Extended Abstracts of the 2006 International Conference on Solid State Devices and Materials, Yokohama, 2006, pp. 788-789

P-11-4

Biosensing with CN_x multi-wall carbon nanotubes

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Introduction

The excellent electrical properties of carbon nanotubes (CNTs) and the relative simplicity of their fabrication make them one of the most promising candidates for creating electronic devices on a scale smaller than can be achieved with silicon [1]. However, many important issues such as controlled nano-patterning of CNT structures, contacts with micro-sized electrodes, reliable methods to selectively control their chirality-dependent electrical properties remain to be solved before CNTs based electronics becomes a reality. In recent years, hybrid systems that combine CNTs electrical properties with the recognition, specificity and catalytic properties of proteins, enzymes, DNA, antibodies, etc., have been proposed. This strategy is expected to produce new bioelectronic systems such as biosensors, field-effect-transistors and self-assembled nanocircuitry.

The mechanism of CNT biosensor detection is based on changes in conductance caused by the adsorption of biomolecules. Our approach has been to utilize nitrogen-doped multi-wall CNTs (CN_x MWNTs) to overcome the difficulties of biomolecular recognition using un-doped CNTs: CN_x MWNTs electrical properties are independent of chirality[2] and their large-diameter structure is rich in defects, particularly hydrophilic nitrogen sites.

A successful "bio-CNT" interface requires not only good adhesion of a biomolecule to the CNT, but also conservation of its structure and functionality. Previous reports of protein functionalization of CNTs, have only demonstrated biomolecule coverage, but the effect of adsorption on protein conformation and activity has not been addressed. Here, we demonstrate the functionalization of CN_x MWNTs with metal-containing proteins of different sizes (from 12 kDa to 440 kDa). In addition, through the use of antibodies, UV-visible spectroscopy and circular dichroism (CD) spectroscopy, we show proteins are not denatured or decoiled by the adsorption process. Finally we build a biosensor device based on CN_x MWNTs that can sense protein adsorption and antibody binding.

2. Results

Before creating a bio-NT conjugate, the initial problem of the poor solubility of carbon NTs in aqueous solution must be resolved. It has been shown that NT solubility can be improved by acid oxidation, which introduces acidic functional groups onto un-doped SWNTs[3] and MWNTs[4] and also CN_x MWNTs[5]. It has been proposed that these groups may assist in the anchoring of biological species to the CNT surface[6].

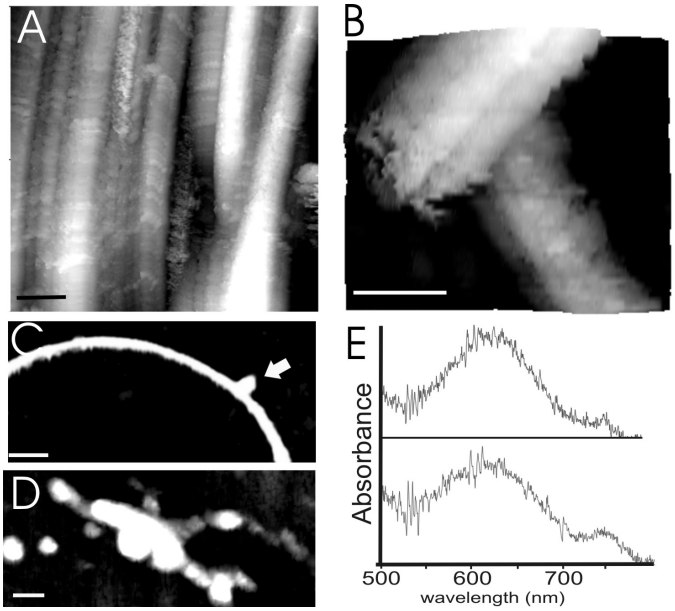

Figure 1. (A) STM height image of a bundle of raw CN_x MWNTs of different diameters dispersed in ethanol. (B) STM height image of acid treated CN_x MWNTs. (C) AFM height image of raw CN_x MWNTs functionalized with azurin proteins showing a very sporadic binding (indicated by arrow). (D) Acid-treated CN_x MWNTs functionalized with azurin, showing a uniform coating. Scale bar is 100nm in (A-D). (E) UV-visible spectra of free (top) and CN_x MWNTs adsorbed (bottom) azurin, demonstrating that the electron environment of the metal ion of the protein is conserved.

The effect of acid treatment on the structure CN_x MWNTs can be seen in Fig 1. Fig. 1A shows a height STM image of a bundle raw CN_x MWNTs before acid treatment. In Fig. 1B, the effect of the acid treatment on the tubes is visible in the increased number of defects on the surface. The adsorption of proteins (azurin) on raw tubes is very sporadic (Fig. 1C), whereas the acid treated tubes are extensively coated (Fig. 2D). The improved adsorption of the proteins to

acid-treated tubes is due to additional interactions between the proteins and carboxylic acid (-COOH) groups on the CN_x MWNT generated by the oxidation process. These interactions are most likely to be hydrogen bonds, but the possibility of covalent amide linkages between protein $-NH_2$ moieties and -COOH groups of the CN_x MWNTcannot be excluded. Additional physical factors specific to CN_x MWNTs, may also contribute to protein binding *e.g.* their larger diameters, compared to un-doped tubes, and pyridine-like nitrogen defects on the nanotube surface.[7]

To demonstrate the conserved functionality of the proteins adsorbed to CN_x MWNTs, CN_x MWNTs coated with different proteins were incubated with specific IgG antibodies. AFM images demonstrate binding of the antibodies to the protein epitope indicating that the external areas of the protein are unaltered during adsorption to CN_x MWNTs. CD spectra of protein adsorbed onto CN_x MWNTs show no differences with the spectra obtained for proteins in solution, suggesting that no major changes in the secondary structure of the proteins is induced during the adsorption. UV-visible spectroscopy (Fig, 1E) demonstrates that the electronic environment of the metal ion of the proteins is conserved. From the combined results of these three techniques, we can conclude that the entire protein retains its general conformation and activity when adsorbed to CN_x MWNTs, making these nanotubes suitable components for future biosensor devices.

Finally, we have succeeded in developing a biosensor device based on CN_x MWNTs. A network of CN_x MWNTs is deposited onto glass and subsequently connected to metal electrodes utilizing a method based on electroless plating techniques[8]. The device successfully detects protein adsorption and subsequent antibody binding.

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