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## Surface Modification Approaches for Electrochemical Biosensors

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### 1. Introduction

Electrochemical biosensors are transducers that convert biological information into electrical information. Electrochemical biosensors provide qualitative and quantitative information (Wang 1999) on the existence and concentration of the target compounds in the analyte in the form of current (amperometric biosensor) or voltage (potentiometric biosensor).

A typical amperometric biosensor consists of three components: the analyte, the transduction element (electrode and conductive nanomaterials) and the biorecognition element (enzyme) (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., ; Shi et al., 2010). During biosensor operation, target compound in the sample is specifically recognized by the enzymes immobilized on the electrode. Electrooxidative intermediate is produced by this enzyme-substrate interaction. The produced electrooxidative intermediate is oxidized or reduced by the voltage applied on the biosensor, and current proportional to substrate concentration is generated and recorded. By calibrating the biosensor using solutions with known concentration, the relationship between measured current and substrate concentration is obtained. The sensitivity and specificity of the sensor is ensured by the high selectivity of enzymes.

Considering the functional mechanism of biosensors, surface modification of the electrode is vital to biosensor performance. The most straightforward and also widely used approach is to immobilize enzymes on the electrode with a polymer layer. However, this method has two major limitations. One is that the activity of the enzymes can be affected by structural change due to the polymer layer, and affected by the pH of the layer (Zou et al., 2008). The other is that the thickness of the polymer layer cannot be precisely controlled, so the response time and sensitivity of the biosensor could be affected (Li et al., 1996). To overcome these limitations, some groups used polymers with neutral pH such as silicate sol-gel for enzyme immobilization to preserve enzyme activity (Salimi et al., 2004) while some groups used electric methods such as cyclic voltammetry to control layer deposition (Llaudet et al., 2005; Smutok et al., 2006). Furthermore, to obtain better performance, nanomaterials including carbon nanotubes (CNTs) and metal nanomaterials are often involved in surface modification (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., ; Shi et al., 2010). Since different modification approaches result in quite distinct biosensor performance, problems with evaluating and comparing different approaches, and sorting out the optimal ones have arisen. To solve this problem, a standardization method which evaluates the performance of biosensors constructed by different approaches is needed.

In this chapter, followed by a comprehensive literature review of surface modification approaches, a tentative protocol for comparing different approaches will be discussed.

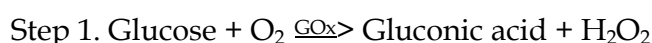
## 2. Immobilization approaches for enzymes

As was mentioned previously, enzymes are the biorecognition element of biosensors. Biosensors function based on the highly selective enzyme-substrate interactions. Thus, the enzymes immobilized on electrode determine the target compound, the activity of the enzymes determines the sensitivity, and the selectivity of the enzymes determines the specificity of the biosensors. As a result, it is important to develop proper enzyme immobilization approaches with high enzyme loading and well-preserved enzyme activity.

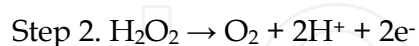
### 2.1 Enzyme based biosensing

Enzymes are usually immobilized on the electrode by polymer encapsulation or covalent linking (McLamore et al., 2010b; McLamore et al., 2011; Rickus et al., 2002; Shi et al., 2010). During biosensor operation, when analyte solution diffuses into the enzyme layer, a series of biochemical and electrochemical reactions will take place. Take the *de facto* enzyme glucose oxidase (GOx) as an example. GOx based biosensors function through the following steps:

In the first step (biorecognition), GOx converts glucose into  $\text{H}_2\text{O}_2$  and gluconic acid. The main purpose of this step is to produce the electrooxidative intermediate  $\text{H}_2\text{O}_2$ , because glucose cannot be directly electrooxidized. Because the enzyme-substrate interaction in this step is specific to glucose, biorecognition step ensures the selectivity of the biosensors.



In the second step (transduction), an electric potential is applied to the electrode. The value of the potential is determined by the type of electrode used, and the type of the electroactive intermediate produced in *step 1*. In this particular example, for measuring  $\text{H}_2\text{O}_2$  with a Pt electrode, the potential used is usually +500 mV-+800 mV (McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010). The main purpose of this step is to measure the concentration of  $\text{H}_2\text{O}_2$  by measuring current.



Since the concentration of  $\text{H}_2\text{O}_2$  is proportional to glucose according to *step 1*, glucose concentration can be determined. By modifying the electrode with conductive nanomaterials, the electron transfer rate during electrooxidizing  $\text{H}_2\text{O}_2$  can be significantly increased. So the biosensor will have increased sensitivity, which is the reason why surface modification with nanomaterials is important to biosensor performance.

