Chapter 8
Hybrid Metallic Nanoparticles: Enhanced Bioanalysis and Biosensing via Carbon Nanotubes, Graphene, and Organic Conjugation

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Abstract Composite materials, incorporating noble metal and metal oxide nanoparticles, have attracted much interest as active substrates for biosensor electronics. These nanoparticles provide a viable microenvironment for biomolecule immobilization by retaining their biological activity with desired orientation and for facilitating transduction of the biorecognition event. Herein, we discuss various methods for fabrication of metal and metal oxide nanoparticle composite materials

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and their applications in different electrochemical biosensors. The materials are organized by the corresponding component with the nanoparticles, i.e. carbon-based composites, polymers, and DNA. The performance of hybrids is compared and examples of biosensing apparatus are discussed. In all cases, the engineering of morphology, particle size, effective surface area, functionality, adsorption capability, and electron-transfer properties directly impact the resultant biosensing capabilities. Ultimately, these attractive features of metal and metal-oxide hybrid materials are expected to find applications in the next generation of smart biosensors.

Keywords Carbon nanotubes • DNA • Graphene • Metal oxide nanoparticles • Metallic nanoparticles • Polymer

8.1 Introduction

Hybrid metallic nanoparticles offer unique opportunities for designing powerful electrochemical bioassays and biosensors, and add a new dimension to such assays and devices. Metal and semiconductor nanomaterials, one-dimensional nanotubes and nanowires have rapidly become attractive labels for bioaffinity assays, offering unique signal amplification and multiplexing capabilities. The coupling of different metallic nanoparticle-based platforms and amplifications processes have dramatically enhanced the intensity of the analytical signal and led to the development of ultrasensitive bioassays and biosensors (Wang 2007). The orders of magnitude of amplification afforded by such metallic nanoparticle-based schemes opens up the possibility of detecting a plethora of agents and markers more rapidly and at lower detection limits. These highly sensitive biodetection schemes might provide an early detection of biomarkers, toxins, pathogens, and diseases in agricultural, food, and medical systems by using ultrasensitive bioelectroanalytical protocols unachievable with standard electrochemical methods.

Applications of biosensors are developed mainly for environmental and bioprocess monitoring, agriculture, bioterrorism, as well as medical and food biosensor systems. The presence of unsafe levels of chemical compounds, toxins, and pathogens in food constitutes a growing public health problem that necessitates new technology for the detection of these contaminants along the food continuum from production to consumption. The recent attention to food safety and regulatory issues towards consumer welfare is of utmost concern. While traditional techniques that are highly selective and sensitive exist, there is still a need for simpler, more rapid, and cost-effective approaches to food safety evaluation. Biosensors offer advantages over current analytical methods. In addition to their good selectivity, low cost, and portability, they have the ability to measure samples with minimal sample preparation required. Microbial metabolism, antibody, and DNA/RNA-based biosensors display promise for use as electrochemical biosensors used in food safety applications (Arora et al. 2011).

8.2 Carbon–Metal Hybrids

The combination of highly conductive carbon nanomaterials and highly catalytic metallic nanoparticles has led to new leaps in electrochemical biosensor performance. Carbon–metal hybrid carrier-molecules and carbon nanotube (CNT)– or graphene–metallic nanoparticle-based electrodes have all greatly enhanced the sensitivity, linear sensing range, and limit of detection of electrochemical biosensors. These nanomaterials display a high degree of catalytic activity, conductivity, and biocompatibility that act in a synergetic manner to improve biosensor performance. These unique material properties and the design and fabrication of biosensors that incorporate CNTs, graphene, and metallic nanoparticles will be thoroughly discussed. The subsequent functionalization and resultant interaction with biorecognition agents (e.g., peptides, proteins, and nucleic acids) for use as biosensors will be reviewed including those associated with health care, environmental monitoring, security surveillance, food safety, and biodefense.

8.2.1 Carbon–Metal Hybrids as Carrier Labels in Biosensing

Better understanding of synthesis routes for nanostructured materials has opened new avenues for developing biosensing platforms. The use of carbon/inorganic hybrid nanostructures as carrier labels in bioanalysis offers very elegant ways of interfacing biomolecule recognition events with inherent signal amplification. Particular attention is given to hybrid nanostructures involving carbon and inorganic nanomaterials. Elegant advances for tagging in biosensing based on such hybrids of carbon/inorganic-nanomaterial heterostructures are underlined along with future prospects and challenges.

Ultrasensitive detection of biomolecules is required in a variety of societal areas including clinical diagnostics, food safety, and environmental protection. Many efforts have been devoted to accomplishing ultrasensitive and even single-molecule detection by using signal amplification based on polymerase chain reaction (PCR) and mass spectrometric (MS) techniques. Although these methodologies have adequate sensitivity, they are destructive and often suffer from time-consuming derivatization, high cost, and the need for professional operation. With the emergence of nanotechnology and nanoscience, nanomaterial-based signal amplification holds great promise for achieving high sensitivity and selectivity for in situ or online detection of biomolecules, due to the use of rapid analysis procedures and easy miniaturization. The applications of nanomaterials in bioanalysis can be classified into two categories according to their functions: nanomaterial-modified transducers to facilitate bioreceptor immobilization or improvement of transducers' properties, such as low-background signals and high signal-to-noise ratio, and nanomaterial–biomolecule conjugates as labels for signal amplification. In particular, nanomate-

rial labels are showing the greatest promise for developing ultrasensitive bioanalysis strategies (Liu and Lin 2007). In these approaches, the nanomaterials usually act as catalysts to trigger the detectable signal or as carriers for both large loading of signal molecules and the accumulation of reaction products (Lei and Ju 2012).

Bioconjugates integrating nanomaterials with the catalytic and recognition properties of biomolecules have led to advanced electrochemical biosensors with ultrahigh sensitivity and multiplexed capability (Wang 2003; Pingarrón et al. 2008; Merkoçi 2010; Yáñez-Sedeño et al. 2010). These biofunctional nanomaterials can not only produce a synergic effect among catalytic activity, conductivity, and biocompatibility to accelerate the signal transduction, but also provide amplified recognition events by high loading of signal tags, leading to highly sensitive and specific biosensing (Lei and Ju 2012). The range of nanomaterials used in biosensors is wide and depends on the specific assay and application. Due to the diverse properties of different nanomaterials, in many situations the coupling of two different nanoscale materials offsets the insufficiency of each individual nanomaterial to fulfill the growing requirements of emerging sensing devices. Moreover, it also endows the resultant nanohybrid material with a greatly enhanced performance, superior to that observed when a single nanomaterial is used.

