9 Carbon Nanotubes for Electrochemical and Electronic Biosensing Applications

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Abstract The structure of carbon nanotubes (CNTs), a type of macromolecular systems, can be thought as rolling up a graphene sheet along certain directions. The unique properties of CNTs, such as very high surface/volume ratio, high chemical stability, high electro-catalytic activity and high charge transfer efficiency, make CNTs a very suitable material for biosensor applications. It is due to these advantages that a variety of CNT related biosensors have been developed since discovery of CNTs. In this chapter, recently reported CNT-based electrochemical and electronic biosensing applications are summarized.

9.1 Introduction

Biosensors, a subtype of sensor devices, are designed to characterize biological properties of various biomaterials from monitoring the physical and chemical signals in the biological materials. Biosensors, typically, consist of sensing elements and signal transducers. The elements selectively sense the analytes, while the transducers convert chemical and biological variations into appropriate physical signals, such as electrical and optical signals, whose change corresponds to the analyte concentration. Biosensors have two characteristics. One is that the sensing elements are attached with biological materials, such as single-strand DNA (ssDNA), proteins (enzymes, antibodies, etc.), cells and so on. The other is that biosensors are used to track the biological processes ((Balasubramanian and Burghard, 2006) and references therein).

Carbon nanotube (CNT) is a type of macromolecular systems. The structure of

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CNTs can be thought as rolling up a graphene sheet along certain directions. The unique properties of CNT have drawn a lot of research attention in various biosensor applications. For example, CNTs have a high surface/volume ratio. Thus, the surface area of CNT-modified electrodes for electrochemical detection would be increased. Moreover, it also exhibits very high chemical stability and hardly reacts with analytes. It has also been found that the CNT can enhance electro-catalytic activity and promote electron-transfer reactions for a wide range of electroactive species. CNT-modified electrodes can alleviate surface-fouling and immobilize important biomolecules. CNT has a 1-D structure that enables efficient charge transfer between the surface-anchored biomolecules and CNTs. Therefore, the conductivity of CNT, especially semiconducting SWNT, is found to be remarkably sensitive to the surface adsorbates. In addition, CNTs are easily functionalized with biorecognition layers which selectively react with bio-analytes. These properties make CNT a very suitable material for electrical biosensors, including electrochemical biosensor and electronic biosensor ((Katz and Willner, 2004; Wang, 2005b) and references therein).

The aim of this review is to cover recently reported applications integrating CNTs into electrical biosensing systems. This chapter is organized as follows. Various design principles of CNT based biosensors are described in the second section. In the third section, a collection of CNT-based electrochemical biosensors are presented, followed by the CNTFET-based electronic biosensors in the fourth section. Finally, the future prospects are discussed.

9.2 Design Principles of CNT-Based Biosensors

Biological species could be detected by a variety of classical methodologies, such as fluorescence and spectrometry with high sensitivity and selectivity. However, these techniques are of complicated systems and difficulties to miniaturize. These drawbacks could be overcome by electrical biosensing techniques. In last decade, CNTs have been utilized as electrodes or transducers to perform electrical biosensing. In the following discussion, we classify CNT-based biosensors into several categories according to different configurations.

9.2.1 CNTs as Modifiers of Electrode Surfaces

The common used electrodes in electrochemical biosensors included carbon electrodes (glassy carbon, graphite, activated carbon, etc.), metallic electrodes (Au, Pt and so on), conducting polymer electrodes, metal oxides electrodes, different composite electrodes, etc.. Some of these electrodes are of a poor sensitivity and stability, long response time and high overpotential for electron transfer reactions

in electrochemical sensor applications. Some of them are very expensive such as noble metal electrodes. In contrast, CNTs, both non-oriented and oriented, with their fast electron transfer and anti-surface fouling properties, could overcome most of these disadvantages.

9.2.1.1 Non-Oriented Modification

Non-oriented modification means that CNTs on the electrode surface are randomly distributed. Casting a CNT suspension onto the support electrodes is the simplest and mostly used method. Because CNTs are insoluble in most solvents, the first step of processing CNTs into a thin films on electrodes is to get a homogeneous CNT suspension, some of which were prepared by dissolving CNTs in a solution of concentrated sulfuric acid (Musameh et al., 2002) or Nafion (Deo et al., 2005; Hocevar et al., 2005; Luong et al., 2005; Tsai et al., 2005; Wang et al., 2003a; Wu et al., 2006). The latter one has extra benefits like improved anti-interferent ability and mechanical strength due to the good cation exchange, discriminative and biocompatibility properties of Nafion, a perfluorosulfonated and negatively charged polymer. Moreover, with the aid of surfactants, such as dihexadecylphosphate (DHP) (Wu and Hu, 2003, 2006; Wu et al., 2006) and sodium dodecylbenzenesulfonate (SDBS) (Wu et al., 2006), CNTs were easily dispersed into the aqueous solution. In addition to aqueous media, other solvents such as *N*, *N*-dimethylformamide (DMF) (Liu and Lin, 2005, Wang et al., 2002; Wang et al., 2004, Wei et al., 2006; Zeng et al., 2006), chloroform (Zhang et al., 2006) and acetone (Jin et al., 2006; Wu et al., 2002) were also used to prepare CNT suspension. Further to this, oxidized CNTs after long-time acid treatment, however, could be directly stabilized in aqueous solution (Lawrence and Wang, 2006; Zhu et al., 2005) because of the electrostatic repulsion induced by the negatively charged chemical groups on MWNTs (Zhu et al., 2005). In the above processes, ultrasonication was usually used to assist effective dispersion of CNTs. After that, a small volume of CNT suspension was drop-casted on the polished glassy carbon electrode (GCE) or metallic electrodes.

Applying the chemical vapor deposition (CVD) method could also fabricate an electrode with non-oriented CNT modification (Tang et al., 2004). Another simple preparation method involves gently rubbing the electrode surfaces on filter papers coating with purified MWNTs (Salimi et al., 2005).

9.2.1.2 Oriented Modification

Assembling oriented CNTs on electrodes is another way to fabricate CNT-modified electrodes for electrochemical detections. Most of these modifications were originated from the CNT growth process, which not only improves the electrical contact between CNTs and the conducting electrodes, but also makes CNTs free of impurities caused by surfactants or binders.

Direct growth of vertically aligned CNT arrays on graphite or gold substrate

could be immediately utilized for electrical biosensing (Tang et al., 2006; Ye et al., 2004). But some of such electrodes were further functionalized with biomaterials for biological detection (Roy et al., 2006; Wang et al., 2004).

To improve the signal-to-noise ratio and detection limits, nanoelectrode array (NEA) based on low-density aligned CNTs were developed for detecting of glucose (Lin et al., 2004) and DNA (Koehne et al., 2003; Koehne et al., 2004; Li et al., 2003). The CNTs were firstly grown on the substrate with controlled spacing and density (Fig. 9.1). Then CNT NEAs were insulated between each other with spin-coated epon epoxy resin or deposited SiO_2. Subsequently the protruding parts of CNT NEA were removed by polishing process. The exposed tips of CNT NEA were functionalized and covalently immobilized with GOx or probe ssDNA. In this setup, the aligned CNT NEA exhibited high elecrocatalytic activity and fast electron transfer rate (ETR) because the edges of the nanotubes are exposed (Li et al., 2002).

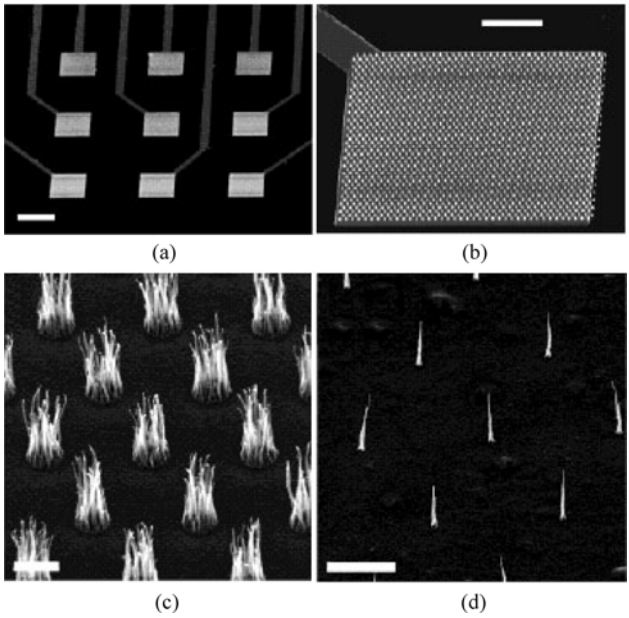

Figure 9.1 SEM images of CNT nanoelectrode array: (a) 3×3 electrode array. (b) array of CNT bundles on one of the electrode pads. (c) and (d) array of CNTs at UV-lithography and e-beam patterned Ni spots, respectively. The scale bars are 200, 50, 2 and 5 μm, respectively. Reprinted from (Li et al., 2003) with permission

Post-synthesization transfer of the CNT array method was also applied to fabricate oriented-CNT modified electrodes (Gao et al., 2003; Wang et al., 2003a; Wang et al., 2003b). Briefly speaking, a thin layer of metal (such as Au) was deposited on the top surface of as-synthesized aligned CNT array and then the substrate which supported the CNT to grow was etched away.

Another way to prepare vertically aligned CNT array was the self-assembly technique (Liu et al., 2005; Patolsky et al., 2004). The carboxylized CNTs generated from oxidative scission were covalently immobilized on a cysteamine monolayer coated gold electrodes via amide bond in a manner of perpendicular orientation.

9.2.2 CNT-Based Composite Electrodes

Carbon paste and composite electrodes, fabricated by mixing carbon powder with mineral oil or other binders, have been used in electrochemical detection for years. After the application of CNTs in this field, CNT-based composite electrodes could be prepared in a similar manner by mixing CNTs powder with a number of binders, e.g. mineral oil, bromoform, Teflon, polymers like Polypyrrole (PPy) and chitosan (CHIT) and so on. The CNT-based composite electrode was fabricated by casting the resulting composite on the top of a support substrate (Fig. 9.2(a)) (Zhang and Gorski, 2005a; Zhang and Gorski 2005b), or packing it into a glass capillary or a Teflon tube (Fig. 9.2(b)) (Luque et al., 2006; Wang and Musameh, 2003b; Yang et al., 2006).

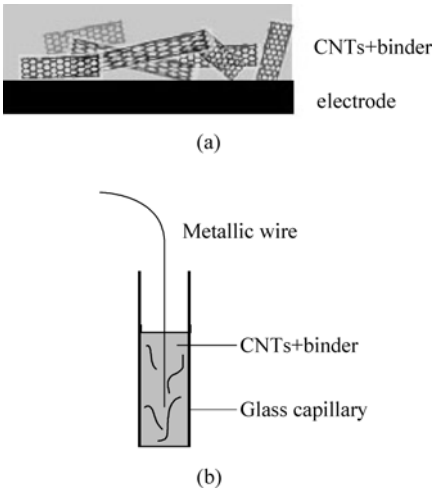

Figure 9.2 Schematic drawing of two types of CNT composite electrodes: (a) composite of CNTs and binder was casted on the top of a support electrode. (b) composite was packed into the electrode cavity of a glass capillary or a Teflon tube

The first CNT-based composite electrodes used bromoform as binder and then was packed into a glass tube (Britto et al., 1996). The CNTs are usually mixed with mineral oil at a certain ration of weight percentage to form CNT-based composite electrodes (Luque et al., 2006; Ly, 2006; Pedano and Rivas, 2004; Rubianes and Rivas, 2005). Similarly, the CNT/Teflon composite was prepared

in dry state by hand-mixing CNTs and Teflon, and the resulted composite was then packed firmly into the cavity of a glass sleeve (Wang and Musameh, 2003b). Moreover, the composite electrode using PPy as the binder was made by cyclic voltammetry (CV) in the mixed solution of pyrrole and MWNTs (Cheng et al., 2005). CHIT was also used as a binder (Zhang and Gorski, 2005a, 2005b). The MWNTs were solubilized in the matrix of such hydrophilic ion-conducting biopolymer. In some biosensing applications mentioned above, the CNT based composite was further mixed with biological molecules (enzymes for example) to obtain biomolecule functionalized CNT-based composite electrodes (Luque et al., 2006; Ly, 2006, Rubianes and Rivas, 2005; Wang and Musameh 2003b).

Furthermore, a layer-by-layer (LBL) technique was another method to fabricate CNT-based composite electrode. It was reported that alternate drop casting of carboxylized MWNT suspension and electropolymerization of neutral red (NR) assembled five layers of homogeneous and stable MWNTs and poly(neutral red) (PNR) film on a GCE (Qu et al., 2006).