### 2.2 Enzyme immobilization approaches

One of the most widely used approach for immobilizing enzymes is to entrap enzymes within polymer layers. The layer containing enzymes can be deposited on electrodes by cast-and-dry, or electropolymerization. Many polymers have been reported for such applications, including nafion (Fortier et al., 1992; Vaillancourt et al., 1999), polypyrrole (Branzoi & Pila 2008; Ekanayake et al., 2007), polytyramine (Situmorang et al., 1999) and silicate sol-gels (Llaudet et al., 2005; Rickus et al., 2002; Salimi et al., 2004).

Nafion is a negatively charged sulfonated tetrafluorethylene copolymer, which possesses a strong surface adhesion to electrode surface and a low swelling capability in aqueous media (Gong et al., 2005; Liaw et al., 2006; Wang et al., 2003b). Thus, nafion is quite appropriate for enzyme immobilization. Biosensors based on nafion/enzyme composite for the detection of glucose and other compounds have been reported (Fortier et al., 1992; Vaillancourt et al., 1999). One noticeable advantage of nafion over other polymers is that the negative charges repel the diffusion of many negatively charged compounds such as ascorbate and acetaminophen into the layer (Ni et al., 1999), significantly enhancing biosensing selectivity. Polypyrrole (PPy) is a conductive polymer mainly made up of pyrroles. Polypyrroles can be formed through electropolymerization using cyclic voltammetry, resulting in a uniformly doped PPy film with positive charges on electrode surface (Schuhmann 1991; Schuhmann & Kittsteiner-Eberle 1991; Schuhmann et al., 1990). One advantage with PPy is that enzymes with negative charges can be absorbed into PPy layers via electrostatic forces (Gao et al., 2003). Another advantage is that the thickness of the PPy layer can be quantitatively controlled by controlling the number of cycles during cyclic voltammetry. The selectivity of polypyrrole film can be enhanced by the addition of various counter ions (Sadik 1999; Teasdale & Wallace 1993; Zotti 1992). Biosensors based on PPy for versatile sensing applications have been reported (Dumont & Fortier 1996; Ekanayake et al., 2007; Umana & Waller 2002). Excellent reproducibility in amperometric response and resistance towards high temperature have been reported for PPy over a number of polymers including polyaniline, poly(aniline/p-phenylenediamine), polyindole, and poly(o-phenylenediamine) (Dumont & Fortier 1996). The major disadvantage with PPy is that the layer is most stable under pH range of 5.5-6.0 (Dumont 1996), which may greatly lower the activities of certain enzymes that favor basic pH, such as glycerol kinase (optimal pH=9.8) and glycerol-3-phosphate oxidase (optimal pH=8.1), both of which are used in adenosine-3-phosphate (ATP) sensing (Llaudet et al., 2005). In addition, Schuhmann et al. reported that the enzyme loading capability of PPy was low (Schuhmann 1991), which may result in a low biosensor sensitivity.

Silicate sol-gels are polymers formed by ethyl esters of orthosilicic acid, among which tetraethyl orthosilicate (TEOS) and tetramethyl orthosilicate (TMOS) are most commonly used in the immobilization of enzymes (Llaudet et al., 2005; Salimi et al., 2004; Yang et al., 1998). The hydrolysis and condensation of sol-gels at low temperature (usually 4 °C) generate a 3-dimensional polymer matrix of silica, which can entrap enzymes (Rickus et al., 2002). Biosensors based on sol-gel approach for the detection of glucose (Salimi et al., 2004), ATP (Llaudet et al., 2005) and other compounds with linear response range covering physiological concentrations have been reported. One advantage of sol-gel immobilization is that enzymes are entrapped within the matrix with no covalent linking involved, thus enzyme activity may be better preserved. Another advantage is that the porous structure of sol-gel matrix facilitates the diffusion of substrates into the matrix and provides space for the interaction between substrates and enzymes. However, since immobilization approaches based on sol-gels require dip coating and the distribution of dissolved enzymes in the sol-gel is not uniform, the thickness of the layer and the amount of loaded enzymes may vary a lot, affecting the reproducibility of biosensors.

Other polymers such as chitosan (Kang et al., 2007; Miscoria et al., 2006) have been used for enzyme immobilization as well. Some approaches directly entrap enzymes in the polymer. The common drawback with these approaches is the relatively low efficacy of enzyme loading that often results in inconsistency in amperometric response and reduced sensitivity

during long-term biosensor operation (Schuhmann 1991; Schuhmann & Kittsteiner-Eberle 1991). Thus, cross-linking agents have been combined with polymer layers for better enzyme loading. These agents include glutaraldehyde (GA) (via  $\text{NH}_2$ - bond) (Guerrieri et al., 1998), 1-ethyl-3-(3-diamino)propyl-carbodiimide (EDC) and *N*-hydroxysulfosuccinimide (NHS) (via  $-\text{COOH}$  bond) (Limbut et al., 2006), and 3-mercapto-1-propanesulfonic acid (MPS) (via  $-\text{SR}$  bond and electrostatic forces) (Miscoria et al., 2006). Increased amperometric sensitivity has been reported for biosensors when cross-linking agents are used for enzyme immobilization (Guerrieri et al., 1998; Miscoria et al., 2006). Some agents such as GA (McLamore et al., 2010b) and thiol linker [dithiobis (succinimidyl undecanoate)] (Claussen et al., 2009) can directly link enzymes to the electrode surface with no polymer layer involved, providing alternatives to polymer immobilization.

