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Preparation of Nucleic Acid Functionalized Carbon Nanotube Arrays

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ABSTRACT

A vertically aligned carbon nanotube (CNT) array is fabricated as a nanoelectrode platform for biosensor development. Prior to chemical functionalization, metal catalyst particles at the ends of CNT are removed and the closed ends are opened. We find that the oxidative treatment for generating the chemical functional groups at the opened ends of the CNT compromise the mechanical stability of the nanotubes, often leading to total collapse of the aligned CNTs. To solve this problem, we have developed a new approach for filling the gaps between CNTs with a spin-on glass (SOG). Results from the coupling of nucleic acids to the CNT arrays suggest that the SOG enhances the reactivity by providing structural support to the CNTs. The SOG also covers the length of the sidewalls of CNTs, leading to a less hydrophobic interface and thus may aid in improving the chemical reactivity.

Carbon nanotubes (CNTs) have been proposed as transducer materials for application as biosensors.[1] One of the challenges facing the implementation of CNT arrays as selective and ultrasensitive biosensors is the addition of chemical functionalities while preserving their inherent chemical and electrical properties.[2] For multiwalled CNT arrays, maintaining the desired vertical orientation facilitates their implementation as electrochemical sensor transducers. Several nanoscale materials exhibit enhanced electrochemical sensitivity over their larger scale analogues. For example, gold nanoelectrode ensembles are capable of detection limits as much as 3 orders of magnitude lower than conventional gold disk microelectrodes (>10 nm diameter).[3,4] As such, it is expected that further materials development and electrode downsizing from the micro to nanoscale will increase biosensor detection sensitivity limits.

Electrochemical studies using CNTs as electrode elements have been reported[5] as well as electrochemical-initiated chemical functionalization of the sidewall of CNTs.[6] There are also few examples of small molecules reacting with carboxylic acid groups at the oxidatively opened ends of single wall CNT, including primary alkylamine in CNT dispersion[7] and benzylamine, as well as ethylenediamine group for the CNT as chemical force microscopy tips.[8] An interesting CNT ring structure was observed and attributed to the coupling between the terminal carboxylic acid groups at the two open ends of carbon nanotube.[9]

Here we present an approach for the attachment of nucleic acids to oxidatively opened ends of CNT arrays, an important step in the development of a general platform for detecting biological macromolecules. In addition to necessary steps such as opening the closed CNT ends and removing metal catalyst at the CNT tips, a novel and critical step is the deposition of a spin-on glass (SOG) film inside hydrophobic CNT arrays.

The multiwalled CNT arrays were grown using a plasma enhanced chemical vapor deposition process described previously.[10,11] Briefly, a 10 nm film of iron catalyst was deposited on silicon substrate by ion beam sputtering technique. Growth was accomplished at a substrate temperature of 900 °C for 10 min with 20 sccm of C_2H_4 and 80 sccm of H_2, under 3 Torr of total pressure and with 100 W of inductive and 200 W of capacitive power. Electron microscopy (SEM and TEM) analysis shows that the CNTs are relatively straight and that the ends are closed with metal-encapsulated catalyst and covered by amorphous carbon. After extensive oxidative pretreatment to render the CNT ends with carboxylic acid end groups for nucleic acid attachment, we found some problems. Notably, the mechanical stability of the aligned CNTs was often compromised, and quite frequently, total collapse of the CNTs arrays did occur. Additionally, even after extensive oxidative pretreatment, the CNT arrays were not highly reactive to nucleic acids under aqueous conditions. The mechanical instability also precludes using the vertically oriented CNT array as an ultrasensitive nanoelectrode platform for biosensors. This led us to explore the integration of gap-filling materials to improve the mechanical rigidity as well as to enhance nucleic acid coupling efficiency.

Figure 1 shows the fabrication steps necessary to achieve chemically active, yet mechanically stable CNT arrays for

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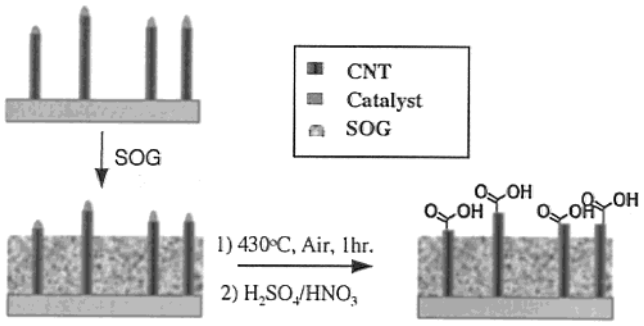

Figure 1. Fabrication and pretreatment of carbon nanoelectrode arrays for functionalization.