9.2.3 Nanoparticles Decorated CNT-Based Electrodes

Due to their unique size and shape, nanoparticles (NPs) frequently exhibit unusual physical and chemical properties, such as large surface-to-volumeratio and promotion of the electron transfer. Another important characteristic of some nanoparticles, such as Pt, is the bio-compatibility, which could preserve the biological activity of attached biological molecules. On the other hand, transition metals, especially noble metals, display high catalytic activities for many chemical reactions. Due to these reasons and easy miniaturization to nanoscale dimensions, metallic nanoparticles have been used for chemical/biochemical sensing applications. Further incorporation with CNTs, which have a large surface area, a high ETR and the electrocatalytic activity as well, makes the nanoparticle and CNT modified electrodes achieve a higher sensitivity.

The main techniques have been employed to immobilize metallic nanoparticles on CNT-modified electrodes including: direct adsorption of preformed nanoparticles (Hrapovic et al., 2004; Luque et al., 2006; Male et al., 2004; Wu and Hu, 2005; Yang et al., 2006), chemical deposition from metal salt solutions (Shi et al., 2005), or electrodeposition from metal salt solutions (Fei et al., 2005, Tang et al., 2004). Among them, electrodeposition method is much more efficient as it was capable to control the density and size of metallic nanoparticles through adjusting applied potential (Day et al., 2005, Tang et al., 2004).

In direct adsorption method, the Pt nanoparticles, which could rapidly oxidize H_2O_2 produced from enzymatic reaction, were prepared elsewhere. Subsequently they were dissolved in a Nafion solution (Hrapovic et al., 2004) or a silicate sol (Yang et al., 2006), which were then mixed with CNTs. In contrast, the chemical deposition and electrodeposition of Pt nanoparticles were normally fabricated by

the reduction of H_2PtCl_6: after immersing the CNTs or CNT-modified electrodes in H_2PtCl_6 solution, a reductive chemical $Na_2S_2O_4$ (Shi et al., 2005), or a specific potential (Fei et al., 2005, Tang et al., 2004), was applied, respectively.

Cu nanoparticles prepared through the reduction of copper dodecyl sulfate ($Cu(DS)_2$), were embedded in Nafion and SWNTs composite on the surface of a Cu electrode as shown in Fig. 9.3 (Male et al., 2004). Moreover, a biosensor with Cu particles in the manner of direct adsorption was prepared by mixing enzyme, Cu particles and MWNTs with mineral oil to form a composite electrode for glucose detection (Luque et al., 2006). This glucose biosensor also used Ir particles, instead of Cu, for the preparation of the electrode in a similar manner.

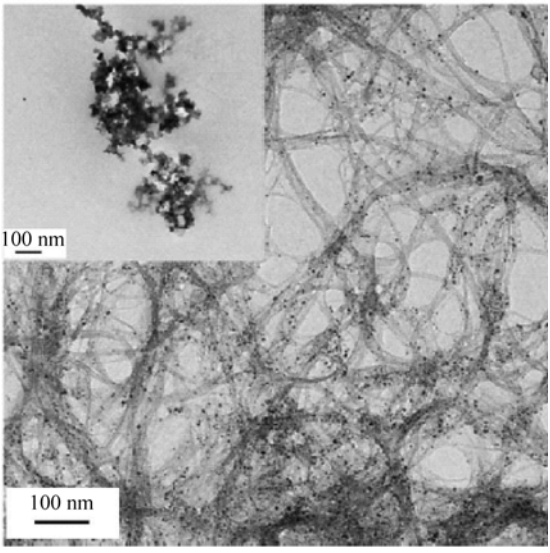

Figure 9.3 TEM image of SWNT decorated with Cu nanoparticles, prepared by reduction of $Cu(DS)_2$. (Inset) TEM image of Cu nanoparticles in the presence of Nafion. Reprinted from (Male et al., 2004) with permission

An MWNT modified Au electrode with Au-colloids adsorption was prepared by solubilizing MWNTs in the DHP which was dispersed in colloid Au aqueous solution for the determination of cytochrome c(Wu and Hu, 2005).

9.2.4 CNTs as Key Sensing Elements

So far, we have discussed using CNTs as the microelectrode modifier for electrochemical detection purpose. In these devices, CNTs mainly work as a role of transducer. However, CNTs, especially SWNTs, could also function as key sensing elements in the detectors with different structures.

Electronic biosensor with CNTFET structure, a type of CNT-based biosensors, has a similar configuration to MOSFET. However, in CNTFET, SWNTs are utilized as the conducting channel, instead of silicon channel. As the SWNT conducting channel of CNTFET is open to the environment, it is found to be very sensitive to the variation of environmental conditions. The SWNTs across the source and drain could be implemented through direct growth using CVD technique or AC dielectrophoresis technique (Li et al., 2004; Li et al., 2005). These SWNTs were individual semiconducting nanotube bundles (Besteman et al., 2003; Bradley et al., 2004; Star et al., 2003), or SWNT networks (Bradley et al., 2005; Star et al., 2006). For the detection purpose, SWNTs working as sensing part were exposed to detection species, while a back gate (silicon or gold) (Kojima et al., 2005; Star et al., 2003) or a liquid gate (Chen et al., 2003, 2004) was used to measure the device responses to detection species.

In order to achieve higher sensitivity for specific species, the SWNTs in most of CNTFETs were functionalized with biological molecules, such as antibodies for specific recognition, ssDNA array for detection of DNA hybridization and enzymes for the study of enzymatic reaction, etc. As shown in Fig. 9.4(a), these biological molecules were observed to non-covalent adsorb on the SWNTs (Bradley et al., 2005; Byon and Choi, 2006; Chen et al., 2004; Kojima et al., 2005; Star et al., 2004; Star et al., 2006; Tang et al., 2006) via weak interactions such as hydrophobic interaction between the nanotubes and hydrophobic domains of biological molecules (Shim et al., 2002). The immobilization of biological molecules could also be implemented through a linking layer, such as carbodiimidazole-activated Tween 20 (CDI-Tween) (Chen et al., 2003; So et al., 2005), polyethyleneimine/polyethyleneglycol (PEI/PEG) (Rouhanizadeh et al., 2005; Star et al., 2003), 1-pyrenebutanoic acid succinimidyl ester (Besteman et al., 2003; Li et al., 2005), which could be coated on the nanotubes prior to the functionalization (Fig. 9.4(b)). These linking molecules bound one end of their molecule chains to SWNTs through van der Waals interactions, while their other end attached to the biological molecules through amide bond formation.

It has been reported that the CNTFET could also be functionalized at the back gate region instead on SWNTs (Maehashi et al., 2004; Takeda et al., 2005), which might affect the effect potential around SWNTs during experiments (Fig. 9.4(c)).

9.2.5 CNT-Based Biosensors with Immobilized Biological Molecules

In order to improve the sensitivity and selectivity of CNT-based biosensors for detecting of some specific species, a broad spectrum of biological molecules, including DNA and proteins (enzymes and antibodies, etc.), were immobilized on these biosensors. These biosensors can be grouped into two main categories: electrochemical biosensors and CNTFET-based electronic biosensors. In the

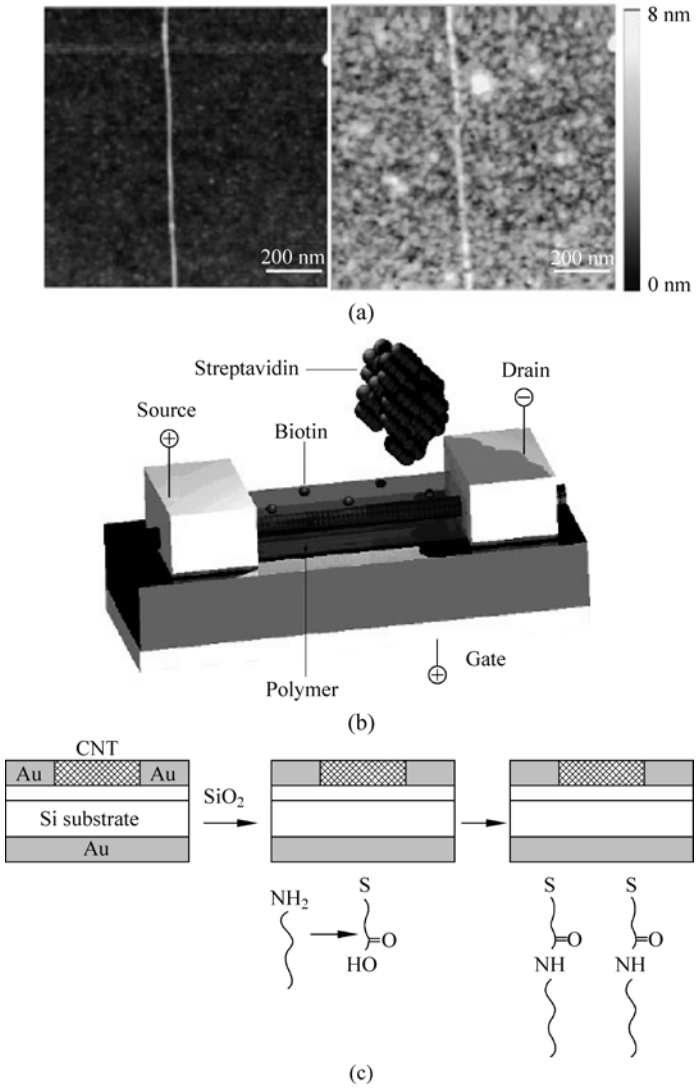

Figure 9.4 (a) AFM images of the SWNT channel of CNTFET before (left) and after (right) the direct adsorption of α-PSA. Reprinted from (Kojima et al., 2005) with permission. (b) Schematic of the CNTFET with a polymeric linking layer. A molecular receptor was used to functionalize such polymer for the recognition of a biomolecule. Reprinted from (Star et al., 2003) with permission. (c) Chemical modification scheme at the gold back gate region of CNTFET for the conjugation with biomolecules. Reprinted from (Maehashi et al., 2004) with permission

former one, CNTs mainly serves as transducer which converts the signals from the active biological molecules to the electrode substrate through electrochemical reactions. While in the latter as described in previous part, CNTs it plays a key role in sensing by a nanoscale transistor built by CNTs. Two techniques developed for immobilizing of probe molecules are covalent attachment and noncovalent attachment including direct adsorption and entrapment method. In the following, a brief overview of various immobilization techniques is given.

9.2.5.1 Direct Adsorption

Noncovalent functionalization of CNTs could be realized through weak interactions, e.g. hydrogen bonding, π-π stacking, electrostatic forces, van der Waals forces and hydrophobic interactions, which could be used to directly adsorb biological molecules on CNTs ((Trojanowicz, 2006) and references therein).

In electrochemical biosensors, pre-CNT-modification method, in other words, mixing CNT and biomolecules first and cast the electrode with the resulting mixture later, is a simple way for the direct adsorption of biological molecules on the electrode surface. A glucose biosensor was demonstrated by using a mixture of MWNTs, Nafion and Glucose Oxidase (Gox) as the GCE modifier (Tsai et al., 2005). A similar process could be seen from these biosensors based on CNT-composite electrode (Ly, 2006; Rubianes and Rivas, 2005). In addition, MWNT, Teflon and GOx were hand-mixed together in the dry state and the prepared composite electrode was used for glucose detection (Wang and Musameh, 2003b).

Biomolecules immobilization could also be achieved by post-CNT-modification, including incubation of the CNT-modified electrode in the biomolecules solution (Tang et al., 2004; Wang et al., 2003a; Wang et al., 2003b; Zhang et al., 2005), or drop cast small volume of the biomolecules solution on the CNT-modified electrode (Deo et al., 2005; Hrapovic et al., 2004; Joshi et al., 2005; Zhao et al., 2005). GOx/PtNP/CNT/graphite electrode for glucose detection was prepared by incubation the PtNP/CNT/graphite electrode in the enzyme GOx solution for 12 h, and then washed carefully with double-distilled water and dried (Tang et al., 2004). Another example was that an MWNT-modified electrode was dropped with 10 μL AChE solution for organophosphorus compound detection (Joshi et al., 2005).

In order to optimize performance of electrodes through adjusting controlled thickness, structural morphology, and biocatalyst loading, the LBL technique was also involved in direct adsorption of biomolecules on CNT-modified electrode. Alternate layers of GOx adsorbed MWNTs could be simply prepared by repeatedly immersing the electrode to MWNT solution and then GOx solution (Huang et al., 2006). This technique could also be applied to achieve alternate electrostatic adsorption of different charged components. MWNTs wrapped by positively charged poly(diallyldimethylammonium chloride) (PDDA) were assembled layer-

by-layer with negatively charged GOx on a chemically functionalized Au electrode (Zhao and Ju, 2006) (Fig. 9.5). Similar structures were presented (Guo et al., 2004; Liu and Lin, 2006).