### 3. Immobilization of nanomaterials

One problem with biosensors based only on polymers and enzymes is the undesired low signal-to-noise ratio, because catalytic ability of enzymes is limited. Consequently, biosensor's amperometric response may be submerged by noise. One of the most commonly used approaches to resolve this problem is to modify biosensors with nanomaterials. Two most commonly used nanomaterials are carbon nanotubes and metal nanomaterials (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010).

Ever since Iijima reported the synthesis method for CNT in 1991 (Iijima 1991), this allotrope of carbon has demonstrated versatile applications in biomedical imaging (Choi et al., 2007b), chemical batteries (Wang et al., 2003a), and biosensing (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010). CNTs have two types: single-walled CNT (SWNT) and multi-walled CNT (MWNT). SWNT is a seamless cylinder formed by rolling-over a one-atom-thick layer of graphite namely graphene (Iijima & Ichihashi 1993) (Fig. 1a), while MWNT has the structure of sheets of graphite arranged in concentric cylinders (Ajayan 1999; Dai 2002) (Fig. 1b). SWNT has a diameter on the order of 1.2 nm (Fig. 1d) while MWNT has a diameter on the order of 10 nm to 20 nm with concentric nanotubes 0.34 nm apart (Ajayan 1999; Dai 2002) (Fig. 1c).

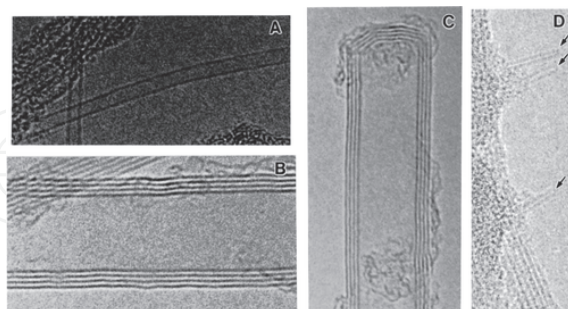


Fig. 1. High-resolution transmission electron microscopy images of typical SWNT (A) and MWNT (B). Closed nanotube tips are also shown in panel C (MWNT tips) and panel D (SWNT tip, shown by arrows). The inner space corresponds to the diameter of the inner hollow in the tube. The separation between the closely spaced fringes in the MWNT (B, C) is 0.34 nm, close to the spacing between graphite planes. The diameter of the SWNT (A, D) is  $\approx 1.2$  nm. Every layer in the image (fringe) corresponds to the edges of each cylinder in the nanotube assembly. (Reprinted with permission from (Ajayan 1999). Copyright (1999) from American Chemical Society)



### 3.1 Electrochemical basis for CNT

STM/STS studies have shown that CNTs consist of both metallic and semi-conductive tubes (Odom et al., 1998; Wilder et al., 1998). Both SWNTs (Wang et al., 2003b) and MWNTs (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010) have been widely used in biosensing. CNTs have been demonstrated to possess the ability to facilitate the electron transfer process during electroreduction and electrooxidation of electroactive species, such as NADH and hydrogen peroxide (Hrapovic et al., 2004; Wang et al., 2003b), and the electron transfer process during enzyme-substrate interaction, even when the enzyme redox center is deeply embedded (Gooding et al., 2003).

Researches have been carried out to explore the underlying mechanism for CNT to enhance biosensor performance. The reasons for CNT to greatly improve biosensor's response are summarized as follows:

First, CNTs enlarge the effective surface area when immobilized on the surface of the electrodes. The electrode impedance is decreased, and the current is increased due to the increase in surface area (Azamian et al., 2002). Another advantage due to enlarged surface area is that more enzymes can be immobilized. MWNTs have been used as a matrix for enzyme immobilization (Shi et al., 2010).

Second, CNTs act as a catalyst that increases electron transfer rate. The carbon atoms at the ends of CNT behave like the edge plane of highly oriented pyrolytic graphite (HOPG) from a mechanistic point of view (Li et al., 2002). When CNTs are pretreated by purifying and refluxing using strong acid such as nitric acid (McLamore et al., 2010a), the tube ends will be connected with oxygenated species, such as carboxylic acids, alcohols and quinines (Gooding 2005; Koehne et al., 2003). The oxygenated tube ends allow efficient electron transfer (Gooding 2005; Koehne et al., 2003), which is the origin for the catalytic ability of CNTs. This underlying mechanism is further supported by comparing peak separation in cyclic voltammogram of potassium ferricyanide between one electrode with aligned SWNTs perpendicular to its surface and another electrode with SWNTs with random orientations. The former has much a smaller separation than the latter, indicating improved electrochemical property (Liu et al., 2005).