Inorganic nanomaterials, such as metal nanowires, nanoparticles, quantum dots (QDs) or inorganic nanocrystals, and carbon nanomaterials, including carbon nanotubes (CNTs), fullerenes or graphene, have received considerable interest in the field of nanoscience owing to their unique physical and chemical properties (in comparison with bulk materials), which offer excellent prospects for enhancing the performance of chemical, biological, and electrochemical sensors (Storhoff and Mirkin 1999; Caruso 2001; Claussen et al. 2011a; Willner and Willner 2002; Wang 2003; Pingarrón et al. 2008; Merkoçi 2010; Yáñez-Sedeño et al. 2010; Pei et al. 2013). When reduced to the nanoscale, such nanomaterials display new and unique size- and shape-dependent properties compared to those they display on a macroscale. A wide variety of inorganic nanoscale materials of different sizes, shapes, and compositions are now available, leading to tunable electronic and optical properties. Particularly attractive are the heteronanostructures of carbon and inorganic nanomaterials (CNTs modified with metal nanoparticles, QDs, semiconductor nanocrystals and metal oxide nanoparticles and graphene–inorganic nanohybrids), which have shown extremely useful integration of the unique properties of these types of nanomaterials. Thus, they exhibit some new functions and superior properties to those of their individual constituents and impart excellent analytical performance to biosensing (Peng et al. 2009; Eder 2010; Wu et al. 2011; Campuzano and Wang 2011) as well as molecular logic paradigms that are well suited for multiplexed biosensing (Manesh et al. 2011; Claussen et al. 2013, 2014). Often a third component, such as ionic liquids or chitosan, plays a key role in the preparation of these carbon–inorganic nanohybrids, acting as effective binder systems, inducing the solubilization of the corresponding carbon nanomaterial, and facilitating its manipulation and functionalization (Zhang et al. 2004; Shan et al. 2010; Zeng et al. 2011).

The power and scope of these nanoheteromaterials can be greatly enhanced by coupling them with immunoreactions and electrical processes (i.e., nanobioelec-

tronics) (Liu and Lin 2007). Commonly used enzyme immunosensors can greatly benefit from the highly enhanced response of the biocatalytic reaction product at the electrode transducers from nanoscale inorganic/carbon amplification platforms carrying multiple tags. Such hybrid nanoarchitectures open up the possibility of detecting ultralow levels of biomarkers that cannot be measured by conventional methods or in connection to a single nanomaterial. This section summarizes recent significant advances and progress in the use of biofunctional carbon/inorganic nanoheterostructures as excellent electronic signal tags in ultrasensitive bioanalysis and biosensing, particularly using bioelectronic affinity assays and electrochemical detection, highlighting some elegant applications. While the new capabilities offered by nanoscale hybrid materials will be illustrated mainly in connection with ultrasensitive electrochemical immunosensors and immunoassays, a wide range of important biomolecules can benefit from similar improvements.

As an example, Zhong et al. (2010) developed a new amplification core–shell nanolabel based on a chitosan-protected graphene nanosheet core and multi-nanogold particles as the shell. Such a nanogold-enwrapped graphene nanocomposite led to ultrasensitive measurements down to 100 pg mL^{-1} of carcinoembryonic antigen (CEA) and convenient assays of serum samples. QD-functionalized graphene sheets (GS) were prepared and used as labels for the preparation of sandwich-type electrochemical immunosensors for the detection of prostate-specific antigen (PSA). The immunosensor displayed a linear response within a wide concentration range (0.005–10 ng mL^{-1}), a low detection limit (3 pg mL^{-1}), and applicability to detect PSA in patient serum samples (Yang et al. 2011). A novel enzyme-free sandwich electrochemical immunoassay for alpha-fetoprotein (AFP) was developed by Tang et al. (2011) using gold nanoparticle-coated carbon nanotubes (CNTs/AuNPs) as nanolabels/nanocatalysts. This highly sensitive approach is based on the catalytic reduction of *p*-nitrophenol (NP) by the CNT/AuNP labels and the redox cycling of *p*-aminophenol (AP) to *p*-quinone imine (QI) by $NaBH_4$ to offer an extremely low detection limit of 0.8 fg mL^{-1}.

Lai et al. (2011) proposed an ultrasensitive multiplexed immunoassay method for tumor markers based on the use as a trace tag of a novel functional CNT/AgNP nanohybrid functionalized with streptavidin and the corresponding biotinylated signal antibody. Through a sandwich-type immunoreaction on a disposable immunosensor array, the high-content AgNPs can be captured on the immunosensor surface to further induce the silver deposition, which greatly amplifies the detection signal (Fig. 8.1). Based on the electrochemical stripping detection of the AgNPs on the immunosensor surface, the proposed simultaneous multianalyte immunoassay method, using CEA and AFP as model analytes, showed an ultrahigh sensitivity with limits of detection (LODs) of 0.093 and 0.061 pg mL^{-1}, respectively, and wide linear ranges over four orders of magnitude.

Han et al. (2012) developed a novel multiple-label method and dual catalysis amplification strategy for the simultaneous detection of free and total PSA (fPSA and tPSA, respectively). AuNP-modified Prussian blue nanoparticles and AuNP-modified nickel hexacyanoferrate nanoparticles decorated onion-like mesoporous graphene sheets, denoted as Au@PBNPs/O-GS and Au@NiNPs/O-GS, respectively, functionalized with streptavidin and biotinylated alkaline phosphatase (AP) were

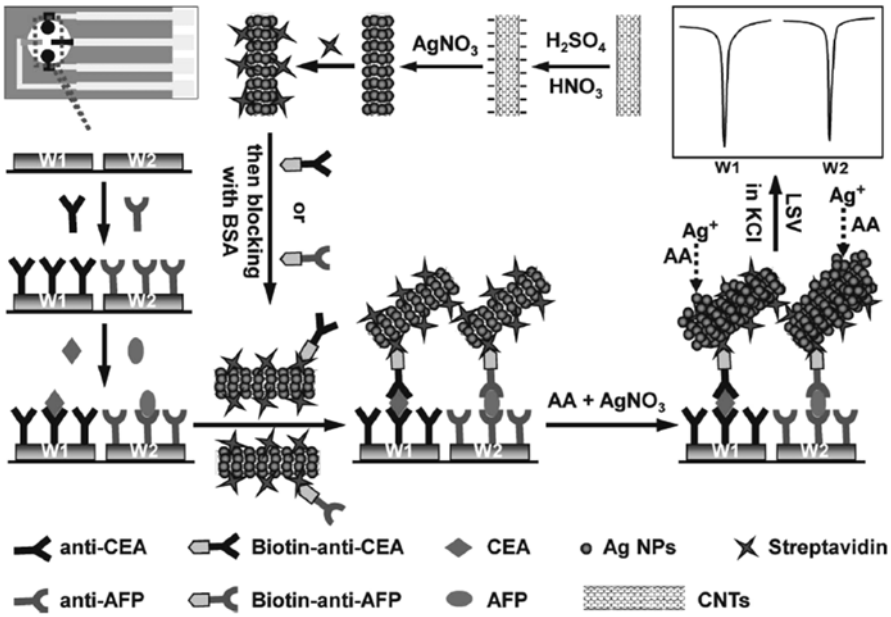

Fig. 8.1 Schematic representation of the multiplexed immunosensor developed based on the use of novel functional CNT/AgNP nanohybrids functionalized with streptavidin and biotinylated signal antibodies as tracer tags, and of the detection strategy by linear-sweep stripping voltammetric (LSV) analysis of AgNPs captured on the immunosensor surface (Reproduced from Lai et al. (2011) with permission). Copyright 2011 Wiley

utilized as distinguishable signal tags to label different detection antibodies. The dual catalysis amplification could be achieved by biocatalysis of AP towards ascorbic acid 2-phosphate (AA-P) to in situ production of ascorbic acid (AA) and then the chemicatalysis of Au@PBNPs/O-GS and Au@NiNPs/O-GS nanohybrids towards AA to generate dehydroascorbic acid (DHA). The linear ranges of the proposed immunosensor were defined from 0.02 to 10 ng mL^{-1} and 0.01 to 50 ng mL^{-1} with LODs of 6.7 pg mL^{-1} and 3.4 pg mL^{-1} for fPSA and tPSA, respectively.

