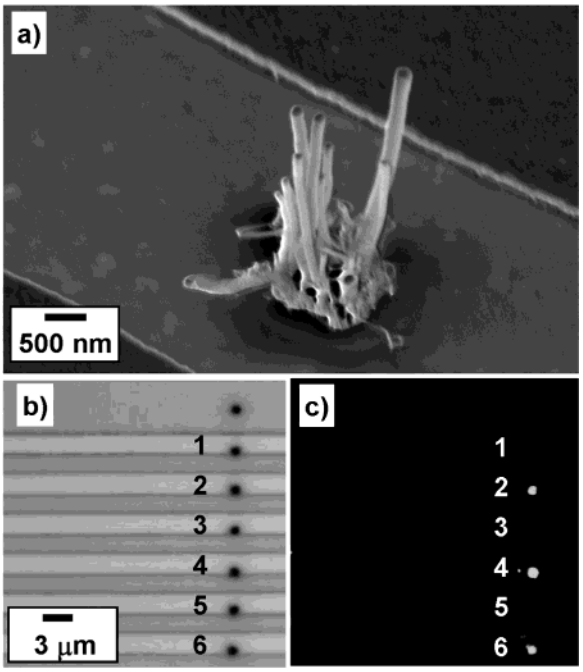

Figure 4. Addressable functionalization of vertically aligned carbon nanofibers. (a) Scanning electron microscope image of ~500 nm diameter region of VACNFs on a 3-micron-wide Mo electrode. (b) White light image showing six Mo electrodes (light horizontal regions) functionalized with ~500 nm diameter circular regions of VACNFs (darker spots). (c) Fluorescence image after VACNFs on electrodes 2, 4, and 6 were functionalized with DNA sequence S1 followed by immersion of the entire chip in complementary, fluorescently labeled DNA of sequence F1.

showing the six Mo electrodes on which carbon nanofiber bundles were grown. The nanofiber bundles appear as dark, resolution-limited fuzzy patches in optical microscopy. The nanofibers on electrodes 2, 4, and 6 were functionalized using the electrochemical modification process, and the remaining three were left alone as a control. Figure 4c shows a fluorescence image after selective electrochemical modification, functionalization with DNA sequence S1,[19] and exposure to the fluorescein-labeled complement F1.[20] As expected, the fluorescence image shows that only those nanofiber bundles that were selectively reduced; virtually no fluorescence is observed for the other bundles on the Mo electrodes or from one bundle grown on the silicon nitride insulator. Thus, we conclude that it is possible to use the method described here to electrochemically address and functionalize VACNFs with biomolecules such as DNA on submicron length scales.

The ability to selectively modify small electrodes with different biomolecules on the same chip without the use of microfluidics has important consequences for the ability to fabricate ultrahigh-density arrays for a wide range of biosensing and diagnostic applications. Electrical control of the functionalization of high-density arrays has the additional possibility of making biochips that can be easily configured during manufacturing under simple software control. We anticipate that this work will enable the fabrication of easily configurable, ultrahigh-density biosensor arrays in a cost-effective manner.

Acknowledgment. This work was supported by the National Science Foundation Grant DMR-0210806, DMR-0094063, and the UW-MRSEC, DMR-0079983. The authors acknowledge the contributions of Jason Simmons, Tami Lasseter, Kevin Metz, and Professor Lloyd Smith of UW-Madison and Dr. Alan Cassell and Dr. Meyya Meyyappan of the NASA-Ames Research Center.

Supporting Information Available: Detailed methods and procedures for the growth and chemical/electrochemical modification of nanotubes and vertically aligned carbon nanofibers. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The sequences linked to the surface are: 5′-HS−$C_6H_{12}T_{15}$ AA CGA TCG AGC TGC AA-3′ (S1), 5′-HS−$C_6H_{12}T_{15}$ AA CGA TCG AGG AGC AA-3′ (S2), 5′-HS−$C_6H_{12}T_{15}$ GC TTA TCG AGC TTT CG-3′ (S3), and 5′-HS−$C_6H_{12}T_{15}$ GC TTA AGG AGC AAT CG-3′ (S4).
(20) The fluorescently labeled sequences in solution that are complementary to those linked to the nanotubes are: 5′-FAM-TT GCA GCT CGA TCG TT-3′ (F1, complementary to S1), 5′-Cy3-TT GCT CCT GCA TCG TT-3′ (F2, complementary to S2), 5′-Cy3.5-CG AAA GCT CGA TAA GC-3′ (F3, complementary to S3), 5′-Cy5.5- CG ATT GCT CCT TAA GC-3′ (F4, complementary to S4).

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