Third, the electrodes are endowed with better wetting properties due to the porous structure of CNTs (Nugent et al., 2001). As a result, analyte solution will diffuse into the CNT bundles with lower friction (Verweij et al., 2007), which contributes to a higher current sensitivity when biosensing is diffusion limited (Cambiaso et al., 1996).

### 3.2 Surface modification approaches using CNTs

#### 3.2.1 Abrasive immobilization

CNT, as an allotrope of carbon, can be attached to carbon electrode surface by non-covalent forces. Salimi et al. prepared glucose biosensor based on abrasive immobilization approach, by gently rubbing the polished basal plane pyrolytic graphite (bppg) electrode surface on a filter paper containing MWNTs (Salimi et al., 2004). Decreased oxidation and reduction potentials for  $H_2O_2$  were discovered compared with bare bppg electrodes, indicating the improvement in electrocatalytic activities of the electrodes due to CNT immobilization (Salimi et al., 2004). In amperometric tests, well-defined response to glucose addition was reported for the bppg/CNT/sol-gel/GOx biosensor while hardly any response could be observed with the bppg/sol-gel/GOx electrodes (Salimi et al., 2004), demonstrating that the low signal-to-noise issue with biosensors based on conventional materials could be resolved by adding nanomaterials. In addition, compared with glucose biosensors with no CNT

involved (Wang et al., 1997; Yang et al., 1998), the analytical parameters (sensitivity, detection limit, response time and linear range) for bppg/CNT/sol-gel/GOx biosensor were comparable or better (Salimi et al., 2004).

### 3.2.2 Immobilization with MPS

(3-Mercaptopropyl) trimethoxysilane (MPS), a silanization reagent with methoxy and thiol functional groups, has been applied to attach MWNTs to electrodes (McLamore et al., 2010a; Zeng & Huang 2004). The thiol groups form covalent bonds to link CNTs to electrodes. Biosensors based on this approach exhibited increased peak current in cyclic voltammetry with potassium ferricyanide, and high sensitivity towards the direct oxidation of IAA, due to the CNTs on electrode surface which facilitated electron transfer. MPS immobilization of CNTs provides an alternative to abrasive immobilization which can be applied to metal electrodes. Desirable reproducibility has been reported for biosensors based on this approach (Zeng & Huang 2004).

### 3.2.3 Immobilization with polymer entrapment

The major obstacle to immobilizing CNTs for biosensing is that CNTs tend to aggregate due to van der Waals forces among tubes. As a result, CNTs are insoluble in almost all solvents (Chen et al., 1998; Star et al., 2001). Since almost all conventional approaches for building enzyme based biosensors rely on polymer layers to entrap enzymes, similar approaches can be developed to immobilize CNT. Researches have shown that many polymer layers can suspend CNT, including nafion (McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010 ; Tsai et al., 2005; Wang et al., 2003b), chitosan (Kang et al., 2007, 2008) and silicate sol-gels (Chen & Dong 2007; Gavalas et al., 2004) .

Nafion is a conductive sulfonated tetrafluorethylene copolymer and its negatively charged layer is capable of suspending CNTs and enzymes. SEM image showed that MWNTs were well dispersed within nafion layer, and formed a conductive network which will facilitate electron transfer during electrochemical reactions (Shi et al., 2010 ) (Fig. 2).

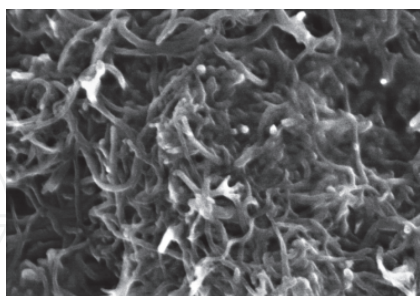


Fig. 2. SEM image for a MWNTs/Nafion layer on a biosensor. (Reprinted with permission from (Shi et al., 2010 ). Copyright (2010) from Elsevier Inc.)