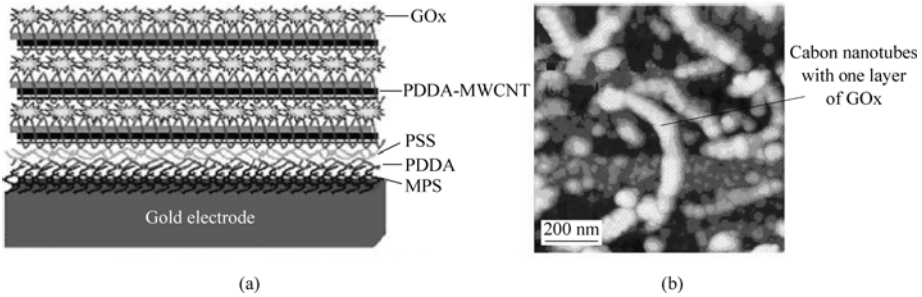

Figure 9.5 (a) Schematic of the multilayer membranes prepared by LBL technique. (b) AFM image of GOx-PDDA·MWNT (LBL)/PSS/PDDA/MPS membranes on gold electrode. Reprinted from (Zhao and Ju, 2006) with permission

Noncovalent adsorption of biological molecules could also be enhanced by using cross-linking molecules. After dropping the enzyme solution on Pt nanoparticle decorated SWNT/GCE, glutaraldehyde was applied on the resulting electrode to crosslink enzyme (Hrapovic et al., 2004). Similarly, GOx was immobilized onto PNR/MWNT multilayers modified GCE by crosslinking enzyme using glutaraldehyde (Qu et al., 2006). Moreover, prevention of the biological molecules from loss could also be implemented by covering a protective thin film say Nafion, which improved the anti-interferent ability of relevant electrodes simultaneously (Tang et al., 2004).

Switch intention to electronic biosensors based on CNTFET structure, biological molecules were found to directly adsorb on the SWNTs if a linking molecule layer was not introduced (Bradley et al., 2005; Byon and Choi, 2006; Chen et al., 2004; Kojima et al., 2005; Star et al., 2004; Star et al., 2006; Tang et al., 2006).

9.2.5.2 Entrapment

Biological molecule entrapment is other method to noncovalently immobilize biological molecules on the electrode surface. It can be achieved in two ways, i.e., encapsulation and electropolymerization.

In encapsulation methodology, biological molecules were immobilized within hydrogels or sol-gel materials while retained their native bioactivity. Hydrogel and sol-gel have excellent properties including high binding capacity with electrodes, large surface area, and an improvement in the electrical communication between electrodes and biological molecules, etc. These positive aspects enhance the sensor response. The encapsulation can be realized by pre-CNT-modification method, which incorporate biological molecules and CNTs into the hydrogel or the sol-gel matrix at first and subsequently casting this composite onto the

support electrode (Fig. 9.6(a)) (Joshi et al., 2005). Two glucose biosensors were prepared in this scheme by encapsulating GOx, as well as CNTs, into a sol-gel matrix (Kandimalla et al., 2006; Yang et al., 2006). Alternatively, in post-CNT-modification method, biological molecules incubated in hydrogel or sol-gel matrix could be brought onto the CNT-modified electrodes (Fig. 9.6(b)), as suggested for a glucose biosensor (Salimi et al., 2004) and cholesterol biosensor (Shi et al., 2005).

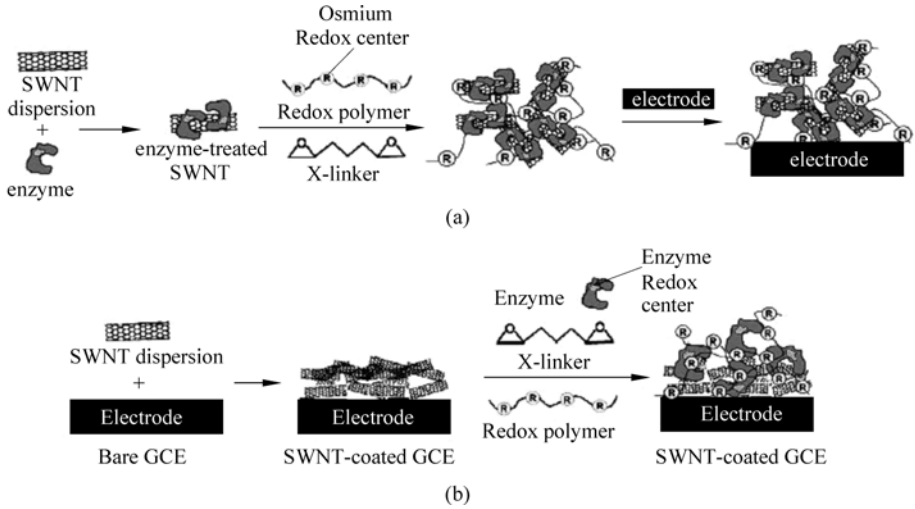

Figure 9.6 Schematic of biomolecules encapsulation with CNT: (a) fabrication of sensor via pre-CNT-modification method. CNTs were first incubated with an enzyme solution, and then the hydrogel or sol-gel was introduced to form a matrix, which is later brought on a substrate. (b) fabrication of sensor via post-CNT-modification method. A film of CNTs was first cast onto a support electrode. Subsequently, a hydrogel or sol-gel composite containing enzyme is casted on such a CNT-coated electrode. Reprinted from (Joshi et al., 2005) with permission

Biological molecules could also be embedded into a polymer matrix simply by mixing them with the monomer. The mixture is then electropolymerized on support electrode. The intimate contact between the biocomponent and polymer enable an efficient signal transduction. A highly sensitive glucose sensor was constructed on an MWNT modified electrode based on the entrapment of GOx on poly-*o*-aminophenol (POAP)-electropolymerized matrix (Ye et al., 2005). PPy was other polymer used for this purpose, since it can be electropolymerized at neutral pH and allow the entrapment of a wide range of biocatalysts. The pyrrole electropolymerization aided GOx immobilization could be performed not only on non-oriented CNT-modified electrode via mixing pyrrole, GOx and oxidized MWNTs (Wang and Musameh, 2005a), but also on aligned CNT arrays (Gao et al., 2003). Figure 9.7 shows SEM images of such a PPy-coated CNT array.

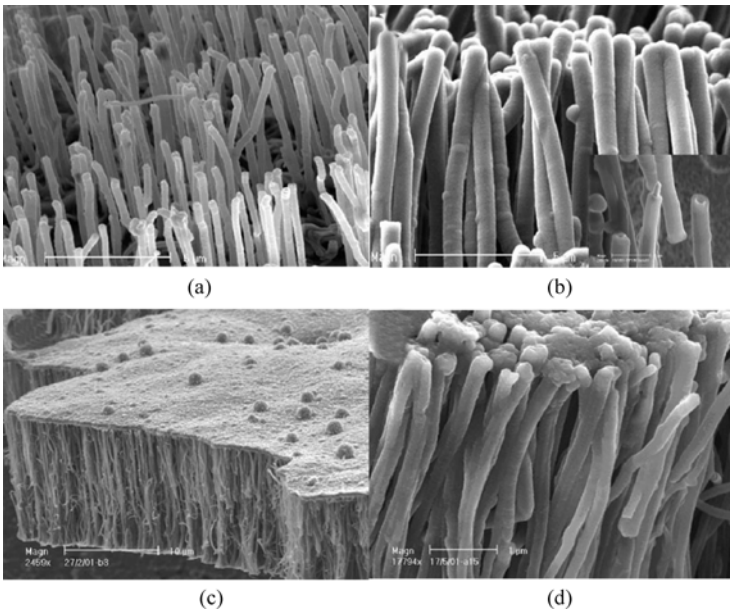

Figure 9.7 SEM images of: (a) pristine CNT array. (b) aligned PPy-CNT coaxial nanowires. (c) PPy only deposited on the top surface of CNTs due to the high density of tube array. (d) polymer formed on both walls and top surface of the CNT array. Reprinted from (Gao et al., 2003) with permission

9.2.5.3 Covalent Attachment

Typically, the biological molecules are randomly distributed on the electrode surface if noncovalent immobilization method is utilized. In addition, the direct adsorption may be not strong so that some biological molecules may loss during analysis. In contrast, covalent attachment method could produce a robust immobilization of biological molecules. And the distribution of biological molecules could also be somewhat controlled by using CNTs as framework. For instance, enzyme GOx was found to covalently attach to the broken tips of vertically aligned MWNTs via amide linkages between amine residues on GOx and carboxylic groups on the MWNT tips in the presence of coupling agents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Lin et al., 2004). Through amide linkage, it has been confirmed that flavin adenine dinucleotide (FAD) covalently bound to the tips of self-assembled MWNT arrays and then apo-GOx was reconstituted on the FAD units. This structure can enhance direct electron transfer between electrode and redox active center of GOx (Liu et al., 2005; Patolsky et al., 2004). Figure 9.8 illustrates the fabrication scheme and relevant AFM images of such an electrode.

In comparison, researchers did not immobilize biological molecules directly on SWNTs through covalent bond in CNTFET-based electronic biosensors. Because they believed that oxidation of SWNTs broke up the aromaticity of benzene units

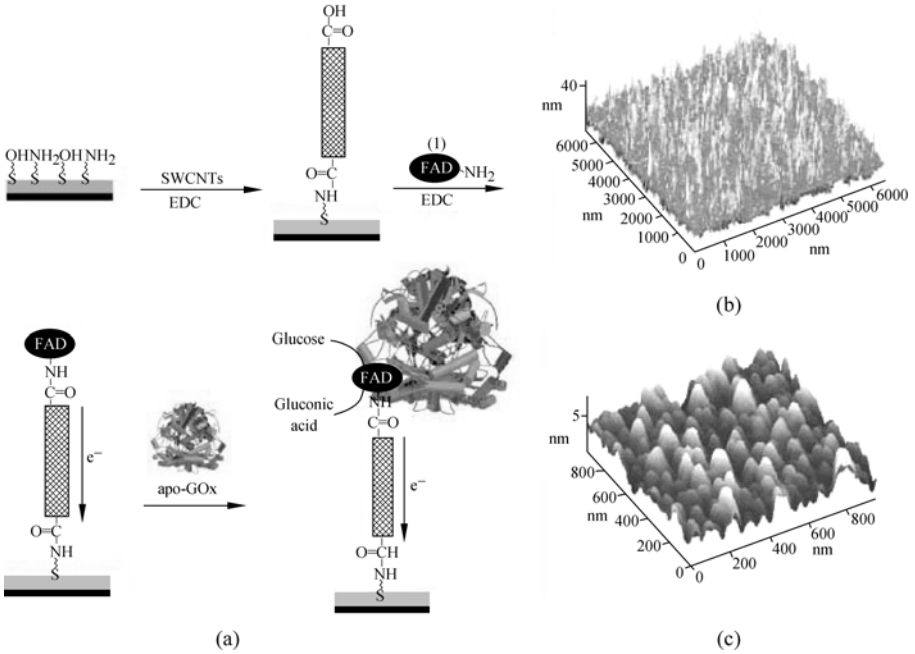

Figure 9.8 (a) Schematic of self-assembled SWNT array and then covalent binding with Gox; (b) AFM image of self-assembled SWNT array through covalently linking to a cystamine/2-thioethanol monolayer functionalized Au electrode after 90 min of coupling; (c) AFM image of the GOx reconstituted on the FAD-functionalized CNTs array. Reprinted from (Patolsky et al., 2004) with permission

of SWNTs and imparted carboxyl and ether substituents on sidewall of SWNTs, which may affect the intrinsic electronic property of SWNT. Instead, biological molecules were covalently attached to the SWNTs via linking molecules, of which other end bound to SWNTs through van der Waals interactions or π-π stacking (Besteman et al., 2003; Chen et al., 2003; Li et al., 2005; Maehashi et al., 2004; Rouhanizadeh et al., 2006; So et al., 2005; Star et al., 2003; Takeda et al., 2005).

9.3 Electrochemical Detection of Biomolecules

Electrochemical techniques of detecting biomaterials in solutions are attractive because of their simplicity and low-cost. In this section we discuss various electrochemical biosensors based on CNTs. In the first part we discuss the assessment criteria for different biosensors. We will present a large number of biosensors to detect various biomolecules in the second part. We also compile glucose biosensors modified by CNT in Table 9.1, and CNT-modified DNA biosensor in Table 9.2. Other biomolecule-functionalized electrochemical biosensors are summarized in Table 9.3. Biosensors without biomolecule-functionalization are summarized in Table 9.4.