Very recently, an ultrasensitive immunoassay for *Shewanella oneidensis* was presented by employing a novel conjugate featuring gold nanoparticles (AuNPs) and antibodies (Ab) assembled on bovine serum albumin (BSA)-modified GO (Ab/AuNPs/BSA/GO) as carrier and a silver enhancement detection strategy in the presence of hydroquinone. This electrochemical immunoassay offers excellent detectability (LOD of 12 cfu mL^{-1}) and a wide range of linearity (from 7.0×10^1 to 7.0×10^7 cfu mL^{-1}) (Wen et al. 2013).

The need for ultrasensitive bioassays and the trend towards miniaturized assays has made the biofunctionalization of nanomaterials one of the more popular fields of research. Although inorganic/carbon nanohybrids are still in an early stage of material science, the use of these nanohybrids as carriers of the signalling molecules for amplification transduction of biorecognition events has taken off rapidly and

will surely continue to expand at an accelerated pace. A wide variety of nanoscaled materials with different sizes, shapes, and compositions have been introduced into biosensing for amplification detection. The judicious coupling of two different nanomaterials has been shown to offer greatly enhanced analytical performance, superior to that observed when a single nanomaterial is used, which is extremely attractive for bioelectronic transduction of biomolecular recognition events. Future efforts will certainly aim at guiding and tailoring the synthesis of nanohybrid materials for meeting specific electrochemical biosensing applications and needs.

The rapid recent progress in electrochemical biosensors based on the use of these bioconjugated nanoscale hybrid materials as tracing bionanotags, characterized by enormous signal enhancement and ultralow LODs, suggests the major impact that such nanobioelectronic sensing devices will have in the near future on important fields, including health care, environmental monitoring, security surveillance, food safety, and biodefense. Many exciting opportunities and challenges thus remain in the development and use of bioconjugated hybrid nanoarchitectures for future bioelectronic sensing applications. For example, a better understanding of their nanofabrication process is necessary to improve the properties of these nanohybrid labels. Furthermore, in order to increase their biocompatibility, a significant direction to explore is a mild biofunctional way to fix the biomolecules on the surface of these bioconjugated hybrid heteronanostructures. Another interesting opportunity for achieving exponential signal amplification is the development of hybrid nanomaterials-based autocatalytic systems, in which each step produces a product that acts both as a template or a stoichiometric trigger and a catalyst (or activates a catalyst) to produce more products. With the demand in life sciences and clinical diagnosis, the ultimate goal of this field is the utilization of nanomaterials which not only enhance the biosensing capabilities compared with conventional platforms, but also bring out new approaches such as miniaturization, reagent-less biosensing, and single-molecule detection.

8.2.2 Graphene–Metal Hybrid Biosensors

There is evidence that graphene and its derivatives can exhibit good electrochemical performance compared with other electrodes such as glassy carbon (Shang et al. 2008), graphite (Shan et al. 2009), or even carbon nanotubes (Alwarappan et al. 2009; Wang et al. 2009). When focusing on graphene-based biosensors, it is noteworthy that few sensors were demonstrated to have actually incorporated pure graphene (Ohno et al. 2009; Guo et al. 2011; Lidong et al. 2012; Ruan et al. 2012); the remainder have employed graphene oxide (and its derivatives) (Alwarappan et al. 2009; Shan et al. 2009; Mohanty and Berry 2008; Lu et al. 2009) or multilayer graphene and related structures (Shang et al. 2008; Lu et al. 2007, 2008; Claussen et al. 2012).

Conjugation of reduced graphene oxide with metal nanoparticles has been recently studied for the development of electrocatalytic platforms in amperometric biosensors (see Table 8.1). The hybridization of reduced graphene oxide with metal nanoparticles helps maintain the interplanar spacing between reduced graphene

Table 8.1 Examples of recently developed graphene–nanoparticle biosensors

Biosensor	Analyte	Sensitivity	Response time	Operating range	Reference
GO decorated with AuNR-antibody conjugates	Transferrin	10.2 nm shift in resonant wavelength at 10 mg/mL transferrin	60 min	0.0375–40 μg/mL	Zhang et al. (2013)
MIP/Gr-AuNPs/GCE	Glycoprotein	0.405 μA/log *M*	8 min	1×10^{-11}–1×10^{-5} g/mL	Wang et al. (2013)
GN-AuNRs-ADH	Ethanol	102 μA/mM cm^2	10 s	5–377 μM	Li et al. (2013)
Nafion/Mb-Gr-Pt/CILE	Trichloroacetic acid	30.08 μA/mM	NR	0.9–9 mM	Sun et al. (2013)
C-SPE/Pt-NPs/RGO/lacc/ Nafion	Caffeic acid	2,147.38 nA/μM	60 s	0.2–2 μM	Eremia et al. (2013)
Pt nanoparticles/Graphene Petals/Ti/SiO_2	Glucose	NR	NR	0.01–50 mM	
GS–CS–PtPd/ChOx	Cholesterol	NR	<7 s	2.2–520 μM	Cao et al. (2013)
(HRP-Pd)/f-graphene-Gr	Hydrogen peroxide	92.82 μA/mM cm^2	<2 s	25–3,500 μM	Nandini et al. (2013)
AgNPs-Pdop@Gr/GCE	Catechol	0.087 μA/μM	NR	0.5–240 μM	Huang et al. (2013)
Ni/Nafion/graphene/GCE	Ethanol	NR	< 5 s	0.43–88.15 mM	Jia and Wang (2013)

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oxide sheets while connecting the nanomaterial network to the electrode surface. Functionalization of electrodes with graphene–nanoparticle composites has shown promising potential for improvement in the electrochemical performance of the sensing devices (e.g. sensitivity, response time, operating range).

The unique properties of graphene–nanoparticle hybrids have been widely used in the fabrication of biosensor systems for food safety applications, to detect toxins (Gan et al. 2013a; Srivastava et al. 2013; Tang et al. 2012; Yang et al. 2013b), pesticides (Oliveira et al. 2013a), allergens (Eissa et al. 2013), ingredients and bioactives (Labroo and Cui 2013; Zhou et al. 2013; Si et al. 2013; Wang et al. 2011), controlled and prohibited substances (Kong et al. 2013; Xie et al. 2012; Lin et al. 2013; Gan et al. 2013b, c; Zhao et al. 2011; Zhang et al. 2011; Cui et al. 2011; Huang et al. 2012; Wei et al. 2012; Wang et al. 2012b; Ma et al. 2013; Ye et al. 2013), and a variety of foodborne pathogens (Hu et al. 2013; Singh et al. 2013a; Chang et al. 2013; Liu et al. 2011a, b; Wan et al. 2011; Jung et al. 2010).