Chitosan is a linear polysaccharide with fine biocompatibility and adhesive capability to chemically modified surfaces. Pretreated CNTs with  $-\text{COOH}$  groups on tube ends could disperse among chitosan containing  $-\text{NH}_2$  groups due to the peptide bonds formed between  $-\text{COOH}$  and  $-\text{NH}_2$  (Kang et al., 2007). Biosensors based on chitosan polymers with CNT and enzymes involved have been reported (Kang et al., 2007, 2008). Similar to other CNT modified electrodes, the oxidation potential for electrooxidative species is significantly lowered (Zhang 2004). A low oxidation potential ensures that interferences such as

acetaminophen and ascorbic acid, that can only be oxidized at high voltages, are excluded, which greatly enhances the selectivity of the biosensors. However, one disadvantage with chitosan is that the peptide bonds formed between CNTs and chitosan eliminate the  $\text{-COOH}$  groups on CNT, which may lower the catalytic ability of CNTs, as the ability mainly comes from the oxidative species at tube ends.

Polypyrrole (PPy) is a highly conductive polymer formed from a number of connected pyrrole rings. Wang et al. reported that "oxidized CNT" together with enzymes could act as combined dopants to form a covalently linked PPy-CNT-Enzyme layer (Wang & Musameh 2005). When electro-oxidized at +650 mV using platinum (Pt) or glass carbon (GC) electrodes as working electrodes, each pyrrole ring will carry one positive charge. With the presence of charge balancing anionic dopants, such as negatively charged enzymes (Kang et al., 2007; Umana & Waller 2002) or  $\text{-COOH}$  modified CNTs (Wang & Musameh 2005), polymer layers with enzymes or CNTs will form on the working electrode surface after electropolymerization (Wang & Musameh 2005). Glucose biosensors based on this approach showed significantly increased response to glucose compared with no MWNT involved. In addition, thanks to irreversibly oxidized PPy's special property to reject electroactive interferences (Malitesta et al., 1990), glucose biosensors based on PPy/MWNT exhibited no response towards uric and ascorbic acids even at +900 mV (Wang & Musameh 2005), showing excellent selectivity. Besides PPy, immobilization approaches based on similar electropolymerization process using polyaniline (PAN) was also reported (Ma et al., 2006). In addition, the auto-assembly linking of negatively charged oxygenated groups on modified CNTs to positively charged polyelectrolyte poly(diallyldimethylammonium chloride) (PDDA) layer with no need of electropolymerization was reported (Mamedov et al., 2002; Rouse & Lillehei 2002).

Silicate sol-gels, including tetramethyl orthosilicate (TMOS) and tetraethyl orthosilicate (TEOS), have been widely used in enzyme immobilization due to the formed porous 3-D matrix structure which physically entraps enzymes (Llaudet et al., 2005; Salimi et al., 2004; Yang et al., 1998). The use of sol-gels to immobilize CNTs on biosensors have been reported by directly dispersing CNTs within pretreated methyltriethoxysilane (MTEOS) (Gavalas et al., 2004), Propyltrimethoxysilane (PTMOS) (Gong et al., 2004) and methyltrimethoxysilane (MTMOS) (Chen & Dong 2007) solutions. Homogeneous suspensions were obtained after ultrasonication and sol-gel/CNT layers were formed on electrodes. TEM image of CNT and the CNT/sol-gel composite (Gong et al., 2004) showed that small MWNT bundles were separated into several independent nanoelectrodes, which greatly increased the contacting area between CNTs and analytes.

#### 3.2.4 CNT paste electrodes

Almost all previously reviewed approaches immobilized CNTs on a substrate electrode, such as glassy carbon (GC), platinum (Pt) and gold (Au). CNTs can be directly packed into a carbon electrode with or without binder materials (Britto et al., 1996; Rubianes & Rivas 2003; Valentini et al., 2003; Wang & Musameh 2003a; Zare et al., 2010) (Wang & Musameh 2003b; Zhao et al., 2003). Britto et al. first reported biosensors based on CNT paste electrode by packing a paste of MWNTs with bromoform into a glass tube for dopamine detection, and the resulted paste electrode showed desirable electrochemical reversibility in cyclic voltammetry compared with conventional carbon electrodes (Britto et al., 1996). Enhanced amperometric response was also reported for CNT paste electrodes compared with carbon paste electrodes (Wang & Musameh 2003b).



