Carbon Nanotube Nanoelectrode Array for Ultrasensitive DNA Detection

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There is a strong need for faster, cheaper, and simpler methods for nucleic acid analysis in today's market for clinical tests. Nanotechnologies can potentially provide solutions to these requirements by integrating nanomaterials with biofunctionalities, and microelectronics. Dramatic improvements in the sensitivity and the high-degree multiplexing can be achieved through the miniaturization enabled by nanotechnologies. Here, we present our study in the development of an ultrasensitive label-free electronic chip for DNA/RNA analysis based on carbon nanotube nanoelectrode arrays.

A reliable nanoelectrode array based on vertically aligned multi-walled carbon nanotubes (MWNTs) embedded in SiO_2 has been fabricated with a bottom-up approach as shown in Fig. 1. These processes result in a planarized surface with only the very end of MWNTs exposed, which behaves as inlaid nanodisk electrodes with the diameter of ~50 nm, as shown in Fig. 2(a). Such MWNT nanoelectrode arrays have shown well-defined nanoelectrode behavior as shown in Fig. 2(b) when the average nanoelectrode separation is controlled over about one micron. The application in electroanalysis has been demonstrated in the trace analysis of small redox molecules as well as the ultrasensitive DNA detection. Characteristic electrochemical behaviors are observed in measuring both bulk and surface immobilized redox species. The detection limit of bulk redox species can reach down to a few nanomolars. The surface immobilized ferrocence with the coverage of less than 1% can be detected using AC voltammetry (ACV).

Primary amine terminated oligonucleotide probes with 18 bases corresponding to a segment of the BRCA1 cancer gene are selectively functionalized to the exposed end of the MWNTs through amide bonds with the –COOH group. Subattomoles of oligonucleotide targets and about a few hundreds of PCR amplicons (~300 bases) have been immobilized at the electrode surface through the specific hybridization as shown in Figs. 3(a) and 3(b). We have demonstrated that such small number of target DNA molecules can be detected using an electrochemical mechanism based on the mediator amplified guanine oxidation. As illustrated in Figs. 3(c) and 3(d), the differential signal between the 1st and 2nd scans is proportional to the number of guanine bases on the electrode surface due to the fact that the guanine oxidation is irreversible. Since the inherent guanine bases are used as the signal moieties, the labeling process can be skipped.

The fabrication method is compatible with semiconductor processes and can be mass-produced into highly integrated devices. We have demonstrated that MWNT nanoelectrode array can be fabricated on individually electrically addressed microcontact pads prepatterned on the surface so that the high-degree multiplex detection can be implemented. This system provides a general platform for rapid molecular analysis in applications requiring ultrahigh sensitivity, high-degree of miniaturization, and simple sample preparations.

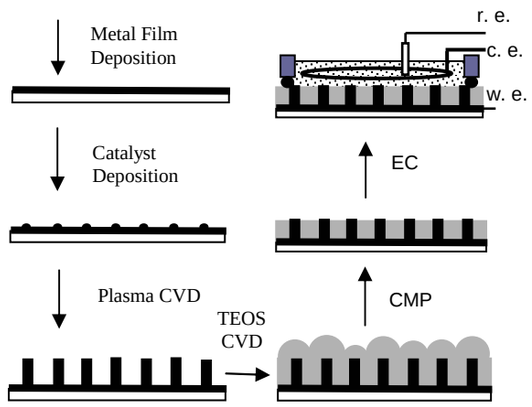

Fig. 1 The procedure for fabricating the MWCNT nanoelectrode array and the setup for electrochemical characterization.

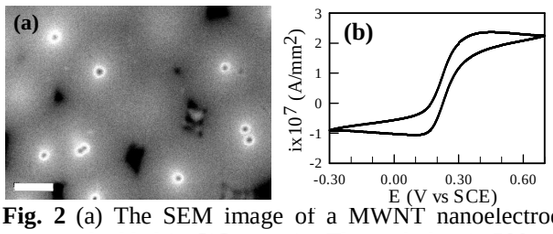

Fig. 2 (a) The SEM image of a MWNT nanoelectrode array embedded in SiO_2 matrix. The scale bar is 500 nm. (b) The cyclic voltammetry of the MWNT nanoelectrode array in 1.0 mM $K_4Fe(CN)_6$ and 1.0 M KCl at a scan rate of 20 mV/s.

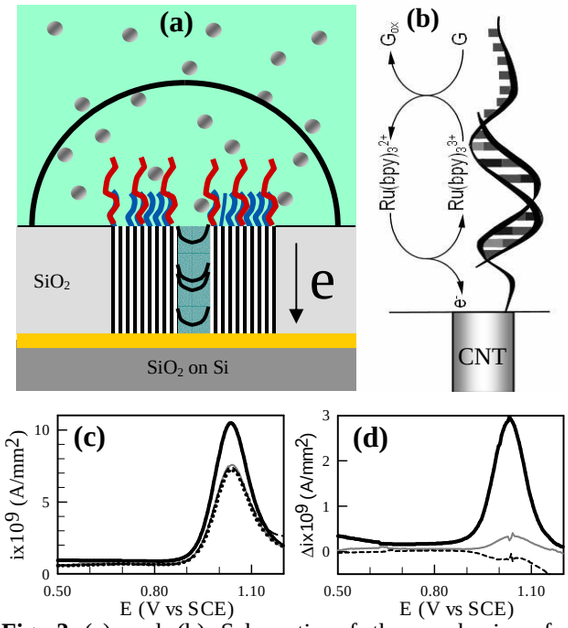

Fig. 3 (a) and (b) Schematic of the mechanism for DNA/RNA sensing using MWNT nanoelectrode array and $Ru(bpy)_3^{2+}$ mediator amplified guanine oxidation. The hemisphere in (a) represents the diffusion layer of $Ru(bpy)_3^{2+}$ mediators. (c) Four consecutive ACV measurements of a low-density MWNT array with hybridized DNA targets in 5.0 mM $Ru(bpy)_3^{2+}$ with 0.20 M NaOAc (pH=5.2) using an AC sinusoidal wave at 10 Hz and 25 mV amplitude superimposed on the staircase DC potential ramp from 0.50 to 1.20 V at 20 mV/s. 1st scan – thick line, 2nd scan – thin grey line, 3rd scan – dotted line, and 4th scan – dashed line. The later three scans are almost completely overlapped. (d) The differential curves representing the signal proportional to the number of guanine bases on the surface: between the 1st and 2nd scans (thick solid line), 2nd and 3rd scans (thin grey line), 3rd and 4th scans (thin dashed line), respectively.