Table 9.1 Summary of CNT-based electrochemical glucose biosensors and their important parameters

No. & Ref. evence	Electrodes	Design principles	Sensitivity $[(mmol/L)^{-1}]$	Linear range [mmol/L]	Res-ponse time [s]	Stability
G1 (Male et al., 2004)	CuNP-SWNT-Nafion/ GCE and CuNP-SWNT-Nafion/Cu	Nanoparticle decoration	256 μA	2.5×10^{-4} – 0.5	10	
G2 (Tang et al., 2004)	Nafion/Gox/PtNP/CNT/ graphite	Nanoparticle decoration, direct adsorption	14 μA	0.1 – 13.5	<5	73.5% after 22 days
G3 (Hrapovic et al., 2004)	Gox/PtNP-SWNT-Nafion/ GCE	Nanoparticle decoration, direct adsorption	2.11 μA	0.0005 – 5	3	
G4 (Wang et al., 2003b)	Gox/MWNT-array/Au	Oriented modification, direct adsorption	1.75 μA	0.05 – 13	<16	96.3% after 1 month, 94.4% after 2 month, and 91.2% after 3 month
G5 (Yang et al., 2006)	Gox/PtNP-Silicate-SGC-MWNTPE	Nanoparticle decoration, direct adsorption	1.11 μA	1 – 25	<15	90% after 3 days, and 80% after 1 month
G6 (Ye et al., 2005)	POAP-Gox/FePc-MWNT/ GCE	Entrapment	0.735 μA	0.0005 – 4	<8	120 days
G7 (Qu et al., 2006)	Gox/PNR-cMWNT (LBL)/GCE	Direct adsorption	0.6 μA	0.05 – 10	<10	Constant in first 17 days, and 85% after 1 month
G8 (Tsai et al., 2005)	Gox-MWNT-Nafion/GCE	Entrapment	0.33 μA	0.025 – 2	<3	
G9 (Gao et al., 2003)	Gox-Ppy/MWNT-array/Au	Oriented modification, Entrapment	0.31 μA	2.5 – 20		70% after 3 days
G10 (Ye et al., 2004)	MWNT-array/GCE	Oriented modification	0.22 μA	0.002 – 11	<10	

Continued

No. & Ref. evence	Electrodes	Design principles	Sensitivity [(mmol/L)$^{-1}$]	Linear range [mmol/L]	Res-ponse time [s]	Stability
G11 (Salimi et al., 2004)	Gox-SGC/MWNT/BPPG	Entrapment	0.196 μA	0.2 – 20	<5	3 weeks
G12 (Wang and Musameh, 2003b)	Gox/Teflon-MWNT	CNT composite electrode, direct adsorption	0.14 μA	2 – 12	25	
G13 (Zhao and Ju, 2006)	Gox-PDDA·MWNT(LBL)/ PSS/PDDA/MPS/Au	Entrapment	44 nA	0.058 – 5	<8	82% after 5 weeks
G14 (Huang et al., 2006)	Gox-MWNT(LBL)/graphite	Entrapment	39 nA	0.5 – 15	6.7	
G15 (Kandimalla et al., 2006)	Gox-FMC-BSA-MWNT-SGC/GCE	Entrapment	18 nA	0.05 – 20	25	96% after 110 days
G16 (Luque et al., 2006)	Gox-CuNP-MWNTPE, Gox-IrNP-MWNTPE	CNT composite electrode, nanoparticle decoration, direct adsorption	8.3 nA (Cu), 7.1 nA (Ir)	1.8 – 12 (Cu), 0 – 20 (Ir)	10 (Cu), 12 (Ir)	Constant in 2 months, and 24% after 6 months
G17 (Lin et al., 2004)	Gox-cMWNT-array/Si	Oriented modification, covalent attachment	~2.5 nA	0.08 – 30	20 – 30	
G18 (Wang and Musameh, 2005a)	Ppy-Gox-cMWNT/GCE	Entrapment	2.33 nA	0.2 – 50	15	

The blanks in the columns indicate that these data were not available in corresponding references. If not indicating 'oriented modification' in the column of 'Design priciples', 'non-oriented modification' is a default design principle.

C carbozylized, BPPG basal plane pyrolytic graphite, BSA bovine serum albumin, FePc iron phthalocyanine, FMC ferrocenemonocarboxylic acid, GCE glassy carbon electrode, Gox glucose Oxidase, LBL layer-by-layer technique, MPS 3-mercapto-1-propanesulfonic-acid, NP Nanoparticle, PDDA poly(dimethyldiallylammonium chloride), MWNTPE MWNTpaste electrode, PNR poly(neutral red), POAP poly-*o*-aminophenol, Ppy polypyrrole, PSS poly(sodium 4-styrenesulfonate), SGC sol-gel composite.

Table 9.2 Summary of CNT-based electrochemical DNA biosensors

Objective	No.	Sensors	Design Principles	Ref.
DNA hybridization	D1	DNR; ssDNA-cMWNT/GCE	Covalent attachment	(Cai et al., 2003a)
	D2	ssDNA-Ppy/cMWNT/GCE	Entrapment	(Cai et al., 2003b)
	D3	$Ru(bpy)_3^{2+}$; ssDNA-cMWNT-array-SiO_2/Si	Oriented modification, covalent attachment	(Koehne et al., 2003; Koehne et al., 2004; Li et al., 2003)
	D4	ssDNA/cMWNT-DNA/CPE	Covalent attachment	(Kerman et al., 2004)
	D5	DNR; cMWNT-Ppy/GCE; ssDNA/RSH/MNP	CNT composite electrode	(Cheng et al., 2005)
	D6	MWNT/GCE; probe-ssDNA-MNP; target-ssDNA-ALP		(Wang et al., 2004b)
	D7	MB; ssDNA-cMWNT-array/Au	Oriented modification, covalent attachment	(Wang et al., 2004)
	D8	DNR; ssDNA-cMWNT-PtNP-Nafion/GCE	Covalent attachment	(Zhu et al., 2005)
	D9	MWNT/GCE; probe-ssDNA1-cCNT-ALP; probe-ssDNA2-MNP		(Wang et al., 2004a)
	D10	MWNT/GCE; probe-ssDNA1/PSS/ALP-PDDA (LBL)/SWNT; probe-ssDNA2-MNP		(Munge et al., 2005)
DNA and nucleic acids	D11	SWNT/GCE		(Wang et al., 2004)
	D12	MWNTPE	CNT composite electrode	(Pedano and Rivas, 2004)
	D13	MB; DNA/MWNT-chitosan/graphite	Direct adsorption	(Li et al., 2005)

If not indicating 'oriented modification' in the column of 'Design priciples', 'non-oriented modification' is a default design principle.

c carbozylized, ALP alkaline phosphatase, CPE carbon paste electrode, DNR daunomycin, GCE glassy carbon electrode, LBL layer-by-layer technique, MB methylene blue, MNP magnetite nanoparticle, MWNTPE MWNTpaste electrode, NP nanoparticle, PDDA poly(diallyldimethylammoniumchloride), PPy polypyrrole, PSS poly(sodium 4-styrenesulfonate), RSH mercapatoacetic acid, ssDNA single-stranded DNA.

Table 9.3 Summary of biomolecules-functionalized CNT-based biosensors

Analyte	Occurrence	Biomolecule functionzlied on electrode	Electrodes	Design Principles	Ref. No.
Cholesterol	Blood	Cholesterol oxidase (ChOx)	PDDA-ChOx(LBL)/ cMWNTs/Au	Entrapment	(Guo et al., 2004)
			ChOx/MWNT/SPE	Direct adsorption	(Li et al., 2005)
			ChOx-SGC/PtNP/ CNT/graphite	Nanoparticle decoration, entrapment	(Shi et al., 2005)
			ChOx/PVA/MWNT-array/Si	Oriented modification, direct adsorption	(Roy et al., 2006)
Choline	Tissues	Choline oxidase (ChOx)	ChOx-SGC/MWNT/ Pt	Entrapment	(Song et al., 2006)
Dopamine	Brain tissue, blood	DNA	DNA/CNTPE	CNT composite electrode	(Ly, 2006)
DNA	Bioassays	ssDNA	See Table 9.2		
Glucose	Blood, body fluids	Glucose oxidase (GOx)	See Table 9.1		
Lactate	Human sweat	Lactate oxidase (LOx)	LOx/cSWNT/GCE LOx/cSWNT/Si/ITO	Direct adsorption	(Weber et al., 2006)
			LOx/CNTPE	CNT composite electrode, direct adsorption	(Rubianes and Rivas, 2005)
Organophos-phate compounds	Agriculture, environment	Acteylcholin-esterase (AChE)	AChE/MWNT/SPE	Direct adsorption	(Joshi et al., 2005)
			PDDA-AChE(LBL) /MWNT/GCE	Entrapment	(Liu and Lin, 2006)
		Organophos-phorus hydrolase (OPH)	OPH/MWNT-Nafion/GCE	Direct adsorption	(Deo et al., 2005)
Phenolic compounds	Water, food products	Polyphenol oxidase (PPOx)	PPOx/CNTPE	Direct adsorption	(Rubianes and Rivas, 2005)
		Tyrosinase (Ty)	Nafion/Ty/SWNT/ GCE	Direct adsorption	(Zhao et al., 2005)
Putrescine	Tissue, pharmacy	Putrescine oxidase (POx)	Glutaraldehyde/ POx/APTES-MWNT-Nafion/GCE	Entrapment	(Luong et al., 2005)

If not indicating 'oriented modification' in the column of 'Design priciples', 'non-oriented modification' is a default design principle.

c carbozylized, APTES γ–aminopropyltriethoxysilane, CNTPE CNT paste electrode, GCE glassy carbon electrode, ITO indium tin oxide, LBL layer-by-layer technique, NP nanoparticle, PDDA poly(diallyldimethylammoniumchloride), PVA polyvinyl alcohol, SGC sol-gel composite, SPE screen-printed electrode.

Table 9.4 Summary of biomolecule-free CNT-based biosensors for various biological molecules

Analyte	Occurrence	Electrodes	Ref. No.
L-Cysteine	Biological system	Pt/CNT/graphite	(Fei et al., 2005)
Cytochrome *c*	Biological system	aSWNT/GCE	(Wang et al., 2002)
		AuNP/MWNT/DHP/Au	(Wu and Hu, 2005)
Dopamine	Brain tissue, blood	CNT/bromoform/glass-tube	(Britto et al., 1996)
		MWNT-Nafion/CFE	(Hocevar et al., 2005)
		PSS/SWNT/GCE	(Zhang et al., 2006)
Folic acid	Tissue, food products	MWNT/Au	(Wei et al., 2006)
Glutathione	Biological system	CNT-array	(Tang et al., 2006)
Indole-3-acetic acid	Plant cells	MWNT/GCE	(Wu et al., 2003)
Lincomycin	Pharmacy	MWNT-DHP/GCE MWNT-SDBS/GCE MWNT-Nafion/GCE	(Wu et al., 2006)
Morphine	Blood, body fluids	MWNT/GCE	(Salimi et al., 2005)
NADH	Living cells	MWNT/GCE	(Musameh et al., 2002)
		TB/MWNT/BPPG AC/MWNT/BPPG	(Lawrence and Wang, 2006)
		TBO-CHIT-MWNT/GCE	(Zhang and Gorski, 2005b)
		AZU-CHIT/MWNT/GCE	(Zhang and Gorski, 2005a)
		CNTEC	(Pumera et al., 2006)
Nitric oxide	Biological system	Nafion/MWNT/GCE	(Wu et al., 2002)
		Myb/MWNT/GCE	(Zhang et al., 2005)
Phenolic compounds	Water, food products	MWNT-Nafion/GCE	(Wang et al., 2003a)
Procaine	Pharmacy	MWNT/GCE	(Wu et al., 2006)
Theophylline	Pharmacy	cMWNT/GCE	(Zhu et al., 2005)
Quercetin	Flavor, pharmacy	MWNT/Ch/graphite	(Jin et al., 2006)
Rutin	Plants	MWNT- β-CD/GCE	(He et al., 2006)
		SWNT/Au	(Zeng et al., 2006)
Thiocholine	Environment	CNT/GCE	(Liu et al., 2005)

a actived, AC azure C, AZU Azure dye, BPPG basal plane pyrolytic graphite, CD cyclodextrin, CFE carbon fiber electrode, Ch choline, CHIT chitosan, CNTEC CNT-epoxy composite, DHP dihexadecylphosphate, GCE glassy carbon electrode, Myb myoglobin, NP nanoparticle, PSS poly(styrene sulfonic acid) sodium salt, SDBS sodium dodecylbenzenesulfonate, TB toluidine blue, TBO toluidine Blue O.