### 3.2.5 Immobilization of aligned CNTs

As has been discussed previously, the catalytic activities of CNTs are mainly due to the carbon atoms at the end of the tubes, especially when tube ends are attached with oxygenated species (Chou et al., 2005; Gooding 2005; Koehne et al., 2003; Nugent et al., 2001). If the CNTs are perpendicular to the electrode surface, carbon atoms at tube ends will be sufficiently exposed and the catalytic activities of CNTs can be further increased. Almost none of the approaches reviewed previously had control over the orientation of CNTs. Take the polymer layer approach as an example, when CNTs are dispersed in the polymer layer, the orientations of CNTs are completely random. Huang et al. developed a method of preparing aligned CNT thin film on a quartz plate which can be easily transferred to other surfaces, such as electrode surface (Huang et al., 1999). Gao et al. developed a glucose biosensor based on this approach using gold electrodes (Gao et al., 2003). Increased glucose sensitivity was reported and a decreased irreversible single oxidation peak was observed compared with glassy carbon electrodes with no CNTs. Yun et al. developed a needle biosensor for  $\text{H}_2\text{O}_2$  based on the same approach with modifications, and enhanced amperometric and voltammetric properties were observed (Yun et al., 2006). Wang et al. reported another CNT alignment approach by microwave plasma enhanced chemical vapor deposition using nickel as a catalyst (Wang et al., 2003c). Resulted CNTs grew densely and vertically along the grain of the catalytic particles (Yudasaka et al., 2009) and the CNTs were aligned straight by virtue of the nickel used, except for few entangled and cross-linked tubes. Wang et al. further reported that by controlling the thickness of the nickel layer, the diameter of aligned CNTs could be controlled (Wang et al., 2003c). Glucose biosensor based on this approach with direct absorption of glucose oxidase by MWNTs has been reported and more than 91% of the initial sensitivity towards glucose remained after three months, indicating good stability (Wang et al., 2003c). Liu et al. developed a self-assembled SWNT alignment method under room temperature, which was much easier to implement compared with Dai and Wang's approaches requiring high temperature (Liu 2000). Derivative CNT alignment approaches have been reported (Chattopadhyay et al., 2001; Kim & Sigmund 2003).

### 3.2.6 Other surface modification approaches using CNTs

Other surface modification approaches using CNTs have been reported, including CNT-nanoelectrode ensembles (NEE) (Lin et al., 2004) and screen-printed CNT (Wang & Musameh 2004), which all provided biosensors with enhanced performance by virtue of the unique structure and properties of CNTs.

### 3.3 Combination of CNTs and metal nanomaterials

Transition metal nanomaterials possess high catalytic activities and facilitate electron transfer for many electrochemical reactions. Metal nanomaterials also enhance the performance of the biosensors by enlarging the effective surface area (Hrapovic et al., 2004). Biosensors incorporating metal nanomaterials, including platinum black (McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010), copper (Xu et al., 2006), silver (Ren et al., 2005), palladium (Claussen et al., 2009; Lim et al., 2005) and gold (Daniel 2004) have exhibited well biocompatibility and enhanced performance. Especially the combination of metal nanomaterials and CNTs for surface modification of biosensors has proved to be feasible and more effective than using either nanomaterial alone (Evans et al., 2002; Hrapovic et al.,

2004; McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010 ; Zou et al., 2008). Increased  $\text{H}_2\text{O}_2$  sensitivity and effective surface area have been demonstrated for Pt black/MWNT/Nafion electrodes over bare electrodes (Shi et al., 2010 ) (Fig. 3).

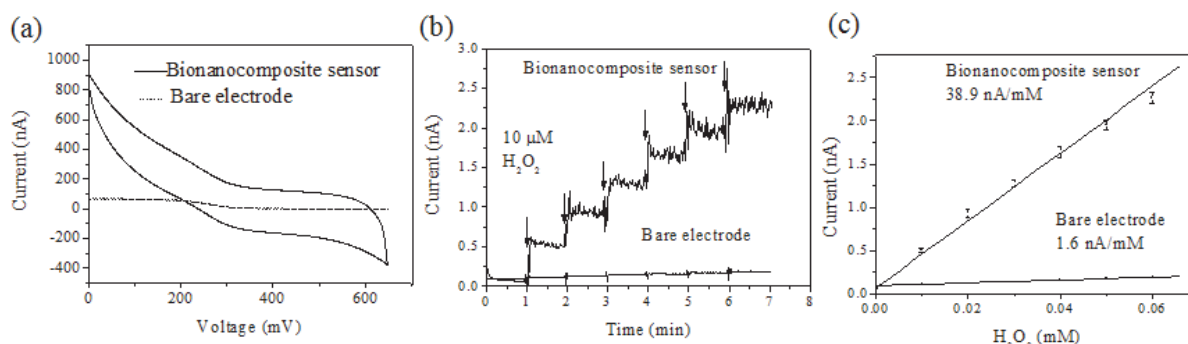


Fig. 3. (a) CV in 4 mM  $\text{Fe}(\text{CN})_6^{3-}$  / 1M  $\text{KNO}_3$  for a bare micro electrode and a bionanocomposite sensor at a scan rate 20mV/s. (Reprinted with permission from (Shi et al., 2010). Copyright (2010) from Elsevier Inc.)

(b) Representative current response to  $\text{H}_2\text{O}_2$  for a bionanocomposite sensor and a bare electrode. (Reprinted with permission from (Shi et al., 2010 ). Copyright (2010) from Elsevier Inc.)