Perhaps some of the most promising graphene-based biosensors have been displayed from those that combine graphene with noble metal nanoparticles (Taguchi et al. 2014). For example, Hong et al. self-assembled positively charged gold nanoparticles (2–6 nm in diameter) onto the surfaces of 1-pyrene butyric acid functionalized graphene (PFG) sheets via a facile chemical mixing technique (Hong et al. 2010). When immobilized onto a glassy carbon electrode, the graphene/gold nanoparticle composite material showed strong electrocatalytic activity and was able to sense uric acid with high sensitivity and a rapid response. Zheng et al. utilized graphene that was covalently functionalized and decorated with palladium nanoparticles immobilized with the enzyme glucose oxidase on a glassy carbon electrode to sense glucose (Zeng et al. 2011). The biosensor had high glucose sensitivity with a linear range from 1.0 μM to 1.0 mM as well as a low detection limit of 0.2 μM ($S/N = 3$). Claussen and coworkers demonstrated the growth of a three-dimensional matrix of graphene petals on a silicon wafer via a chemical vapor deposition process (Claussen et al. 2012). The petals were decorated with platinum nanoparticles of varying size, density, and morphology through a current-pulse electrochemical deposition technique (Fig. 8.2). Likewise the enzyme glucose oxidase mixed with the conductive polymer PEDOT:PSS was electrodeposited onto the biosensor surface for subsequent glucose biosensing. The results demonstrated a robust sensor design that demonstrated exceptional performance with regards to glucose sensitivity (0.3 μM detection limit, 0.01–50 mM linear sensing range), stability (shelf life >1 month), and low interference from electroactive species typically found endogenously in human serum samples.

8.2.3 Carbon Nanotube–Metal Nanoparticle Biosensors

An important property of carbon nanotubes (CNT) for electrochemical detection is their ability to promote electron transfer in electrochemical reactions (Gooding 2005; McCreery 2008; Katz and Willner 2004). CNTs are commonly referred to as rolled-up graphene sheets, and both allotropes have a meshwork of

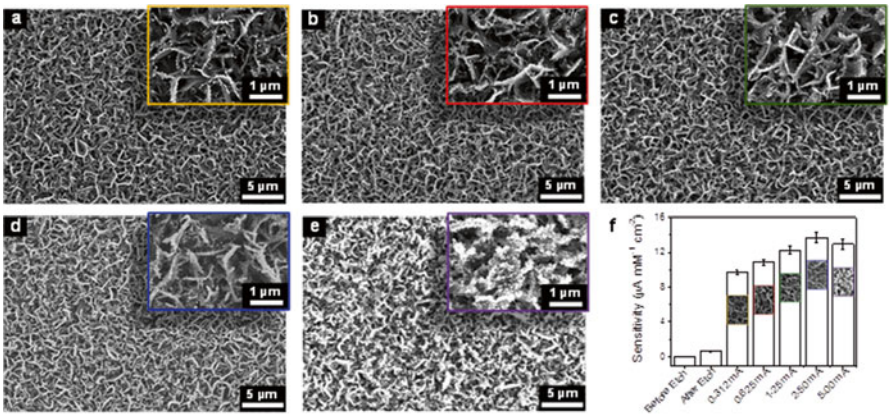

Fig. 8.2 Field emission scanning electron microscopy micrographs of platinum nanoparticles electrodeposited on multilayered graphene petal nanosheets (MPGNs) grown on a silicon wafer. Current pulses (500 ms) of (**a**) 312 µA (*orange*), (**b**) 625 µA (*red*), (**c**) 1.25 mA (*green*), (**d**) 2.5 mA (*blue*), and (**e**) 5.0 mA (*purple*) were used to electrodeposit Pt nanoparticles of distinct size and density onto the MGPNs. (**f**) *Bar graph* displaying the H_2O_2 sensitivity of the MGPN electrode (before and after the oxygen plasma etch) and the PtNP-MGPN electrodes. *Errors bars* show standard deviations for three different experiments. The MPGNs with the most needle-like Pt nanostructures (**d**) had the highest H_2O_2 sensitivity and highest glucose sensitivity in subsequent analysis (Reproduced from Claussen et al. (2012) with permission). Copyright 2012 Wiley

sp^2-hybridized carbon atoms (Yang et al. 2010b). CNTs have high aspect ratios, high mechanical strength, high surface areas, excellent chemical and thermal stability, and rich electronic and optical properties (Ajayan 1999). The properties of CNTs can also be capitalized on by combining them with other functional materials, such as conducting polymers or metal nanoparticles, in order to enhance their electrochemical sensing performance (Dai 2007). Table 8.2 shows some examples of carbon nanotube nanoparticle-mediated biosensors for the quantification of analytes such as glucose, glutamate, xanthine, t-DNA, ethanol, and D-amino acid.

The combination of excellent conductivity, good electrochemical properties, and nanometer dimensions has seen CNTs used frequently for the detection of diverse biological structures such as DNA, viruses, antigens, disease markers, and whole cells. In food systems particularly, there have been many reports of CNT-based electrochemical biosensors for the detection of toxins (Singh et al. 2013b; Palaniappan et al. 2013; Temur et al. 2012; Yang et al. 2010a), pesticides (Oliveira et al. 2013b; Cesarino et al. 2012; Liu et al. 2012), allergens (Liu et al. 2010; Cao et al. 2011), ingredients (Antiochia et al. 2013; Monosik et al. 2012a, 2013; Ziyatdinova et al. 2013; Wang et al. 2012a), controlled and prohibited substances (Batra et al. 2013; Kim et al. 2012a, b; Monosik et al. 2012b), microbial metabolism markers (Lim et al. 2013; Park et al. 2012), and a variety of foodborne pathogens (Yang et al. 2013a; Li et al. 2012; Pandey et al. 2011; Garcia-Aljaro et al. 2010; Zelada-Guillen et al. 2010).

The ability to control the position and density of carbon nanotube arrays and the size, density, and morphology of nanoparticles on a biosensor surface is

Table 8.2 Examples of previously developed carbon nanotube–nanoparticle biosensors

Biosensor	Analyte	Sensitivity	Response time	Operating range	Reference
GOx/Pd-Au-SWCNT/PAA	Glucose	5.2 μA/mM cm^2	5 s	1.3–50 mM	Claussen et al. (2009)
GOx/Pt-SWCNT/PAA	Glucose	70 μA/mM cm^2	8 s	LOD = 380 nM	Claussen et al. (2010)
GluOx/Pt-SWCNT/PAA	Glutamate	27.4 μA/mM cm^2	NR	0.05–1,600 μM	Claussen et al. (2011c)
GOx/MPA/Au nanodisks/PAA	Glucose	0.47 nA/mM cm^2	NR	1–21 mM	Claussen et al. (2011a)
Pt/MWNT/Nafion/GO	Glucose	531 pA/mM	0.88 s	0.01–17.5 mM	McLamore et al. (2011)
XO-CD/pAuNP/SWNT/GCE	Xanthine	2.47 A/M cm^2	5 s	0.05–9.5 μM	Villalonga et al. (2012)
GluOx/cMWCNT/AuNP/CHIT	Glutamate	155 nA/μM cm^2	2 s	5–500 μM	Batra and Pundir (2013)
CGi + p-DNA	t-DNA	NR	NR	100 fM to 1 μM	Ko et al. (2011)
CNT-Ni hybrid	Ethanol	14.81 μA/μM cm^2	<10 s	50–600 μM	Chen and Huang (2010)
DAAO/c-MWCNT/CuNPs/PANI/Au	D-amino acid	54.85 μA/mM cm^2	2 s	0.001–0.7 mM	Lata et al. (2013)

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challenging. Pang et al. demonstrated how CNT arrays can be grown from titania nanotubes with subsequent uniform decoration of Pt nanoparticles (~3 nm) (Pang et al. 2009). After immbolization of the enzyme GOx, the biosensor sensed glucose with a high sensitivity of 0.24 μA mM^{-1} cm^{-2} and a linear sensing range of 0.006–1.5 mM. Zhao et al. developed aligned CNT arrays by chemical vapor deposition on a silicon wafer. A thin film of gold (200 nm) was subsequently deposited onto the CNTs and the Au-CNTs were peeled from the Si floor with the assistance of 10 % hydrofluoric solution (Zhao et al. 2007). Platinum nanoparticles were electrodeposited onto the CNTs to improve electrocatalytic performance while GOx and Nafion were drop-casted onto the Pt nanoparticles/CNTs to transform the electrode into a glucose biosensor. The biosensor was able to sense glucose within a linear sensing range from 0.010 to 7 mM and a fast response time within 5 s. Fisher and coworkers demonstrated how low-density single-walled CNT arrays can be developed from a porous anodic alumina template (Franklin et al. 2008; Claussen et al. 2009). In particular, the team demonstrated how the spacing of Pt nanospheres electrodeposited concentrically around single-walled CNT arrays and functionalized with the enzyme GOx can dramatically change glucose biosensing from 300 μM to 15 mM with a theoretical glucose detection limit of 74 μM ($S/N = 3$) to a linear sensing range of 100 μM to 20 mM and a detection limit of 5.8 μM ($S/N = 3$) (Claussen et al. 2011b).