9.3.1 Assessment Criteria of Sensors

When designing various CNT-based biosensors for practical application, several measurement parameters are of importance. Detection range is the most essential one, which determines the sensitivity, specificity and dynamic range required by the sensors. For example, normal level of glucose in human vein is 3.89 – 6.11 mmol/L and thus the fasting plasma glucose level at or above 7.0 mmol/L is diagnosed as diabetes. Because of this, a practical glucose sensor should cover a detection range from a few μmol/L to 15 mmol/L. A cholesterol sensor should be sensitive in the range of 50 – 400 mg/dL (1.3 – 10.4 mmol/L), because the desirable level of LDL cholesterol is considered to be less than 100 mg/dL (2.6 mmol/L) and a patient who has a level of larger than 240 mg/dL (>6.2 mmol/L) of cholesterol is running a high risk of heart disease. As some biomolecules, like enzymes, degrade during storage, a biosensor with high stability is a vital characteristic. Of course, specificity, sensitivity, accuracy, response time and reproducibility, etc, are also very critical to electrochemical sensors.

9.3.2 Electrochemical Biosensors

9.3.2.1 Glucose

Glucose is the principal circulating sugar in the blood and the major energy source of the body. A high fasting blood sugar level is an indication of prediabetic and diabetic conditions. As a result, a tight monitoring of blood glucose level is one of the most frequently performed routine analyses in clinics and hospitals.

Conventionally, enzyme GOx is used in biosensors to detect the concentration of glucose. GOx catalyzes the oxidation of β-D-glucose into D-glucono-1,5-lactone, while oxygen (O_2) acts as final electron acceptor and then is reduced as hydrogen peroxide (H_2O_2), which is electrochemically detectable. The concentration of glucose is determined by monitoring the resulting charge and current through enzyme.

We compiled the design principles and performance characteristics of reported CNT-based biosensors in a descent order of sensitivity in Table 9.1, where the performance of a variety CNT-based glucose sensor are listed. One should note that the total electrode current was referred in comparison of the sensitivities because the surface area of electrodes was seldom reported in most of papers.

One can see that four of the top five most sensitive glucose biosensors were decorated with metallic nanoparticles. The sensor with Cu Nanoparticle (G1) exhibited a highest sensitivity of 256 $\mu A/(mmol \cdot L^{-1})$ as shown in Fig. 9.9(a). But the linear range from 2.5×10^{-4} to 0.5 mmol/L, was much lower than clinical detection level of glucose. Pt nanoparticle-decorated sensor G2, G3, and G5 showed preferable linear detecting range. Figure 9.9(b) illustrated the response of sensor G5 to glucose with and without Pt nanoparticle attachment. In addition,

both G2 and G3 used a pre-selective membrane Nafion to improve the selectivity of such sensors. The difference is that G3 utilized Nafion as solubilizing agent of CNTs, while a thin Nafion layer was coated on the surface of CNT-modified electrode in G2. Compared with another nanoparticles decorated CNT-based glucose biosensors (G1, G3, G5 and G16), G2 showed a superior performance with relatively high sensitivity and clinically compatible detection range.

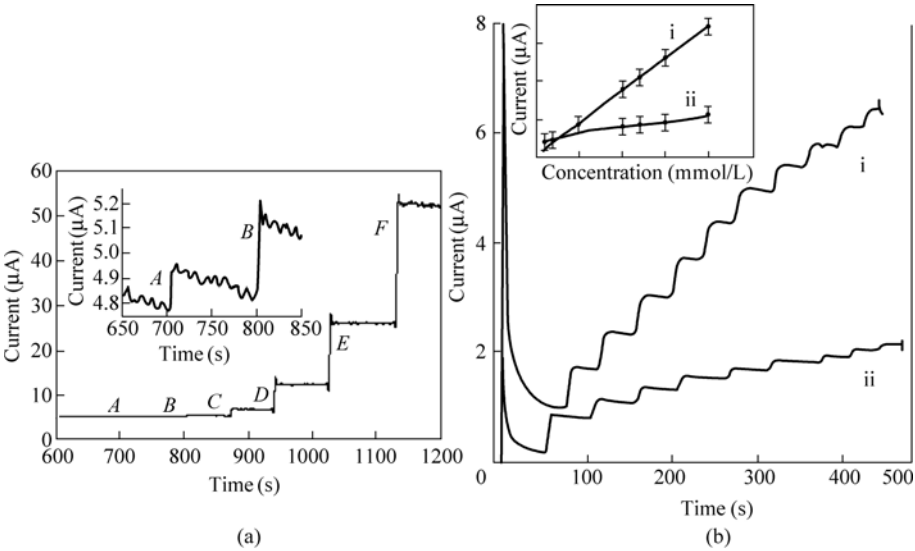

Figure 9.9 (a) The performance of sensor G1 with CuNP-SWNT-Nafion/GCE (refer to Table 9.1) in amperometrically detecting glucose of different concentrations, 0.5, 1.0, 5, 20, 50, 100 μmol/L at 0.65 V vs. Ag/AgCl (3 mol/L NaCl). (Inset) addition of 0.5 μmol/L and 1.0 μmol/L of glucose, respectively. Reprinted from (Male et al., 2004) with permission. (b) Real-time monitor of successive addition of 2 mmol/L glucose at the biosensors (i) with Pt nanoparticles and (ii) without Pt nanoparticles measured at 0.1 V vs. SCE. (Inset) the calibration curve for the glucose addition. Reprinted from (Yang et al., 2006) with permission

Immobilization of GOx on the electrode surface using entrapment methods (electropolymerization and encapsulation) was also commonly adopted. G6 immobilized GOx on a poly-*o*-aminophenol (POAP) electropolymerized electrode surface. GOx was immobilized in a Nafion matrix in G8. Both G9 and G18 entrapped GOx within an electropolymerized PPy film, but G18 exhibited a much lower sensitivity. This could be attributed to the fact that the MWNTs were in direct contact with the substrate of G9, while the CNTs are just suspended and loosely contacted with the substrate of G18. G11 and G15 both encapsulated GOx into a sol-gel composite, and the resulted sensitivities were 196 and 18 nA, respectively. In G13 and G14, LBL technique was utilized to entrap GOx with CNTs at the surface of the electrode. In this category of sensors, G11 is more practical for clinical application.

In term of the response time, Pt Nanoparticle-decorated sensors S2 and S3 demonstrated the fastest response of less that 5 s with high sensitivity. Some biosensors prepared by entrapment of GOx, say G8 and G11, also showed a fast response. However, the sensors without GOx, such as G1 and G10, had relatively slow response.

9.3.2.2 Cholesterol

Cholesterol is a fatty lipid that is a primary component of the cell membranes and a precursor to steroid hormones. Cholesterol is also found in the blood circulation of humans. Its concentration in the blood plasma can influence the pathogenesis of some conditions, such as the development of atherosclerotic plaque and coronary artery disease. In this sense, the determination of cholesterol concentration in clinical diagnosis is essential. To prepare amperometric cholesterol biosensor, Guo and coworkers utilized LBL technique to deposit cholesterol oxidase (ChOx) onto carboxylized-MWNTs/Au electrode (Guo et al., 2004). The device detection range for cholesterol measurement was from 0.2 up to 6 mmol/L. A biosensor based on MWNTs/screen-printed-carbon electrodes was also used to detect cholesterol in the range of 100 – 400 mg/dL (Li et al., 2005). Moreover, by immobilizing ChOx with sol-gel on Pt nanoparticles decorated CNT/graphite electrodes, Shi et al. developed a sensor for the detection ranging from 4 to 100 μmol/L (Shi et al., 2005). In addition, vertically-aligned MWNTs modified with polyvinyl alcohol (PVA) was reported to detect cholesterol (Roy et al., 2006).

9.3.2.3 Choline

Choline, often classified in the vitamin B complex, is a natural amine and essential for cardiovascular and brain function and for cellular membrane composition and repair. An amperometric sensor for choline detection has been demonstrated by immobilization of choline oxidase within aqueous sol-gel-based composites on an MWNTs coated Pt electrode (Song et al., 2006). The choline sensor exhibited a wide measurement range (5 – 100 μmol/L), fast response (< 8 s) and low detection limit (0.1 μmol/L).

9.3.2.4 L-Cysteine

Cysteine is one of the 20 amino acids commonly found in animal proteins. Its L-stereoisomer participates in the biosynthesis of mammalian protein. Cysteine is often used to produce of various flavors. Therefore, determination of L-cysteine is very important in food, pharmaceutical and personal care industries. But due to slow electron transfer, conventional electrodes are not satisfactory. In contrast, Pt decorated MWNT/graphite electrodes were capable of sensing L-cysteine with a large detection range of 0.5 – 100 μmol/L (Fei et al., 2005).

9.3.2.5 Cytochrome *c*

Cytochrome *c*, or cyt *c*, is a highly conserved protein, can be found in plants,

animals, and many unicellular organisms. It is also a small heme protein found loosely associated with the inner membrane of the mitochondrion. However, detection of cyt *c* always faces difficulty because of the non-effective detection attributed to the protein denaturation at electrode surface and subsequently extremely slow electron-transfer kinetics. A CNT based cyt *c* sensor was demonstrated with activated SWNTs film modified GCE (Wang et al., 2002). The peak current of such sensor increased linearly with the concentration of cyt *c* in the range of from 30 to 700 μmol/L. In addition, colloid Au (diameter of 20 nm) immobilized MWNTs/Au electrode was prepared for the same purpose (Wu and Hu, 2005).

9.3.2.6 Dopamine

Dopamine is a kind of chemicals naturally produced in the body. In human brain, dopamine functions as a neurotransmitter and a neurohormone. Thus it is essential to the normal functioning of the central nervous system. Dopamine is also used as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood pressure. Obviously, precise determination of dopamine in brain tissues is demanded in clinical diagnoses. The first reported biosensor using CNTs modified electrode was a dopamine biosensor by Britto et al. (Britto et al., 1996). Figure 9.10(a) depicts the differential pulse voltammetry (DPV) response of this sensor for various concentrations of dopamine. In addition to this, CNT-modified electrodes, combined with negatively charged pre-selective membrane, including Nafion (Hocevar et al., 2005) and poly(styrene sulfonic acid) (PSS) (Zhang et al., 2006), were used to detect dopamine in the presence ascorbate and ascorbic acid. Hocevar et al. invented a disposable dopamine microsensor utilizing MWNT and Nafion modified carbon fiber microelectrode to achieve a linear detection range from 2 to 20 μmol/L and a detection limit of 70 nmol/L towards dopamine (Hocevar et al., 2005). The dopamine sensor based on PSS/ SWNT/GCE exhibited good performance, such as a large determination range (16 nmol/L – 600 μmol/L) and a low detection limit (8 nmol/L) (Zhang et al., 2006). Moreover, a DNA immobilized CNT composite electrode was used to detect dopamine with an ultra small detection limit of 0.021 nmol/L (Ly, 2006) (Fig. 9.10(b)). Polyphenol oxidase (PPOx)-modified CNTPE was also used to detect dopamine with a detection limit 1 μmol/L (Rubianes and Rivas, 2005).

9.3.2.7 Folic acid

Folic acid (FA) is one of forms of a water-soluble vitamin B that is important for the formation of red and white blood cells. It occurs naturally in food and can also be taken as supplements. An FA sensor with MWNTs/Au electrodes was developed by Wei and coworkers (Wei et al., 2006). Under optimized conditions, the voltammetric sensor responded linearly to the concentration of FA from 0.02 – 1 μmol/L.