(c) Average linear regression result for averaged current versus molar concentration of  $\text{H}_2\text{O}_2$ . (Reprinted with permission from (Shi et al., 2010 ). Copyright (2010) from Elsevier Inc.)

Due to the high hydrophobicity of CNTs, most metal nanomaterials would not attach to CNTs via physical absorption. Hrapovic et al. linked Pt nanoparticles to SWNTs using the charge interaction between Pt and nafion-suspended SWNT, where Pt was positively charged and nafion was negative. A uniform layer containing Pt-CNT was formed (Hrapovic et al., 2004) (Fig. 4a). Kang et al. reported glucose biosensors based on a uniform Pt-CNT-chitosan film because the amino group of chitosan facilitates the dissolving of both Pt nanoparticles and  $-\text{COOH}$  modified CNT. Pt nanoparticles and CNT were dispersed in chitosan sol-gel as shown in the TEM image (Kang et al., 2008) (Fig. 4b). Electrodeposition of Pt and Au nanoparticles on CNT modified electrodes using  $\text{H}_2\text{PtCl}_6$  and  $\text{HAuCl}_4$  as Pt and Au source for glucose biosensing has also been reported, and SEM image showed that the porous MWNT film provided an ideal matrix for the distribution of Pt nanoparticles (Kang et al., 2007; Zou et al., 2008) (Fig. 4c).

The combination of metal nanomaterials and CNTs in biosensor surface modification integrates the catalytic capabilities of both nanomaterials and has been proved to be more effective in enhancing the biosensor's performance than using either material alone, including amperometric response, detection limit, linear range and stability (Claussen et al., 2009; Claussen et al., 2010; Hrapovic et al., 2004; McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2010 ).

### 3.4 Attaching enzymes to nanomaterials

Various surface modification approaches based on CNTs and metal nanomaterials have been reviewed in the previous sections. A good question is how to immobilize enzymes on

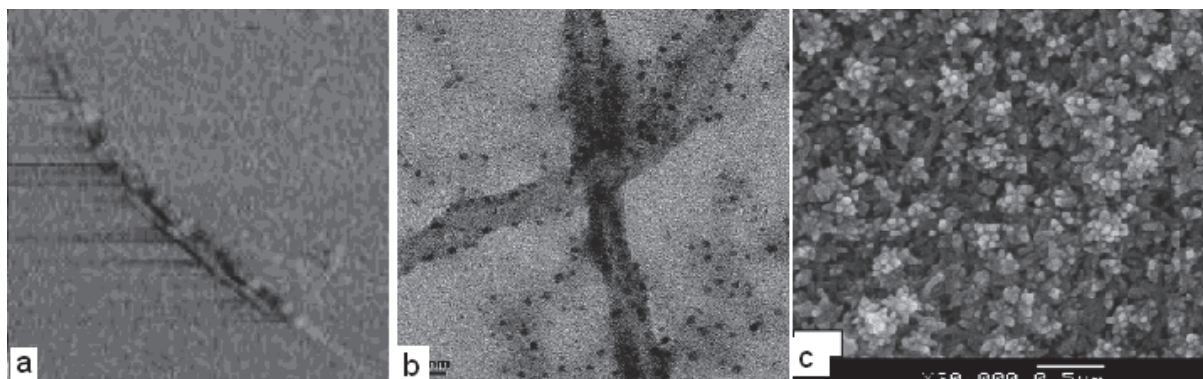


Fig. 4. (a). AFM tapping-mode phase image (size,  $1\ \mu\text{m} \times 1\ \mu\text{m}$ ; data scale, 20 nm) of one SWCNT in the presence of Pt nanoparticles. (Reprinted with permission from (Hrapovic et al., 2004). Copyright (2004) from American Chemical Society)  
 (b). TEM image of CNT-PtNP-CS-MTOS sol-gel. (Reprinted with permission from (Kang et al., 2008). Copyright (2007) from Elsevier B.V)  
 (c). SEM image of a Pt/MWNTs/GC electrode. (Reprinted with permission from (Zou et al., 2008). Copyright (2007) from Elsevier B.V)

