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Nanomaterial-Based Biosensor as an Emerging Tool for Biomedical Applications

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Abstract—The combination of nanomaterials and biological sensing elements to selectively recognize chemical or biological molecules has resulted in the development of novel nanobiosensors. Nanobiosensors offer several important advantages over conventional biological procedures, and could have a significant impact on humankind. Hence, the momentum toward building miniaturized, reliable, sensitive, and selective sensing instruments has focused on combining nanomaterials with biomolecules for detection of a wide range of analytes. In this article, we present an overview of the various nanomaterial-based biosensors that utilize different biological recognition elements for biomedical applications. In this review, several types of nanomaterial-based biosensors along with their applications are discussed, including the latest developments in the field of nanobiosensors for biomedical applications.

Keywords—Nanomaterial-based biosensor, Nanomaterials, Biomolecules, Biomedical application.

INTRODUCTION

Significant advances in the nanotechnology have paved the way for the induction of a large number of new materials and devices of preferable properties for numerous applications. Controlled fabrication and manipulation of nanomaterials, from either a ''bottom-up'' or ''top-down'' approach, have allowed for the rapid development of nanometer-scale devices. Furthermore, the unique chemical and electrical properties of nanomaterials have enabled the development of new and improved sensing devices.[103,138] A variety of nanomaterials with different sizes, shapes, and unique properties, such as nanotubes (NTs) and nanowires (NWs), have been exploited for biosensing.[9,22,48] Recently, the integration of biomaterials into nano-scale electrical devices offers significant advantages for the detection of chemical or biological species over conventional methods. Most biological processes involve electrostatic interactions and charge transfer, which are directly detected by nanomaterials-based electronic devices. Eventually, these types of biosensor will be the most suitable for biological sensing. This article reviews new advances in the development of nanobiosensors, and explores reported biomedical applications for detection of several biological targets, antigens associated with diseases, and cellular signaling inside individual living cells.

THE CONCEPT OF NANOMATERIAL-BASED BIOSENSOR

A biosensor is a device that can detect an analyte and typically involves the combination of a biological recognition component (biological part) which acts as the primary transducer, and a physicochemical detector component (non-biological part, signal amplification, and transduction) which acts as the signal conversion unit. Many different biomolecules can be used as recognition elements depending on the target analyte and application.[103] The biological recognition elements can be nucleic acids, such as DNA, aptamers, and PNA, proteins, such as enzymes and antibodies, and even whole cells, such as microorganisms, neurons, and tissue slices. Similarly, a diverse range of nanomaterials can be used as secondary transducers for efficient transport of electrons because of their unique and sensitive electrical properties.[20,29,132] The nanomaterial in secondary transducers acts as an interface, measuring the physical change that occurs,

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because of the biochemical interactions then transforming that signal into a measurable output. Thus, the integration of highly sensitive nanomaterial-based platform with biomolecules can lead to high-performance biosensors. As mentioned above, the role of the two components in the biosensors is to detect the signal from the change in a reaction and produce signals in the form of electrical, thermal, or optical signals, which can be converted digitally for further processing.[132] The other two properties important to the performance of the sensor are its selectivity and sensitivity.[112,117] The sensitivity, which reflects the intrinsic detection capability of the biosensor, is the magnitude of the sensor signal change in response to the change in analyte concentration. The selectivity of a biosensor depends upon the choice of biological receptor. Thus, this selectivity can be modified when nanomaterial-based sensors are associated with diverse biological recognition elements.

Figure 1 shows a diagram of a nanobiosensor system based on diverse nanomaterials and biomolecules. This type of nanobiosensor recognizes the change in the charge transport properties of a nanomaterial when biomolecules bind to some analyte, and the change can be correlated quantitatively to the concentration of the analyte.