8.3 Polymer–Metal Hybrids

As stated, the integration of bio-recognition and signal transduction elements has been an important area of biosensor research for several decades. Similar to the incorporation of carbon nanostructures, the functional properties (electronic, optical, and magnetic) and relative stability when bound to biological molecules (peptides, proteins, and nucleic acids) of metallic and metal oxide nanoparticles (MNPs) make them attractive candidates for integrated biosensors. Proteins, peptides, and antibodies have been utilized to conjugate MNPs for use in a wide array of biosensors to detect and amplify various small-molecule signals. To conjugate biomacromolecules with MNPs, residual thiol groups are often reacted with MNPs to form metal–sulfide bonds (Dreaden et al. 2012), or electrostatic interactions are exploited for physisorption of biomacromolecules to the surface of the MNP. Both methods result in the random placement of the biomacromolecule on the surface of the MNP and may also result in reduced bioactivity; therefore, MNPs composed with tailored, functional polymer coatings have emerged as a popular substrate for precision bio-conjugation. Encapsulation of MNPs within polymer matrices avoids the deleterious effects of MNP aggregation; moreover, polymers provide a versatile, functional platform for attaching organic moieties and biomacromolecules with various signal readout strategies, e.g. electrochemical, enzymatic, colorimetric, fluorometric, magnetic, and chemiluminescent. Both polymer conjugates of noble metal and metal-oxide MNPs are currently being explored as biosensors, and this section

will focus on advances in the synthesis of polymer–MNP hybrids and their use in the bio-recognition component of electrochemical biosensors.

8.3.1 Polymer–Metal Hybrid Synthesis

Firstly, reproducible sensor materials require controlled MNP growth, particle size distribution, and particle–polymer interactions. There are many well accepted ex situ and in situ routes for the synthesis of polymer–MNP hybrids that include both standard engineering polymers and advanced conducting polymers (Sperling and Parak 2010; Shaidarova and Budnikov 2008). The polymer–MNP hybrid can be generated by compounding powders of MNPs with common melt-processed polymers such as poly(styrene), poly(ethylene) or poly(methyl methacrylate) (ex situ). Alternatively, the polymer–MNP hybrid can be formed by precipitation of the MNP from metal or metal-oxide precursors dissolved within a swellable polymer or polymer solution, such as poly(acrylic acid), poly(vinyl alcohol), polyaniline, polypyrrole or poly(vinylpyrrolidone) (in situ) (Ferey 2008; Ramesh et al. 2009; Njagi and Andreescu 2007). A schematic for the synthesis of polymer–nanoparticle hybrids is shown in Fig. 8.3.

The ex situ approach has two steps: (1) bulk synthesis of MNP powder and (2) dispersion of MNP powder throughout a polymer matrix, often by compounding with a polymer melt. The bulk synthesis of noble metal and metal-oxide nanoparticle powders are direct processes and have been reviewed elsewhere (Shi et al. 2013). The more nuanced in situ synthesis of polymer–MNP hybrids can take many forms. In this route, MNPs are synthesized inside a polymer matrix by either decomposition or reduction of precursors dissolved into the polymer film/monolith or polymer solution. Polymer–MNP hybrids prepared by the reduction of metal salts in the presence of stabilizing polymers have tightly controlled size and size distribution; thus, the in situ preparation method has been the route of choice for biosensor materials (Rao 2012; Antonietti et al. 1995; Spatz et al. 1996). For instance, $HAuCl_4 \cdot 4H_2O$ gives stable gold MNPs upon refluxing in methanol/water in the presence of poly(vinylpyrrolidone) and NaOH. In poly(acrylamide), $AuCl_4$ can be directly reduced by $NaBH_4$. Reduction of metal ions in the presence of these polymers results in the conjugation of the metal cations with the ligand, and this dramatically limits the MNP size and controls size distribution (Daniel and Astruc 2004). Synthesis of metal-oxide nanoparticles in solutions of polymer stabilizers also aids to the control of crystallinity and oxidation. For example, the reduction of Fe^{2+} and Fe^{3+} can be driven toward either Fe_2O_3 or Fe_3O_4 by altering poly(acid) concentration and constituents (Daniele et al. 2013; Qi et al. 2013; Zhang et al. 2012). Other common polymers used to stabilize MNPs are hydrophilic and biocompatible polymers, such as poly(ethylene glycol), poly(ethylene oxide), poly(lactic-c*o*-glycolic acid), poly(vinyl alcohol) and poly(acrylic acid). These in situ methods and selected polymers for the synthesis of polymer-MNPs have been extensively studied for both optical and electrochemical biosensor applications; therefore, the remainder of this section will concentrate on in situ generated polymer–MNP hybrids with attached bio-recognition agents and their utilization as biosensors.