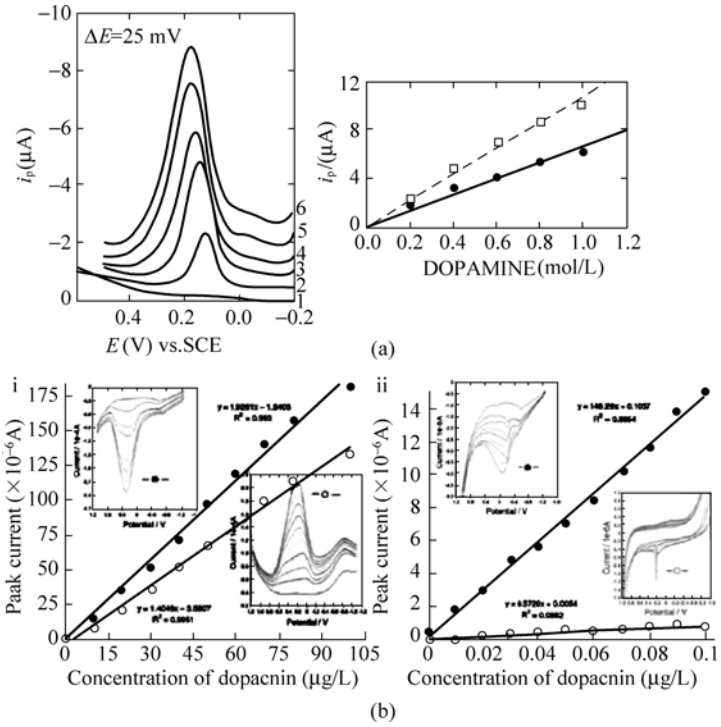

Figure 9.10 (a) DPV responses (left) and the corresponding calibration plot (right) of dopamine in PBS at a MWNT composite electrode. Marks from No. 1 to 6 corresponded to 0, 200, 400, 600, 800, 1000 mmol/L addition of dopamine. Reprinted from (Britto et al., 1996) with permission. (b) the calibration curve of square-wave (SW) stripping voltammetry of dopamine at various concentrations: (i) 0, 10, 20, 30, 40, 50, 60, 70, 80 and 100 mg/L; and (ii) 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1 and 0.11 μg/L at optimum conditions. (Inset) (i) anodic and cathodic SW stripping voltammograms; (ii) SW anodic and CV voltammograms. Reprinted from (Ly, 2006) with permission

9.3.2.8 Glutathione

Glutathione (GSH) is a tripeptide of the amino acids glycine, cystine, and glutamic acid existing widely in plants and animal tissues and constructing reduced and oxidized forms in biological oxidation-reduction reactions. A well-aligned CNT arrays grown directly on graphite substrate was used as working electrode to detect GSH (Tang et al., 2006). It had excellent electrochemical activity and good anti-fouling property for direct electrochemical oxidation of GSH, with a response time of 5 s, a low detection limit of 0.2 μmol/L and a high sensitivity of 254.8 nA/(μmol · L^{-1} · cm^2).

9.3.2.9 Indole-3-acetic acid

Indole-3-acetic acid, also known as IAA, is generally considered to be the most

important native auxin, promoting elongation of stems and roots. An amperometric sensor for IAA was prepared by coating MWNTs onto GCE (Wu et al., 2003). The linear response with the concentration of IAA and the detection limit was 0.1 – 50 μmol/L and 0.02 μmol/L, respectively.

9.3.2.10 Lactate

Lactate is constantly produced during normal metabolism and exercise. It is one of several molecules and ions in human sweat. Thus determination of lactate would help us to monitor human physiological conditions. GCE and silicon/indium tin oxide (Si/ITO) substrate modified with lactate oxidase (LOx)-immobilized carboxylized SWNTs was used to detect lactate respectively (Weber et al., 2006). It had a linear response in the range of 1 – 4 mmol/L and 10 – 50 mmol/L, respectively. Lactate could also be detected using LOx modified CNT paste electrodes with a detection limit of 300 μmol/L (Rubianes and Rivas, 2005).

9.3.2.11 Lincomycin

Lincomycin is an antibiotic derived from cultures of the bacterium *Streptomyces lincolnensis,* used in the treatment of certain penicillin-resistant infections. The detection of lincomycin by GCE modified with MWNTs, which were stabilized by DHP, SDBS and Nafion, respectively, was demonstrated. (Wu et al., 2006). The MWNT-DHP/GCE was found to have a linear response from 0.45 to 150 μmol/L and a detection limit of 0.2 μmol/L.

9.3.2.12 Morphine

Morphine is an extremely powerful opiate analgesic drug and the principal active agent in opium. Determination of morphine in biological samples is meaningful to monitor drug concentration and therapeutic level in humans. An MWNT/GCE based morphine electrochemical sensor with a linear detection range of 0.5 – 150 μmol/L, a calculated detection limit of 0.2 μmol/L, and a sensitivity of 10 nA/(μmol/L) has been reported (Salimi et al., 2005).

9.3.2.13 NADH

β-Nicotinamide adenine dinucleotide (NADH) is of importance in living cells because NAD^+/NADH couple is a cofactor system for several hundreds of dehydrogenase enzymes. Conventional electrodes determining oxidation of NADH, however, are highly irreversible and poorly stable because of a large overpotential and surface fouling by the reaction products. These problems were overcome by utilizing MWNT/GCE sensors (Musameh et al., 2002). The overvoltage of NADH oxidation was lower by ~0.5 V compared to a bare GCE, and more that 90% initial activity remained after 60 min stirring in NADH solution. Furthermore, the synergistic effects of CNTs and redox mediators (RM) towards the NADH oxidation were reported in several papers (Lawrence and Wang, 2006, Zhang and

Gorski, 2005a, 2005b). Zhang and Gorski. covalently attached RM Toluidine Blue O (TBO) and Zure dye (AZU) to polysaccharide chain of chitosan (CHIT) and interspersed with MWNTs. Such TBO-CHIT-MWNT and AZU-CHIT-MWNT film electrode decreased the overpotential for the mediated process, while amplified the NADH current by several tens of times and reducing the response time to ~5 s (Zhang and Gorski, 2005a, 2005b). Lawrence and Wang. adsorbed the phenothiazine dyes (toluidine blue and azure C) on the MWNTs modified basal plane pyrolytic graphite (BPPG) electrode, which promoted low potential, sensitive and stable determination of NADH (Lawrence and Wang, 2006). An MWNTs-epoxy composite electrode was also found to have a better performance toward NADH than graphite composites electrode (Pumera et al., 2006).

9.3.2.14 Nitric oxide

Nitric oxide (NO) is an important signaling molecule in the body of mammals including humans. Its biological functions at low concentrations are as signals in many diverse physiological processes such as blood pressure control, neuro-transmission, learning and memory. Applying Nafion/MWNTs/GCE, the NO could be detected linearly in the range of $2\times10^{-7}-1.5\times10^{-4}$ mol/L with a detection limit of 8.0×10^{-8} mol/L (Wu et al., 2002). The Nafion thin film was used to eliminate the interference from anions, especially nitrite. The same group also reported to utilize myoglobin (Myb) functionalized MWNTs/GCE for the same purpose (Zhang et al., 2005). Such a sensor was based on the electrochemical reduction of NO, hence avoided the possible interference of oxidizable substance in solution. The performance of Myb/MWNTs/GC based biosensor was nearly the same with previous one.

9.3.2.15 Organophosphate compounds

Organophosphate (OP) compounds are a diverse group of chemicals used in domestic and industrial settings, including insecticides, nerve gases, etc. Accordingly, early and rapid detection of these toxic agents in food and environment is on a growing demand in homeland security and healthcare. During the past decade, biosensors based on inhibition of acetylcholine esterase (AChE) were widely used for detecting OP compounds. For instance, a disposable biosensor for OPs was developed based on the enzyme AChE functionalized MWNTs modified screen-printed electrodes (SPE) (Joshi et al., 2005). The mechanism of the OP sensor is to evaluate the inhabitation of the AChE by OP compounds, which was determined by measuring the electro-oxidation current of thiocholine generated by the AChE catalyzed hydrolysis of acteylthiocholine (ATCh). MWNTs acted as physical immobilization matrix for enzyme, while exhibited electro-catalytic activity toward thiocholine. The overpotential for thiocholine oxidation was found to be as low as 0.2 V without using a mediating redox species, and the detection limit for paraoxon is 0.5 nmol/L (0.145 ppb). Moreover, utilizing the

direct biosensing route of enzyme organophosphorus hydrolase (OPH), which catalyzed the hydrolysis of OP compounds, an OPH-functionalized MWNT-Nafion/GCE was developed to detect OP compounds amperometrically (Deo et al., 2005). Such sensor could detect as low as 0.15 μmol/L paraoxon and 0.8 μmol/L methyl parathion with sensitivities of 25 and 6 $nA/(\mu mol \cdot L^{-1})$, respectively. Recently, Liu et al. demonstrated a self-assembled AChE-functionalized MWNT/GCE to detect OP compounds (Liu and Lin, 2006). AChE was kept bioactively in a sandwich-like structure, where AChE molecules were embedded in two PDDA layers on the surface of MWNTs. Low oxidation overvoltage (+0.15 V) and detection limit as low as 0.4 pmol/L paraoxon with a 6-min inhibition time was achieved by such CNTs-modified electrode.

9.3.2.16 Phenolic compounds

Phenolic compounds are the hydroxy derivatives of benzene and its condensed aromatic systems. Not only are they naturally occurring compounds distributed in many plants, vegetables and food products, but also some of them are toxic. Unfortunately the detection of phenolic compounds using traditional solid electrodes were challenged by surface fouling attributed to the polymeric oxidation products. Wang and coworkers have overcome the problem by using MWNT-Nafion/GCE (Wang et al., 2003a). The stability of this sensor was largely enhanced with 85% of initial activity remaining after 30 mins. in phenol solution, compared to complete inhibition of the redox process within 6 mins at the bare surface. Enzyme was also utilized in some CNT-modified biosensors. For example, SWNTs were used as supporting material of enzyme tyrosinase in an amperometric sensor (Zhao et al., 2005). The electrode detected the catalyzed oxidation of phenolic compounds in the presence of oxygen, with a sensitivity of 155 $\mu A/(mmol \cdot L^{-1})$ for phenol. The sensor was also used to detect benzoic acid with a linear response in the range of 2.5 μmol/L – 12.4 μmol/L. In addition, Polyphenol oxidase (PPOx) was reported to functionalize a CNTPE-based sensor to detect various phenolic compounds, including phenol, catequeine, etc. (Rubianes and Rivas, 2005).

9.3.2.17 Procaine

Procaine is the first injectable man-made local anesthetic drug. Although procaine is rarely used today, procaine has the advantage of reducing bleeding without leading to the euphoric and addictive qualities, unlike other local anesthetics. Wu et al. utilized MWNT-film modified GCE for voltammetrical procaine detection (Wu et al., 2006). The reporeted detection limit is 200 nmol/L.

9.3.2.18 Putrescine

Putrescine is a foul-smelling ptomaine produced in decaying animal tissue by decarboxylation of ornithine. It can be used for many analytical and clinical applications, such as cancer marker, bacterial diagnosis and etc. An amperometric

biosensor was constructed on a γ-Aminopropyltriethoxysilane (APTES)-modified MWNT/GCE (Luong et al., 2005). Compared to classical electrodes, direct electron transfer between putrescine oxidase (POx) and the MWNTs modified GCE surface without using mediators was achieved in such a biosensor, where APTES was not only used to assist to solubilize MWNTs, but also served as an immobilization matrix for POx. The sensor exhibited a detecting range of 0.5 – 250 μmol/L.

9.3.2.19 Theophyllin

Theophylline (TP) is used to prevent and treat wheezing, shortness of breath, and difficulty breathing caused by asthma, etc. AGCE casted with carboxylic MWNTs was utilized to detect TP (Zhu et al., 2005). The biosensor showed a linear response to the TP concentration in the range 3×10^{-7} – 1.0×10^{-5} mol/L with an estimated detection limit of 0.05 μmol/L.

9.3.2.20 Quercetin

Quercetin, one kind of flavonoids, forms the 'backbone' for many other flavonoids such as the citrus flavonoids rutin and hesperidin. Quercetin has significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. In addition, quercetin shows various positive effects to human health in combating or helping to prevent cancer, prostatitis, heart disease, etc.. Thus monitoring quercetin concentration is of interest in many aspects. Jin et al. applied MWNTs-modified paraffin-impregnated graphite disk using choline (Ch) bond and catalyzer (MWNT/Ch/WGE) to detect quercetin (Jin et al., 2006). Under the optimum conditions, the sensor showed sensitivity up to 40 μmol/L with a detection limit of 4.8 nmol/L and a signal lose of 20% after two month storage.

9.3.2.21 Rutin

Rutin is a citrus bioflavonoid found in many plants, especially in black tea and apple peels. In humans, rutin can attach to the to the iron ion Fe^{2+}, which prevents Fe^{2+} from binding to H_2O_2 and creating a highly reactive free radical which may cause cell damage. Therefore, rapid, sensitive and reliable methods are welcomed to determine rutin in drug analysis. Combining GCE with β-cyclodextrin (β-CD) incorporated MWNTs, He et al. reported to obtain a detection range of rutin form 4.0×10^{-7} – 1.0×10^{-3} mol/L and estimated the detection limit to be 2.0×10^{-7} mol/L (He et al., 2006). Electrochemical behavior of rutin was investigated voltammetrically at a SWNTs modified gold electrode. The sensor exhibited a linear response in the range of 2.0×10^{-8} – 5.0×10^{-6} mol/L with a detection limit as low as 1.0×10^{-8} mol/L (Zeng et al., 2006).