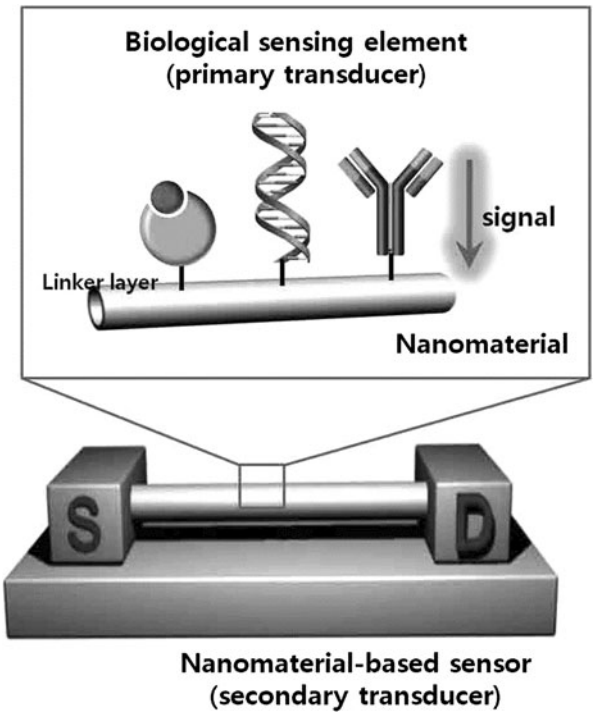

FIGURE 1. Schematic illustration of a nanobiosensor consisting of biomolecules, linker layer, nanomaterials, electrode, and substrate. S and D represent the source and drain electrodes, respectively. The electronic components are required to monitor current flowing through the nanomaterial. The overall sensing process involves (1) the specific interaction between analyte and biomolecule, (2) signal transduction to nanomaterials-based sensor, and (3) electrical readout.

Recently, the field-effect-transistor (FET) platform has been widely used for high-performance biosensors. This type of biosensor has several important features for the detection of chemical or biological species. First, the nanomaterial, which is in a transistor configuration, serves as the conducting channel either as a single or network of nanomaterials between the source and drain electrodes.[111] The principle of detection involves charge transfer from the analyte–biomolecule interaction to the nanomaterial. If a charge transfer occurs, the threshold voltage or current will become either more positive (electron withdrawing) or more negative (electron donating).[4,20,29] Second, the nanomaterials are typically located on the surface of the supporting substrate and are in direct contact with the biomolecule which can detect a target analyte.[6,24,53] FETs readily change their conductance upon binding of charged target molecules to biomolecules anchored to the device's surfaces. Finally, all of the electrical current flows through the nanometer-scale cross section of the nanomaterials.[26] All these remarkable characteristics lead to a FET device's configuration that is extremely sensitive to minute variations in the surrounding environment.

However, the field effect is dependent on many factors, such as Debye length, nanomaterial size, adjustment of source–drain bias voltage, and gate voltage, surface chemistry for functionalization of nanomaterial, and so on.[29,106] For example, the charged molecule under detection must have an efficient electrical field length (Debye length), which can be controlled by the ionic strength within the solution.[2] Thus, a careful choice of buffer solution is needed to improve the performance of FET sensors. However, currently, there are insufficient experimental data and theoretical simulations on field-effect-based devices.[139]

In recent years, giant magnetoresistance (GMR) sensors have shown great potential for biomolecule detection. Magnetoresistance is defined as the change in the resistance of a material in response to an externally applied magnetic field.[31,110] The GMR effect is related to the fact that the spin of electrons has two different values (called the spin-up and spin-down) crossing magnetic–nonmagnetic–magnetic multilayer structure, where the parallel or antiparallel alignments of the magnetic layers can be engineered.[110,124] An applied magnetic field is used to change the relative orientation of the magnetizations of the two magnetic layers.