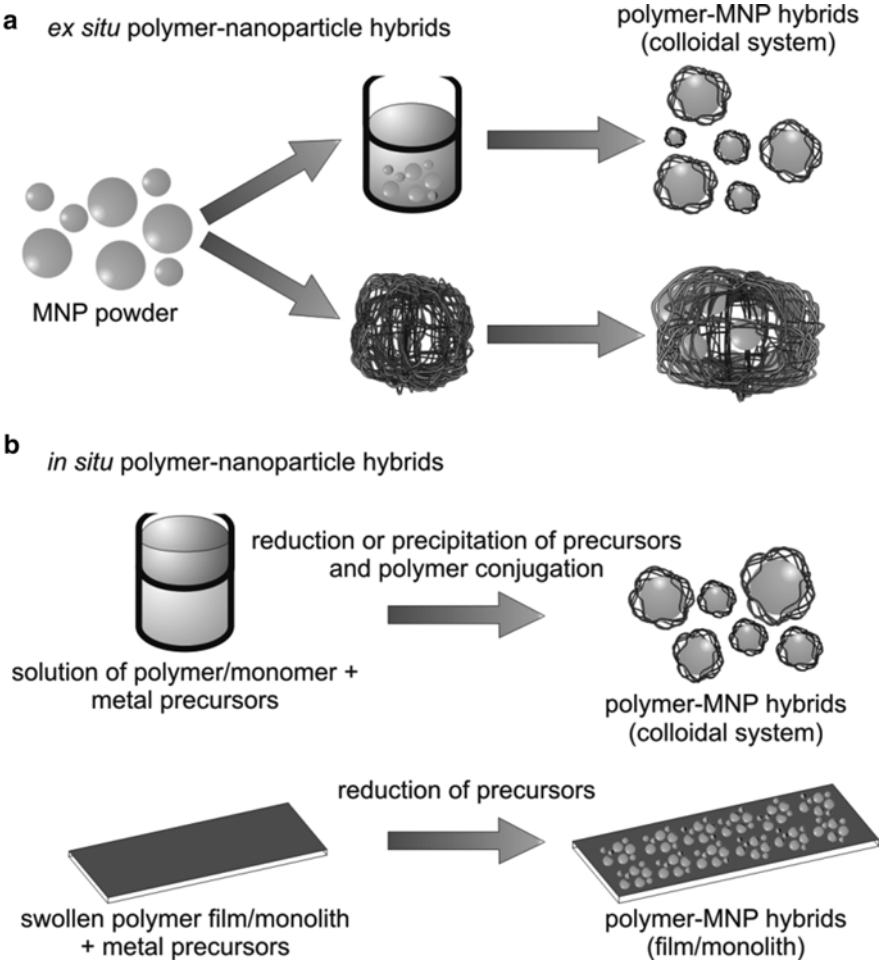

Fig. 8.3 (**a**) Ex situ and (**b**) in situ synthesis of polymer–nanoparticle hybrids. The ex situ system relies upon synthesis of metal nanoparticle powders or dispersions that can be subsequently incorporated into polymer matrices by either solution-based ligand exchange or compounding into bulk polymers. The in situ system most often uses reduction of precursor salts to form metal nanoparticles with a polymer-capped surface or within a bulk gel matrix

8.3.2 Polymer–Noble Metal Hybrids

The unique physicochemical properties of noble metals at the nanoscale have led to the development of a wide variety of biosensors, such as (1) nanobiosensors for point-of-care disease diagnosis, (2) nanoprobes for in vivo sensing/imaging, cell tracking, and monitoring disease pathogenesis or therapy monitoring, and (3) other nanotechnology-based tools that benefit scientific research on basic biology. Gold MNPs are among the most extensively studied nanomaterials and have led to the development of

substrates for the conjugation of bio-recognition agents. The earliest gold–MNP hybrid utilized single-strand DNA. In 1996, Mirkin et al. described the first use of gold MNPs as biosensors. Functionalized with thiol-modified ssDNA probes, the gold MNPs would form a cross-linking network upon detection of a complementary ssDNA–MNP hybrid (Mirkin et al. 1996). This cross-linking network lead to the aggregation of the gold NPs causing a bathochromic shift in the surface plasmon resonance. More recent explorations of DNA–MNP hybrids will be assessed in Sect. 8.3.3.

Although similar surface plasmon resonance methods have shown that direct detection of biomolecules and analyte interaction is possible, many have benefited from the electroactive or catalytic properties of NPs as reporters for electrochemical biosensing with unprecedented levels of sensitivity. Noble metal–polymer hybrids have been used as biosensors for detection of analytes including, but not limited to, glucose (Wei et al. 2012; Crespilho et al. 2006), dopamine (Prakash et al. 2013), hydrogen peroxide (Kim et al. 2010; Muraviev et al. 2006), cholesterol (Yang et al. 2006; Ansari et al. 2009), and urea (Kozitsina et al. 2009). Several authors have described the development of amperometric-based biosensors, which are usually more suited for mass production than potentiometric biosensors. In this approach the working electrode is usually a noble metal MNP covered by the bio-recognition component, which enables the amperometric signal. For example, Kim et al. (2000) developed a disposable immuno-chromatographic sensor for on-line quantitative determination of human serum albumin. The polymer–MNP sensor used gold MNPs in a polyaniline matrix (a conducting polymer) for signal generation. The immunoassay was a membrane strip sensor, where the reaction between the conjugate and analyte took place and was carried up into a membrane that contained the immobilized antibody. The secondary antigen–antibody reaction formed a "sandwich complex" at the electrode and the gold–polyaniline polymer–MNP hybrid generated a conductimetric signal. Yin et al. used gold NPs over a surface of poly(styrene-acrylic acid) nanospheres, which served as a matrix to conjugate alkaline phosphatase, to detect the tumor necrosis factor. Omidfar et al. (2012) have also developed a high-sensitivity electrochemical human serum albumin sensor based on human serum albumin MNPs (electrochemical label) within a PVA monolith (polymer matrix) which exhibited high sensitivity and excellent stability.

Ranging from surface plasmon, infrared spectroscopy, and fluorescence to traditional electrochemical methods, noble metal polymer–MNP hybrids are providing a new horizon for biosensing and bioanalyses in clinical diagnostics and biological research; furthermore, the new range of biocompatible polymers systems is providing hybrids and biosensors that can be utilized in complex biosystems.

8.3.3 Polymer–Metal Oxide Hybrids

In contrast to noble metal polymer–MNP hybrids which are commonly utilized as cast films for charge transport purposes, metal-oxide nanoparticles (MONPs) are most often employed as colloidal systems and exhibit varying electrochemical

properties, ranging from nanocatalysis to semiconduction. Synthesized by the in situ precipitation of metallic precursors in the presence of stabilizing ligands, MONPs have been prepared with a variety of inorganic chemistries, e.g. Fe_2O_3, Fe_3O_4, TiO_2 (Chen et al. 2001; Lee et al. 2007), SiO_2, and ZnO.

Of the available MONP chemistries, the magnetic properties of Fe_2O_3 and Fe_3O_4 have been exploited across the gamut of biosensors, which include pollution detection (Xu et al. 2012; Horak et al. 2007), disease diagnostics and therapeutics (Sandhu et al. 2010; Veiseh et al. 2010), blood analyses, bioimaging (Lee and Hyeon 2012), and chemical and biological separation. Similar to noble metal hybrids, controlling the particle size and size distribution are important; however, unlike noble metal nanoparticles, the shape, crystal structure, and defect distribution in the polymer–MONP hybrids dramatically affects their electrochemical properties. For example, iron-oxide MNPs have magnetic properties directly correlated to their particle size and crystal structure. To tailor these properties, numerous synthetic approaches have been developed for the generation of Fe_2O_3 and Fe_3O_4 polymer–MONP hybrids (Kievit and Zhang 2011; Netto et al. 2013; Sandhu et al. 2010). Due to the importance of the crystal structure/oxidation state to subsequent properties, the choice of polymer ligand is critical for the stability and functionality of the polymer–MONP hybrids. The methodology used is based on direct precipitation of iron salts inside the pores of the porous polystyrene seed and was pioneered by Ugelstad et al. (1973), similar to the deposition of noble metal MNPs in polymer monoliths. The particles obtained exhibit a narrow particle size distribution with a good magnetic separation, which are critical parameters. Hydrophilic magnetic latexes were first reported by Kawaguchi et al. using acrylamide as the initial monomer, and more recently Lee et al. have modified nanoparticle surfaces with PVA by precipitation of iron salts in PVA aqueous solution to form a stable dispersion. They found that the crystallinity of the particles decreased with increasing PVA concentration, while the morphology and particle size remained almost unchanged. This phenomenon has been shown to be a result of metal–organic chelation and in situ ligand exchange, and it is a critical factor in controlling polymer–MONP hybrid morphology.