9.3.2.22 Thiocholine

Thiocholine (TCh) is one of the products in acetylcholinesterase (AChE) catalyzed hydrolysis of thiocholine ester (AChE). It is used to monitoring the AChE inhabitation, which is a biomarker for the toxic effect of some pesticides, such as

organophosphorus compounds (OPs). Thus, the analysis of TCh is demanded for pollute control monitoring. The major drawbacks of using GC, CP, and bulk metal electrodes for TCh detection is the high oxidation overpotential, which leads to cause high background current and interference from other electroactive compounds. However, using an MWNT modified GCE (Liu et al., 2005), the enzymatically generated TCh was detected at lower oxidation overpotential (0.15 V) and higher sensitivity. Under optimal batch condition, the detection limit was 5×10^{-6} mol/L. Furthermore, by applying constant-potential flow injection analysis, the detection limit was greatly improved to 3×10^{-7} mol/L.

9.3.2.23 DNA

DNA is a genetic material in cells that holds the inherited instructions for growth, development, and cellular functioning. The development of an effective DNA-sensing system, therefore, is vital in these studies. Besides traditional DNA detection techniques such as fluorescent, radiochemical and chemiluminscent methods, electrochemical methods are attractive for their simple and low-cost solutions to DNA analysis ((Gooding 2002) and references within).

The use of CNT in electrochemical DNA sensors has recently attracted many researchers. A range of sensors in the literature are compiled in Table 9.2. These CNT-modified sensors are utilized to detect DNA (Li et al., 2005; Pedano and Rivas, 2004; Wang et al., 2004) and DNA hybridization (Cai et al., 2003a, 2003b; Cheng et al., 2005, Kerman et al., 2004; Koehne et al., 2003, 2004; Li et al., 2003; Munge et al., 2005; Wang et al., 2004a; Wang et al., 2004b; Wang et al., 2004; Zhu et al., 2005), respectively.

One of commonly used methods to prepare CNT-modified sensors for DNA hybridization detection was to attach ssDNA onto carboxylized CNT-modified electrode (including D1-D4, D7, D8), which was allowed to monitor a current signal when the complementary sequence (target) hybridizes with the immobilized ssDNA (probe). The first reported such sensor (D1) was based on a ssDNA-modified MWNT/GCE. A 24-based complementary ssDNA was detected using DPV in the presence of the electroactive intercalator daunomycin (DNR). The average anodic current of DNR was linear with the concentration of complementary ssDNA in the range of 0.2 nmol/L to 50 nmol/L with a detection limit of 0.1 nmol/L. The sensor D1 also exhibited a good selectivity towards the oligonucleotide sequences with a mismatch of a few bases. The hybridization of less than 10^6 DNA targets can be measured by combining the MWNT nanoelectrode array (MWNTNEA) electrodes (D3) with $Ru(byp)_3^{2+}$ mediated guanine oxidation (Li et al., 2003). When detecting the long single-stranded PCR amplicon with a large number of inherent guanine bases, the detection limit was further lowered to under ~1000 target DNAs (Koehne et al., 2003). Sensor D8 utilized CNTs to promote electro-transfer reactions and platinum nanoparticles for its high catalytic activities, achieving a detection limit of 0.01 nmol/L for target DNA, while

employing DNR as an indicator (Fig. 9.11). Moreover, using methylene blue (MB) as a redox indicator, a DNA-hybridization sensor (D7) was fabricated on self-assembled MWNTs with covalently attached probe DNA, as shown in Fig. 9.12.

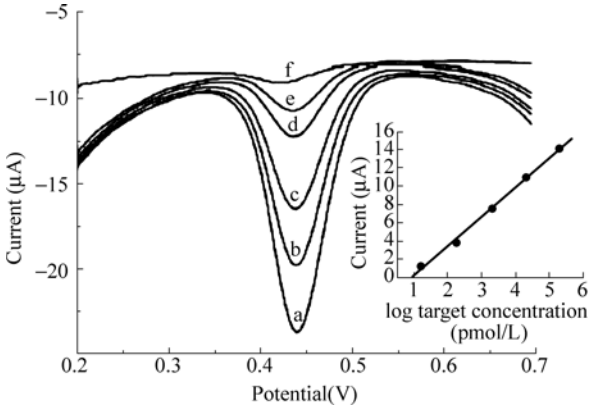

Figure 9.11 DPV voltammograms of Pt nanoparticles decorated ssDNA-MWNT/GC electrodes for different concentrations of target DNA: (a) 2.25×10^5 pmol/L; (b) 2.25×10^4 pmol/L; (c) 2.25×10^3 pmol/L; (d) 2.25×10^2 pmol/L; (e) 2.25×10^1 pmol/L; (f) 0.0 pmol/L. (Inset) the corresponding logarithmic calibration plot. Reprinted from (Zhu et al., 2005) with permission

Furthermore, DNA-hybridization sensors fabricated with previous method were also developed in the absence of indicator. D2 detected DNA sequences on a complementary DNA-doped PPy film on MWNT/GCE with by AC impedance measurement. When hybridization occurred, a decrease of impedance value was observed. D4 attached carboxylized MWNTs onto a CPE using a hybridization assay, and the probe DNA was further covalently adsorbed on the MWNTs. Such sensor provided a larger surface area for DNA immobilization and consequently exhibited a detection limit down to 10 pg/mL.

Magnetite nanoparticle (MNP) modified CNT-based sensors were prepared for DNA hybridization detection. In D5, A MWNT/PPy-modified GCE detected the DNA hybridization by capping the ssDNA probe on mercapatoacetic acid (RSH)-coated MNPs, which exhibits unique hybridization selectivity and effective discrimination ability in mismatched DNA sequences. The degree of hybridization corresponded to the reduction peak current of the indicator DNR. The detective limit of target DNA was as low as 2.3×10^{-14} mol/L. Wang and his group also developed enzyme-based electrochemical sensors (D6, D9, and D10) for the purpose. The DNA hybridization applied enzyme alkaline phosphatase (ALP) as tag and MNP as the collector for specific DNA sequences in D6. The hybridization information could be acquired from the enzymatically liberated product on a MWNT/GCE. Furthermore, CNTs played a dual-amplication role in both recognition and transduction events in sensors D9 and D10. The CNTs were not only loaded

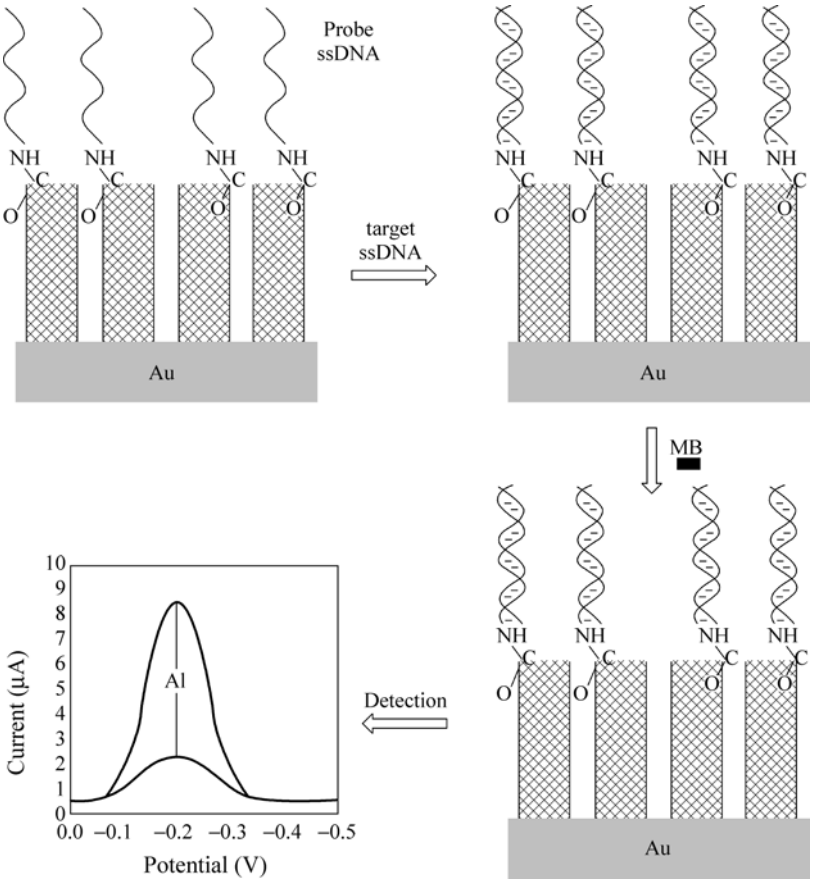

Figure 9.12 Schematic of the preparation of ssDNA-MWNT-array modified Au electrodes and the detection of target ssDNA sequences in the presence of an indicator, methylene blue (MB). Reprinted from (Wang et al., 2004) with permission

with various enzyme ALP tags and used as DNA labels during hybridization detection, but also used to modify the GCE and accumulate the product of the enzymatic reaction. Such sensor could detect DNA down to 80 copies (5.4 amol/L). The difference between D9 and D10 was that the ALP tracer was immobilized on CNTs by LBL method in D10.

The DNA could be detected on CNT modified electrodes, involving SWNT/GCE (D11), MWNTPE (D12), and DNA/MWNT-chitosan/graphite electrode (D13).

9.3.2.24 Others

Other species detected with the aid of CNT-modified electrodes include uric acid and norepinephrine (simultaneous detection by a chitosan-MWNT modified GCE (Lu et al., 2005)), cholera toxin (CT) (with anti-CT-immobilized, liposome-and-ploy(3,4-ethylenedioxythiophene)-coated-MWNT-Nafion/GCE (Viswanathan et al., 2006)) .

9.4 Field-Effect Transistors Based on SWNTs

Field-effect transistors (FETs) fabricated with SWNTs as active part of the device have been intensely studied since SWNTs are prospective candidates in molecule electronics. Because the most sensitive elements, pristine or specifically functionalized SWNTs, are worked as exposed conducing channel, the electronic properties of CNTFETs are sensitive to attached specific molecules and its surrounding environment. Therefore, CNTFETs were not only used for various chemical gases, such as NO_2, NH_3, etc. (Bradley et al., 2003, Qi et al., 2003, Zhang et al., 2006), but also sensitive to biorecognition events, biocatalytic processes and so on. The sensing signal from CNTFETs due to these species could be obtained

Table 9.5 Summary of the biosensors based on CNTFETs

Objective	Biomolecule functionzlied on SWNTs	With a linker layer (Y/N)	References
Protein recognition	Biotin, SpA, U1A	Y	(Chen et al., 2003)
	Biotin	Y	(Star et al., 2003)
	hCG	N	(Chen et al., 2004)
	HA	Y	(Takeda et al., 2005)
	α-PSA	N	(Kojima et al., 2005)
	Anti-copper oxLDL	Y	(Rouhanizadeh et al., 2006)
	Thrombin aptamer	Y	(So et al., 2005)
	PSA-AB	Y	(Li et al., 2005)
	SpA, hCG	N	(Byon and Choi, 2006)
DNA hybridization	PNA	Y	(Maehashi et al., 2004)
	ssDNA	N	(Star et al., 2006)
	ssDNA	N	(Tang et al., 2006)
Enzymatic study	GOx	Y	(Besteman et al., 2003)
	Starch	N	(Star et al., 2004)
Protein adsorption	Cytc	N	(Boussaad et al., 2003)
	SA	N	(Bradley et al., 2004)
	SA, IgG	N	(Atashbar et al., 2006)
Others	Cell membrane	N	(Bradley et al., 2005)

α-PSA anti-pig serum albumin, cytc cytochrome c, GOx glucose oxidas, hCG human chorionic gonadotropin, IgG immunoglobulin G, oxLDL oxidized low density lipoprotein, PNA peptide nucleic acid, PSA-AB anti-prostate-specific antigen monoclonal antibody, SA streptavidin, SpA staphylococcal protein A, ssDNA single-stranded DNA.

from two different ways. One is the chemiresistor configuration by monitoring real-time conductance change of CNT during analytes addition. The other way is to monitor the field-effect modulated conductance after introduction of the analytes. This kind of sensors sometimes is referred as chemFET.

In the following, we enumerate various papers utilizing CNTFET in biosensing applications, and also compile these biosensors and some of their aspects in Table 9.5.

9.4.1 Protein Recognition

Some proteins, especially antibodies, that are specific to corresponding antigens, have been utilized as recognition receptors in CNTFET biosensors. Most of exploited biological applications of CNTFETs are to recognize specific protein.