nanomaterial modified electrodes, so that the biosensors have biorecognition capability while the catalytic activities of nanomaterials are preserved. Existing methods include direct absorption of enzymes by MWNTs due to the porous structure (McLamore et al., 2011; Shi et al., 2010), encapsulating enzymes and nanomaterials in the same polymer layer (Chen & Dong 2007; Choi et al., 2007a; Lim et al., 2005; Tsai et al., 2005), depositing multiple layers containing nanomaterials and enzymes (Zou et al., 2008) and attaching enzymes to modified electrodes via cross-linking agents (Claussen et al., 2009; Claussen et al., 2010). Gooding et al. reported a self-assembled attachment approach by incubating CNTs in microperoxidase MP-11 solution in HEPES buffer and showed that the enzymes were attached to the ends of the tubes via covalent bonds instead of being entrapped in the gaps among tubes (Gooding et al., 2003). They further demonstrated that no enzymes were linked to the side walls of CNTs with AFM showing that the number of CNTs was almost the same as the number of MP-11 enzymes (Gooding et al., 2003). Peak current of cyclic voltammetry in PBS for biosensors with MP-11 linked to SWNT was more than three times that of biosensors with no SWNT, demonstrating the electrochemical catalytic activities of CNTs (Gooding et al., 2003). Willner et al. reported similar approaches and linked the enzyme redox active center of flavin adenine dinucleotide (FAD) to the end of CNTs (Fernando et al., 2004). The attachment of enzyme redox center not only provided increased biosensing sensitivity due to enhanced electron transfer, but also allowed the direct electron transfer between enzymes and CNTs, which was the basis for third generation biosensors measuring direct electron transfer. Attaching enzymes to nanomaterials facilitates the “electrical communication” between enzymes and nanomaterials, resulting in improved biosensor response. However, one potential drawback associated this technique is that the structure of enzymes may be changed due to the covalent bonds which link enzymes to CNTs. The catalytic activities of enzymes may be affected due to the structural change.

#### 4. Conclusions

Almost all the literatures reviewed in this chapter reported biosensor performance in terms of amperometric and/or voltammetric response to the target compounds. Due to the large

amount of existing surface modification approaches, problems with evaluating and comparing different approaches, and sorting out the optimal ones have arisen.

For biosensors, two important biophysical factors will affect amperometric response:

1. Enzyme activity. When covalent bonds exist between the enzymes and cross-linking agents, polymer layers, nanomaterials or the electrode surface, the structural change of enzymes will decrease the catalytic activities. 2. Diffusion properties of layer immobilized on the surface of the electrode, including polymers, enzymes and nanomaterials. The ideal case is that the layer is most permeable to target compounds, while most resistant to the interfering compounds that will otherwise generate interference. For surface modification approaches aimed at enhancing the performance of biosensors, such as the immobilization of CNTs, two factors should be considered as well: 1. Enlarged surface area. Both CNTs and metal nanomaterials can enlarge the effective surface area of the electrodes. 2. Enhanced electron transfer rate due to the catalytic ability of nanomaterials.

The complexity arising from the many factors affecting biosensing has posed great difficulty for comparing different surface modification approaches considering analyzing various configurations of membranes, the underlying connections and interactions among components within membranes. Consequently, certain standards should be set up to evaluate the performance of the biosensors. Amperometric response is the commonly used standard. However, different approaches are based on electrodes of different geometric shape, such as disk electrode, wire electrode, and needle electrode, with quite distinct effective surface area. Therefore, it is more reasonable to use the current density (current per surface area) to evaluate the performance of biosensors instead of amperometric response. Current density is defined as:

$$j = i / A \quad (1)$$

Where  $j$  is the current density,  $I$  is the amperometric sensitivity of the biosensor and  $A$  is the effective surface area. For macro biosensors,  $A$  can be determined by cyclic voltammetry with potassium ferricyanide. According to the Randles-Sevcik equation (Bard & Faulkner 2000):

$$i_p = (2.69 \times 10^5) n^{3/2} D^{1/2} C A v^{1/2} \quad (2)$$

where  $n$  is the number of transferred electrons during the oxidation and reduction of potassium ferricyanide,  $D$  is the diffusion coefficient ( $6.70 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$ ),  $C$  is the molar concentration of ferricyanide,  $A$  is the effective surface area ( $\text{cm}^2$ ), and  $v$  is the scan rate ( $\text{V sec}^{-1}$ ). By varying  $v$  and measuring  $i_p$ ,  $A$  can be determined after linear regression. For microelectrodes, surface area can be determined by cyclic voltammetry with potassium ferricyanide as well. The diffusion limited current  $i_{lim}$  is defined as (Heinze 1993):

$$i_{lim} = KnFDcR \quad (3)$$

where  $K$  is the geometric constant,  $F$  is the faradic constant,  $r$  is the radius of the electrode tip, and other constants have the same meanings as in equation (2).

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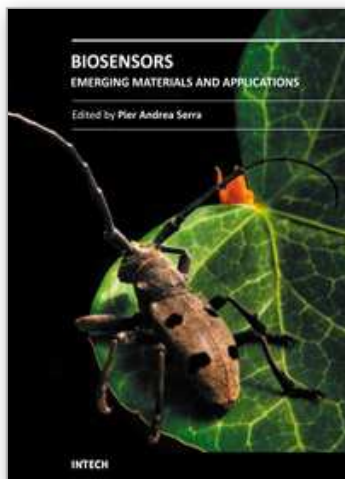


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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 19 different countries. The book consists of 27 chapters written by 106 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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