Various biomacromolecules, such as antibodies, proteins, and DNA, and bioactive small molecules have been covalently incorporated onto the polymer–MNPs, and the possibilities of the chemistries to do so have been as wide-ranging as the array of ligands. Some interesting ligands with regard to biosensors include 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride (EDC), *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP), *N*-hydroxysuccinimide (NHS), and methylene bis-acrylamide (MBA). These coupling ligands are readily utilized for the simple and efficient conjugation of proteins as bio-recognition agents. More recently, the incorporation of "clickable" moieties into the polymer–MNP matrix has led to a broader range of conjugation ligands (Ge et al. 2013; Daniele et al. 2013; Zhou et al. 2008; He et al. 2009; Liu et al. 2009).

Enzymes, such as glucose oxidase, hydrolase, horseradish peroxidase, creatinase, lactase, and lactate dehydrogenase have been successfully immobilized on the surface of Fe_3O_4 polymer–MNP hybrids via covalent immobilization (Ge et al. 2013; Zou et al. 2010; Peng et al. 2013; Yang et al. 2009; Zhang et al. 2008a, b; Cevik et al. 2012;

Villalonga et al. 2011). An early enzyme biosensor utilizing polymer–MONP hybrids was reported by Rossi et al. (2004), in which GOx was conjugated with Fe_3O_4 through a poly(ethylene glycol) linker. In order to improve the sensitivity of such enzymatic biosensors, some electron mediators or electron promoters were introduced into the biosensing system. Accordingly, an amperometric glucose biosensor was developed by entrapping GOx in chitosan composite doped with ferrocene monocarboxylic acid-modified Fe_3O_4 nanoparticles. With the aid of a permanent magnet, these polymer–MNP hybrids with incorporated GOx were attached to the surface of an electrode and acted as mediator to transfer electrons between the enzyme and the electrode. The large surface area of Fe_3O_4 nanoparticles and the porous morphology of chitosan lead to a high loading of enzyme and increased sensitivity. Zhuo et al. developed a three-layer composite composed of Fe_3O_4 magnetic core, Prussian blue interlayer, and gold shell to fabricate an electrochemical immunosensor by functionalization with bi-enzyme of horseradish peroxidase and GOx (Zhuo et al. 2009). Besides the metal oxides mentioned above, CuO (Li et al. 2011), Bi_2O_3 (Ding et al. 2010), and CeO_2 (Saha et al. 2009) nanoparticles have been reported to be used for GOx immobilization and biosensor design.

It should be pointed out that, in some MONP-based biosensing systems, the recognition mechanism is not based on the direct reaction between enzyme and analyte. Most biological samples exhibit negligible magnetic susceptibility; therefore, magnetic nanoparticle polymer–MNP hybrids can be used for detection of biomolecules and cells based on magnetic resonance effects. Diagnostic magnetic resonance (DMR) technology encompasses numerous assay configurations and sensing principles, and diamagnetic nanoparticle biosensors have been designed to detect a wide range of targets including DNA/mRNA, proteins, enzymes, drugs, pathogens, and tumor cells. The core principle behind DMR is the use of magnetic nanoparticles as proximity sensors that modulate the spin–spin relaxation time of neighboring water molecules, which can be quantified using clinical MRI scanners or bench-top nuclear magnetic resonance (NMR) relaxometers. DMR biosensor technology holds considerable promise to provide a high-throughput, low-cost, and portable platform for large-scale molecular and cellular screening in clinical and point-of-care settings.

Ultimately, polymer–MNP hybrids display a range of beneficial electrochemical attributes; moreover, the tailored functionality of the polymer matrices provides for unique routes for the precision attachment of bio-recognition molecules. In the last decade, these benefits have been successfully employed for biosensing and bioanalysis applications, and continued development of polymer–MNP hybrids and conjugation chemistries will see gains in both biosensor precision and sensitivity.

8.3.4 Polymer–Metal Hybrids for DNA Sensing

As discussed in Sect. 8.2, DNA–MNP hybrids are a unique class of materials that have generated much interest for biosensing applications. Specifically, in the arena of nucleic acid detection, a DNA ligand provides both possible ligation chemistry and

detection elements in one unique package. Detection of nucleic acids, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), is very important in many life sciences for understanding their basic functions and for identifying certain targets (Wang 2000, 2002; Pyun 2012). DNA is one of the most important molecules of life which encodes the genetic information and instructs the biological synthesis of proteins and enzymes through the process of replication and transcription of genetic information in all known living organisms and many viruses. Since its backbone is resistant to cleavage and, furthermore, the double-stranded structure provides the molecule with a built-in duplicate of the encoded information, it becomes indispensable. A sequence of nucleotides, guanine (G), adenine (A), thymine (T) and cytosine (C), are responsible for encoding the genetic information for further generations. Hence, understanding the structural properties of DNA can lead to understanding the origin of many of diseases, the mutation of genes, and the action mechanism of antitumor/antivirus drugs. To date there have been many scientific and commercial attempts to design and prepare DNA detection systems based on different techniques. These systems have found great interest in medical diagnostics, assessment of gene expression, drug discovery, identification of genetic mutations or single nucleotide polymorphisms, forensic, environmental (pollution, pathogen classification), bioterrorism, and food applications. Classical methods for DNA detection are mostly time-consuming and expensive. Thus, large-scale DNA testing/detection requires the development of small, portable, inexpensive, sensitive, selective, fast, and easy-to-use methods. A biosensor which is an analytical device with a biologically active material (DNA) can offer great promises for achieving this goal. Among different types of biosensors, which are classified according to the transducer that is used, electrochemical biosensors are the most commonly used ones because of their sensitivity, selectivity, compact size, low cost of construction, real-time analysis, and simplicity of use (Gooding 2002; Palecek 2002).

Recent advances in nanotechnology have provided great progress for biosensing purposes. Among different technologies, combining nanoparticles and polymer technology has provided enhanced stability and sensitivity. There has been great interest in terms of research on polymer–nanoparticle hybrids for different types of applications in the sensing area. This sub-section mainly focuses on the use and development of polymer–nanoparticle hybrids for electrochemical DNA biosensing. Electrochemical DNA hybridization detection has advanced a long way with the use of nanoparticle materials and polymer modification was successfully used to stabilize the dispersion of nanoparticles on the electrode surface (Muti et al. 2010; Yumak et al. 2011; Fang et al. 2008; Chang et al. 2008; Zhang et al. 2008a, b, 2009; Yang et al. 2007; Sun et al. 2010; Du et al. 2009; Radhakrishnan et al. 2013; Wang et al. 2003).

Hybridization probe biosensors are one of the most crucial improvements in the field of gene-related biomolecule detection. These kinds of biosensors most commonly rely on the immobilization of an oligonucleotide (ODN) probe onto a transducer surface for hybridization with its complementary target sequence (Fig. 8.3).