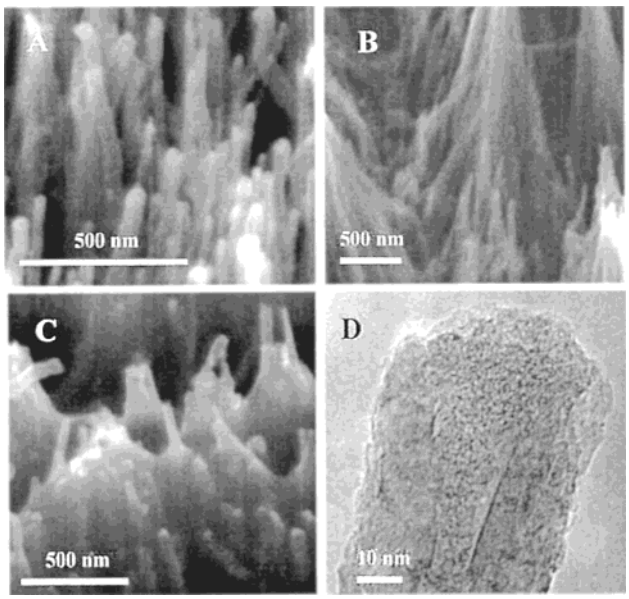

Figure 2. Electron microscope images. (A) Vertically aligned multiwalled CNT arrays with length about 1 um. (B) Collapsed CNT arrays after purification process. (C) CNT arrays with SOG after purification and tip opening process. (D) High-resolution transmission electron microscope image of an opened CNT end.

nanoelectrode sensor development. After CNT growth, a film of spin-on glass was used to fill the gaps between the individual CNTs in the array. The SOG film (Honeywell Microelectronics Auccuglass 512B, with 15 wt % methyl groups bonded to Si atoms in the Si−O backbone) was deposited at a spin speed of 3000 rpm and underwent subsequent thermal curing at 400 °C for 4 h under a positive pressure of argon. Figure 2 shows SEM images of (a) vertically aligned CNT array, (b) collapsed CNT array without SOG, (c) CNT array with SOG after 430 °C air oxidation and acidic treatment, showing CNTs with opened ends, and (d) high resolution TEM image of an open-ended CNT. We found that the SOG film provided structural support to the carbon nanotubes, enabling them to retain their vertical configuration during the purification and tip opening process. The SOG film also serves as a dielectric material, which electrically insulates the individual nanotubes from their neighbors.

Nucleic acid attachment was accomplished (Figure 3) using standard water soluble coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)

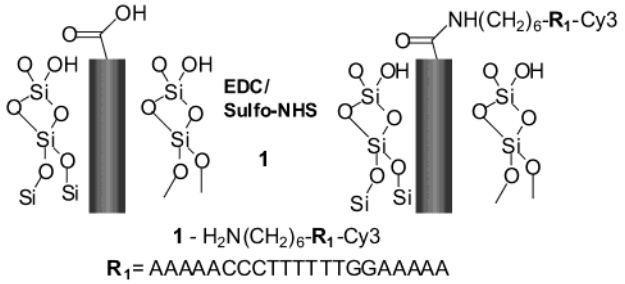

Figure 3. Coupling of nucleic acid to the functionalized CNT arrays.

and *N*-hydroxysulfo-succinimide (sulfo-NHS) (Pierce). In this reaction, the EDC reagent activates the terminal −COOH groups on the ends of the CNTs, forming a highly reactive *o*-acylisourea active intermediate. The EDC-derived ester intermediate rapidly undergoes hydrolysis; however, in the presence of excess sulfo-NHS a more water stable sulfo-succinimidyl intermediate is formed. Subsequently, the succinimidyl intermediate undergoes nucleophilic substitution reaction with the primary amine linker on oligonucleotide, resulting in the formation of an amide linkage.[12] It was calculated that the array contains about 10^{10} CNTs, assuming each CNT occupies 100 nm^2. Reaction was performed with the CNT array on a silicon chip submerged in 50 mL deionized water. Freshly prepared aqueous solutions of sulfo-NHS and EDC (2 mL each containing 1000 equivalents) were added first. A 2 mL aqueous solution of nucleic acid (100 equivalents) was then added to the reaction and the solution was stirred. On the basis of fluorescent intensity, it appeared that the reaction was essentially completed after 2 h.