Chen et al. firstly reported to used CNTFET in such application (Chen et al., 2003). They found the Tween-coated SWNTs exhibited excellence resistance to the non-specific binding (NSB) of various proteins. Thus CNTFETs coated with biotinylated Tween, staphylococcal protein A (SpA)-Tween and U1A antigen-Tween could real-timely monitor the specific binding of streptavidin, IgG and 10E3 down to 1 nmol/L, respectively. The interferences from the NSB of other proteins could be eliminated. Response of such device to 10E3 could be seen in Fig. 9.13(a). Similarly, Star and his coworkers also applied CNTFET devices to detect specific protein binding (Star et al., 2003). Alternatively, a PEI/PEG polymer coating layer had been used as the prohibitor for NSB of proteins and linking molecules between SWNTs and molecular receptor, biotin. The biotin-streptavidin binding events could be observed from the shift of transfer characteristics of the CNTFETs (Fig. 9.13(b)). Another example for label-free detection of biomolecules was to differentiate low density lipoprotein (LDL) cholesterol between the reduced (native LDL) and the oxidized state (oxLDL) on an anti-copper oxLDL antibody conjugated CNT network-based FET, in which a PEI/PEG layer was appeared as the linker (Rouhanizadeh et al., 2006). Furthermore, complementary detection of prostate-specific antigen (PSA) was achieved by utilizing p-type CNTFETs and *n*-type In_2O_3 nanowire based FETs both anchored with anti-PSA monoclonalantibody (PSA-AB) via linking molecules (Li et al., 2005).

The antibody, anti-pig serum albumin (α-PSA), was directly immobilized on SWNTs through NSB without linking molecules (Kojima et al., 2005). Subsequently, the adsorption of antigen, PSA, was investigated from the real-time detection and characteristics change of CNTFETs.

Considering their high specificity, relatively inexpensive and capability of reversible denaturation, aptamers, which are artificial oligonucleotides, were used as an alternative to protein-based sensing elements (So et al., 2005). 15-mer ssDNA aptamer bound to CNTFET via CDI-Tween could specifically attach to blood-

clotting factor thrombin, and the detection limit to aptamer of the biosensor was around 10 nmol/L.

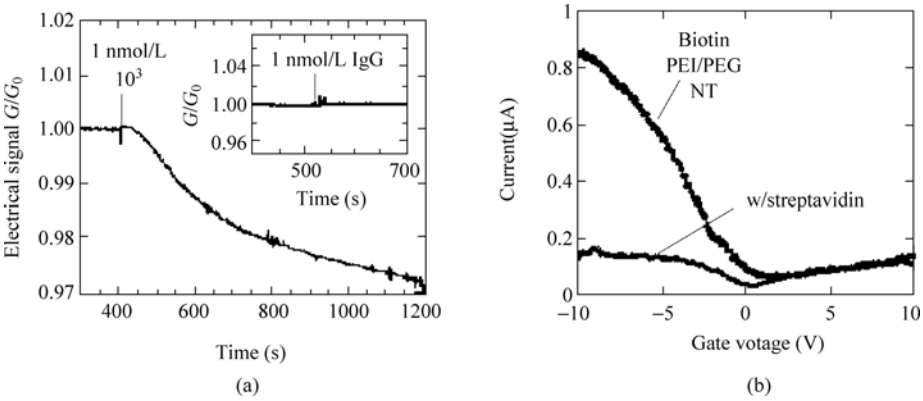

Figure 9.13 (a) The Conductance vs. time curve shown that a recombinant human autoantigen functionalized CNTFET exhibited specific response to $\leqslant$ 1 nmol/L 10^3 mAbs. (Inset) the same device shown selectivity that reject the interference polyclonal IgG at a much larger concentration of 1 μmol/L (Inset). Reprinted from (Chen et al., 2003) with permission. (b) Transfer characteristic change of a biotinylated polymer-coated CNTFET before and after exposing to streptavidin. Reprinted from (Star et al., 2003) with permission

In order to further understand the biosensing mechanism of the specific protein recognition by CNTFET, Chen et al. conducted a series of control experiments to block selected areas of the devices, including only contacts between SWNTs and metallic electrodes, both contacts and exposed SWNTs, or none of such two regions (Chen et al., 2004). These devices were subsequently used for protein recognition. The results revealed that electronic effects occurring at the contacts due to the protein adsorption induced Schottky barrier modulation dominated the electronic biosensing signal. And the contribution from the exposed SWNTs was negligible if the adsorbed species were not highly charged. With this knowledge in mind, Byon and Choi purposefully modified the device geometry to increase the Schottky contact area (Byon and Choi, 2006). This modification was implemented by evaporating metallic source/drain electrode at a tilted angle using a shadow mask. In comparison with the detection limit in the sensing of proteins or protein-protein interactions in previous works (Chen et al., 2003, Chen et al., 2004), which is ca. 100 pmol/L to 100 nmol/L, devices with increased Schottky area could detect proteins as low as 1 pmol/L concentrations.

Alternatively, a different architecture, in which protein receptor hemagglutinins (HA) was immobilized on the back-gate side of CNTFET via linking molecules instead of SWNTs channel, was prepared to detect anti-HA binding (Takeda et al., 2005). The detection limit of anti-HA was estimated to be 5×10^{-8} mg/mL, and was better than enzyme-linked immunosorbent assay (ELISA) system.

9.4.2 DNA Hybridization

Nowadays, the development of genome diagnosis has been a subject with great interest, in which label-free electronic methods, including CNTFET based biosensors, are believed to be a potential way for sensitive, selective and low cost detection of DNA hybridization (Fig. 9.14). The first SWNT transistor used for this purpose was developed in association with covalently immobilized amino modified peptide nucleic acid (PNA) oligonucleotides onto Au surface of the back gate via a self-assembled monolayer (SAM) (Maehashi et al., 2004). This device could effectively detect the complementary DNA with a concentration as low as 6.8 fmol/L. Moreover, Star et al. also utilized CNT network FETs as selective detectors for DNA immobilization and hybridization (Star et al., 2006). The probe DNA was non-covalently adsorbed on the SWNT network instead of the back gate region as reported in ref. (Maehashi et al., 2004). The devices were further used for the label-free detection of DNA hybridization at picomolar to micromolar concentrations. It was also found that the addition of Mg^{2+} during hybridization could improve the sensitivity of DNA detection probably by increasing the extent and overall efficiency of DNA hybridization on SWNTs. The mechanism of DNA detection using CNTFET devices, however, is still under argument. Star et al. suggested a charge-based mechanism (Star et al., 2006). In contrast, Tang and his coworkers demonstrated that DNA hybridization on the contact regions of the CNTFET caused a more significant contribution to the acute electrical conductance change, probably due to the modulation of energy level alignment between SWNTs and Au electrodes (Tang et al., 2006). More experiments are still needed to clarify such issue.

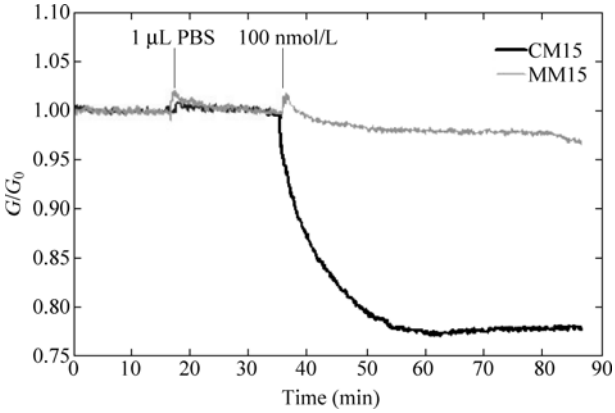

Figure 9.14 Real-time monitoring of 15mer DNA hybridization in PBS on a ssDNA functionalized CNTFET. Two liquid cells were used in parallel for simultaneous drop adding 5 μL of complementary (blue) and mismatched (green) target oligo solutions to 500 μL of buffer. Reprinted from (Tang et al., 2006) with permission

9.4.3 Enzymatic Study

With GOx attached to the nanotube sidewall through linking molecules, a liquid-gated FET device could be used for the detection of glucose (Fig. 9.15) (Besteman et al., 2003). It was observed that the immobilization of GOx induced the transfer characteristics negative gate voltage shift, which was probably due to a change in the capacitance of the tube. Subsequently, the addition of 0.1 mmol/L glucose solution increased the conductance of enzyme functionalized CNTFETs, but the exact mechanism was not explained. Furthermore, an alternative approach was used to detect the enzymatic degradation of starch on CNTFET (Star et al., 2004). Instead of enzyme, the substrate of an enzymatic reaction, starch, was attached to the devices surface directly, leading to a shift in the transfer characteristics of devices. After incubation of these devices in aqueous buffer solutions of enzyme amyloglucosidase (AMG), the I_d - V_g curve almost completely recovered to the trace record before the starch deposition, suggesting that nearly all the starch were hydrolyzed to glucose and then washed away by buffer solution, because the AMG along would not affect the device characteristics and the adsorbed starch could not be washed off by buffer solution along.

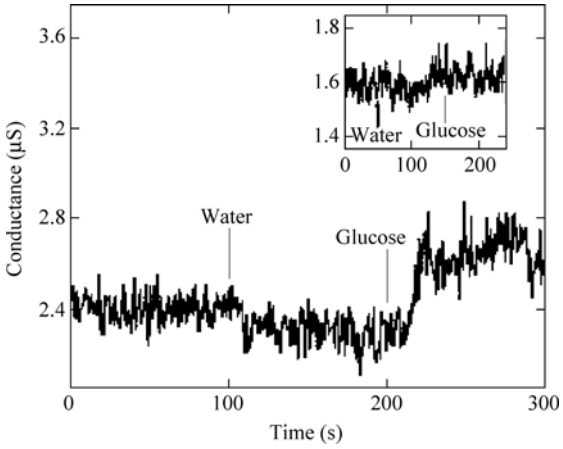

Figure 9.15 Real-time monitoring of the GOx functionalized CNTFET to glucose. Arrows showed the addition of 2 μL milli-Q water and 2 μL 0.1 mmol/L glucose to the device. (Inset) the same measurement on a control CNTFET without GOx. Reprinted from (Besteman et al., 2003) with permission

9.4.4 Protein Adsorption

Noncovalent adsorption of proteins, such as cytochrome *c* (Boussaad et al., 2003) and streptavidin (Atashbar et al., 2006, Bradley et al., 2004), IgG immunoglobulin

G (IgG) (Atashbar et al., 2006), could also be simply detected by SWNT or SWNT-network based FET devices. Such NSB on bare unmodified SWNTs could alter the electrical characteristics of devices. Charge transfer between proteins and the SWNTs may be responsible to the detection process.

9.4.5 Others

Bradley et al. integrated a complex biological system, the cell membrane of Halobacterium salinarum, with CNTFET device, and found that both of them were preserved their properties and functions (Bradley et al., 2005). On the same time, the charge distribution in this biological system was investigated.

9.5 Conclusions and Future Prospects

We have summarized recent advances in the applications of CNTs for biosensing, in which electrochemical biosensing and electronic biosensing were emphasized. The large surface/volume ratio, bio-compatibility and high electrical sensing sensitivity of CNTs are the key advantages for biosensing applications. The marked electrocatalytic reactivity and efficient electron transfer, coupled with the resistance to surface fouling and hence to high stability, pave the way for the construction of a wide range of electrochemical and electronic biosensors with many attractive analytical characteristics.

However, the exploitation of CNTs in the applications of biosensors is still in its infancy. Future efforts should aim at a more in-depth understanding the chemical and physical properties of CNT and new configurations of CNT-based biosensors. More work is needed to settle the dilemma whether the oxygen-containing surface groups on CNTs after oxidants treatment could improve the electrocatalytic ability of CNTs (Day et al., 2004, Musameh et al., 2005). To fabricate CNT NEA in large-scale through vertically aligned CNTs at a low density could have promising potentials as interstitial space between nanoelectrodes could be filled with a passivation layer and only the tips of CNTs are exposed for electrochemical detection in order to minimize the background current (Koehne et al., 2003, Koehne et al., 2004, Li et al., 2003, Lin et al., 2004, Tu et al., 2005). In addition, the open ends and defects in CNTs were found to have similar properties with edge planes of highly ordered pyrolytic graphite (HOPG) and acted as electro-catalytically active sites (Banks and Compton, 2005, Gooding et al., 2003, Nugent et al., 2001). This discovery could be further utilized to improve the electrochemical activity and sensitivity of CNT-modified electrodes. Furthermore, the role of contact between CNTs and the metallic electrodes is believed to play an important role in the CNT transistors and should also be

further investigated, since it was suggested that the modulation of the Schottky barrier might dominate in the response of CNTFET-based biosensor to analyte (Chen et al., 2004, Tang et al., 2006). Due to integrate of biological cell membranes with CNTFETs for monitoring charge distribution in the cell membrane (Bradley et al., 2005), one also needs to study the cytotoxity of CNTs towards biological species. These directions of development could bring a bright prospect to the new generation of CNT-based biosensors for a variety of applications.

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