Muti et al. (2010) fabricated tin oxide (SnO_2) nanoparticles (SNPs)–poly(vinylferrocenium) (PVF^+)-modified single-use graphite electrodes for electrochemical DNA hybridization detection. SnO_2 is a semiconductor and because of its conductive properties, these nanoparticles can be used in several applications (Wang

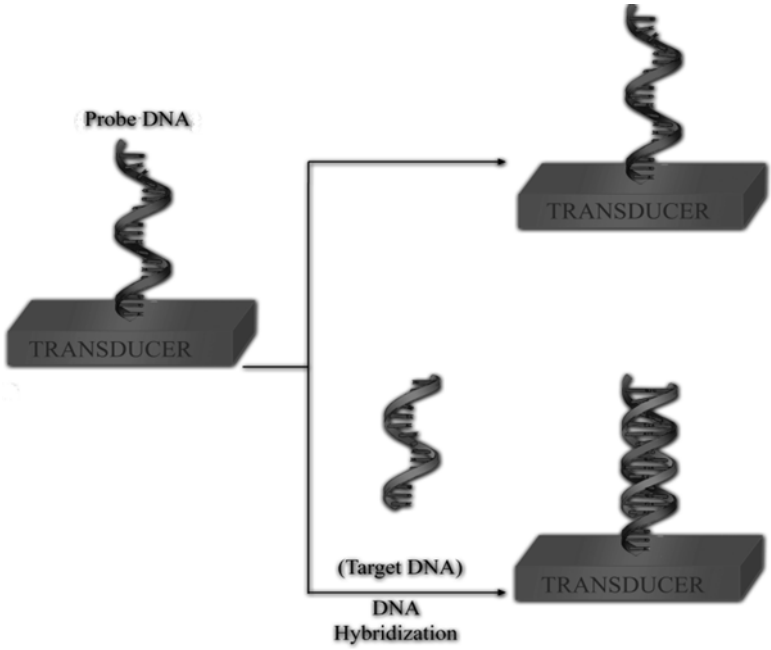

Fig. 8.4 Scheme of an electrochemical hybridization biosensor

et al. 2003; Ansari et al. 2009). In their work, they combined the nanoparticles with a conducting polymer (redox polymer), PVF^{+}. This polymer shows a simple and good electrochemistry because of its ferrocene/ferrocenium groups in its structure. PVF^{+}-modified electrodes have been used for the same purpose previously (Kuralay et al. 2008, 2009) and in the presence of SnO_2 nanoparticles more sensitive results were obtained. Scanning electron microscopy (SEM) was used to differentiate the modifications on the pencil graphite electrodes (PGEs), as well as electrochemical experiments (Fig. 8.4). Electrochemical behaviors of the PGEs were investigated by differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). The change in the guanine oxidation signals was used as the indicator of DNA hybridization. Different modifications in the probe DNA and probe DNA concentration were examined in order to obtain optimum working conditions for improving sensitivity and selectivity. After optimization studies, DNA hybridization was performed in the case of complementary hepatitis B virus (HBV), mismatch (MM), and noncomplementary (NC) sequences. The SNP—polymer-modified PGE showed high selectivity and specificity to its complementary DNA in the concentration range of 20–140 μg mL^{-1} with a detection limit of 1.82 μg mL^{-1}.

Zinc oxide (ZnO) nanoparticles (ZNPs) enriched with PVF^{+} hybrids were used for electrochemical nucleic acid hybridization related to HBV by Yumak et al. (2011) using PGEs as the electrode materials. ZNPs have different applications due to their wide band gap and large excitation energy (Na et al. 2008; Sun et al. 2009). ZNPs (approximately 30 nm) were synthesized by the hydrothermal method

and characterized by X-ray diffraction (XRD), Braun–Emmet–Teller (BET) N_2 adsorption analysis and transmission electron microscopy (TEM). SEM was used to identify different modifications on the PGEs. Electrochemical experiments included DPV and EIS techniques. The change in the guanine signals was evaluated and used as the indicator of DNA hybridization. Various modifications in DNA oligonucleotide types and probe concentrations were examined in order to optimize the electrochemical signals. After the optimization studies, the sequence-selective DNA hybridization was investigated for the cases of a complementary amino-acid-linked probe (target), NC sequences, or target and MM mixture in the ratio of 1:1. The detection limit was calculated as 11.7 μg mL^{-1}.

Besides this work there have been many attempts in this attractive topic. For example, Fang et al. investigated label-free electrochemical detection method for DNA–peptide nucleic acid (PNA) hybridization using ferrocene-functionalized polythiophene transducer and ssPNA probes on a nanogold-modified electrode (Fang et al. 2008). DNA hybridization using gold nanoparticles based on assembly of alternating DNA and poly(dimethyldiallylammonium chloride) multilayer films by layer-by-layer electrostatic adsorption has been studied by Chang et al. (2008). DNA hybridization detection was performed by Zhang et al. using ZNPs, multi-walled carbon nanotubes (MWCNTs), and chitosan hybrids with methylene blue (MB) indicator (Zhang et al. 2008a, b). Electrochemical detection of DNA hybridization based on carbon nanotubes, nano-zirconium dioxide (ZrO_2), and chitosan-modified electrodes was studied by Yang et al. (2007) using glassy carbon electrodes (GCEs) with DPV. The detection limit ($S/N = 3$) was found to be 75 pM. Sun et al. (2010) performed DNA hybridization using nano-V_2O_5, MWCNTs, and chitosan nanocomposite materials modified *N*-hexylpyridinium hexafluorophosphate carbon ionic liquid (CILE) as binder with graphite powder. The electrochemical indicator MB was used to monitor the hybridization event with DPV. The detection limit ($S/N = 3$) was found to be 1.76 pM (Sun et al. 2010). Cationic poly-L-lysine (pLys) and Au–CNT hybrid was used as a DNA hybridization biosensor for detection of the phosphinothricin acetyltransferase (PAT) gene with MB indicator (Du et al. 2009). An electrochemical DNA biosensor based on silver nanoparticles/poly(*trans-3*-(3-pyridyl) acrylic acid) (PPAA)/MWCNTs-COOH-modified GCEs has been prepared by Zhang et al. (2009). The DNA hybridization was monitored using intercalator adriamycin by DPV with a detection limit of 3.2 pM ($S/N = 3$). Polypyrrole-poly(3,4-ethylenedioxythiophene)-Ag (PPy-PEDOT-Ag) nanocomposite films for label-free electrochemical DNA sensing were prepared by Radhakrishan et al. (2013). The detection limit was found to be 5.4 fM.

This sub-section summarizes the importance of polymer–nanoparticle hybrids, mainly in electrochemical DNA hybridization biosensors. A general introduction to the topic has been given, then the applications of these biosensors were presented. The applications have shown that electrochemical DNA detection provided sensitive, selective, reliable, low-cost methods when combined with polymer–nanoparticle technology. These works will definitely be useful for future works in different areas including medicine, pharmacy, forensic applications, environmental monitoring, bioterrorism and food applications.

8.4 Conclusions

The integration of bio-recognition and signal transduction elements has been an important area of biosensor research for several decades. However, recent advances in the fabrication of nanomaterials have greatly improved their performance. Metallic and metal oxide nanoparticles (MNPs), carbon–metal hybrid carrier molecules and carbon nanotube (CNT)– or graphene–metallic nanoparticle materials have all greatly enhanced the sensitivity, linear sensing range, and limit of detection of electrochemical biosensors. These nanomaterials display a high degree of catalytic activity, conductivity, and biocompatibility that act in a synergetic manner to improve biosensor performance. For example, vast improvements in electrical conductivity and catalytic performance have been shown by reducing material size from the bulk to the nanoscale. Furthermore, the use of these nanomaterials creates a unique microenvironment that is well suited for biological stability and biological–inorganic interaction, while the use of covalent (e.g., thiol binding, cross-linking) and noncovalent biofunctionalization schemes with biorecognition agents (peptides, proteins, and nucleic acids) transforms these nanomaterials into highly sensitive probes/electrodes capable of both in vivo and ex vivo biosensing. Such nanostructured biosensors have demonstrated utility in a wide range of fields and applications including those associated with health care, environmental monitoring, security surveillance, food safety, and biodefense. Thus, we envisage that the combination of hybrid metallic nanoparticles with CNTs, graphene, and organic conjugation will continue to improve and transform the field of biosensing for years to come.

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