Confirmation of nucleic acid attachment was accomplished using a 532 nm laser fluorescent microarray scanner to detect the Cy3-labeled DNA molecule. This provides a simple surface characterization technique to probe the efficiency of the coupling reaction. Results for the CNT electrodes with an integrated spin-on siloxane film and without the film are shown in Figure 4. The brighter intensity for the array with the integrated siloxane film (panel 4b) indicates that the efficiency of DNA coupling reaction is higher compared to the case without the siloxane film (panel 4a). Both arrays underwent the same aqueous washing at 90 °C following the coupling reaction, which should remove any noncovalent, weakly bound DNA molecules. We confirmed that the coupling of nucleic acid did not occur on the SOG surface by performing a reaction with just SOG film containing no CNTs, in which no fluorescence signal was detected. For detailed discussion on the chemical coupling of nucleic acid to silicon surfaces, the reader is referred to ref 13. Hybridization to the CNT coupled nucleic acid was also investigated. In this case, CNT array with SOG was coupled to PNA ($H_2N(CH_2)_6$−GCCGATGCACC) and when hybridized with the Cy3-labeled complementary DNA exhibited strong fluorescent signal as seen in panel 4c. These results and other control experiments are summarized in Table 1 and strongly indicate that the coupling occurred via the amide bond formation. The increased reactivity is also imparted by

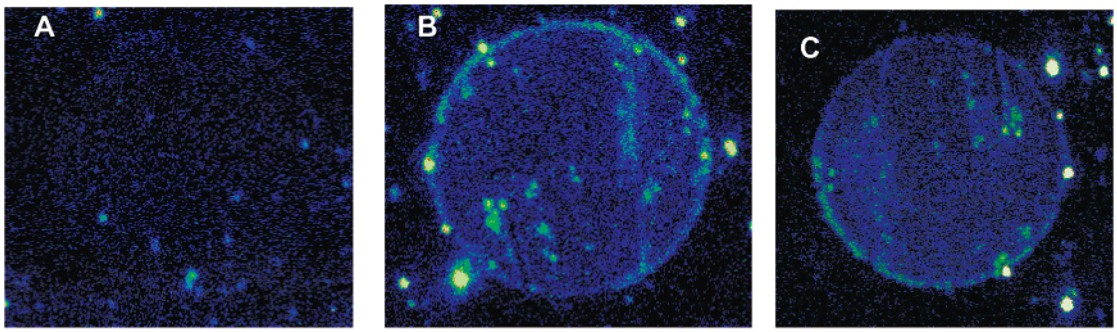

Figure 4. Fluorescently scanned images of CNT arrays. (A) Without SOG. (B) With SOG. (C) Hybridization of Cy3-labeled complementary DNA to coupled PNA on CNT array with SOG.

Table 1: Summary of Fluorescence Data Supporting the Higher Coupling and Hybridization Efficiency with the SOG-Filled CNT Array

	with SOG	without SOG
DNA with linker and Cy3	strong	weak
DNA with Cy3, no linker	weak	weak
DNA with linker + cDNA with Cy3	strong	weak
DNA with linker + noncDNA with Cy3	weak	weak

covering the length of the CNT sidewalls with the SOG, which renders the CNT interface hydrophilic and thus more compatible for coupling chemistry in aqueous solvent.[14] This is confirmed by contact angle measurements, in which SOG-filled CNT array exhibited a contact angle of 11° (vs 80° for the CNT array without the SOG film). The hybridization experiments were also done with DNA with similar success. PNA is preferable for in vivo biosensors as it is not biodegradable. Finally, it is also possible to use SiO_2 as gap-fill material, instead of SOG, as we demonstrated recently for electrode fabrication.[15]

In summary, we have shown that the integration of a SOG film with vertically aligned CNT array provides structural support to the CNT during oxidative treatment for the opening of closed CNT ends. In turn, the improved CNT structure enhances the chemical coupling of nucleic acids to the CNT array.

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