The GMR biosensors can be used to detect and analyze magnetically labeled nucleic acids, proteins, whole cells, or microorganisms.[83,131] The GMR biosensor is based on the detection of the magnetic fringe

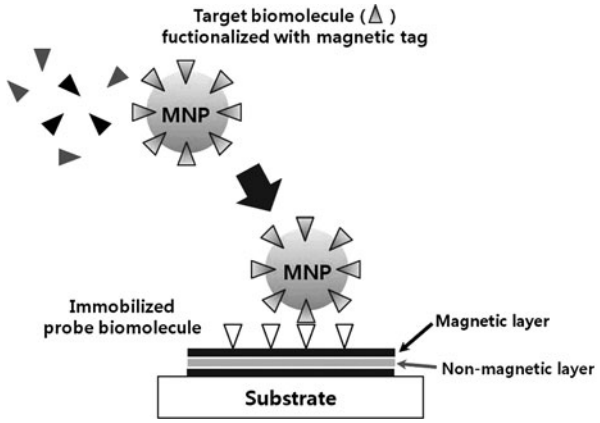

FIGURE 2. Generic illustration for the detection of magnetically labeled biomolecules in GMR sensor. The biomolecule labeled with a magnetic nanoparticle (MNP) tag binds to an immobilized probe biomolecule on the surface of GMR sensor. The changes in the magnetic field resulting from the magnetic tag by specific biomolecular ligand–receptor interactions are detected by the underlying GMR sensor.

field of a magnetically labeled biomolecule (tagged with a magnetic label such as magnetic particle) interacting with a complementary biomolecule that is immobilized on the magnetic field sensors.[32,47,124] The sensors detect the presence of the magnetic labels *via* a change in the resistance (Fig. 2). However, biological samples in blood, urine, serum, etc., naturally contain insufficient amounts of magnetic material for the sensing platform to quantitatively detect. Thus, biomolecules must be functionalized with magnetic tags or carriers such as magnetic microspheres, microbeads, and nanoparticles. In addition, the performance of GMR biosensors for the detection of analytes, such as sensitivity, specificity, dynamic detectable range, and signal-to-noise ratio, can be affected by the properties of the magnetic labels, such as size, shape, magnetic composition, and surface properties. Thus, the magnetic labels should be selected based on the intended application of the GMR biosensor.

NANOMATERIALS FOR SENSING APPLICATIONS

With the many advances in the controlled synthesis of nanoscale materials, novel functional nanomaterial can be created. When scaled down to the nanoscale, most materials exhibit novel properties that cannot be extrapolated from their bulk behavior. Nanomaterials are generally defined as materials that have dimensions ranging 1–100 nm[122]; in other words, intermediate material between the molecular scale and the bulk scale. Various kinds of nanomaterials, such as carbon nanotube (CNT), graphene, conducting polymer nanotube (CPNT), and silicon nanowire (SiNW), are being applied to biosensors because of their unique physical, chemical, mechanical, magnetic, and optical properties, and these nanomaterials have markedly enhanced the sensitivity and specificity of detection.[138] In particular, the advantages of nanomaterials include an increased surface area, electrical conductivity, and connectivity, chemical accessibility, and biocompatibility.[109] Thus, when fabricating an efficient nanobiosensor, one must consider the type of nanomaterial to use, since this factor can dictate the performance of the biosensor. In general, most nanomaterials have been prepared using two basic approaches: One approach is the "bottom-up" approach, which involves the self-assembly of small sized structures into larger structures; the other is the "top-down" approach, where large systems are reduced to smaller sizes to produce multifunctional nanoscale structures.[132] The objective of this section is to briefly describe the characteristics of nanomaterials, such as CNT, graphene, CPNT, and SiNW with regard to biosensing.

Carbon Nanotube and Graphene

Carbon might be the most widely used material in electroanalysis and electrocatalysis. CNTs, which are allotropes of carbon from the fullerene structural family, consist of graphene sheets wrapped into a hollow cylinder with the ends capped or open.[9,91] Multi-walled CNTs (mwCNTs) containing concentric graphite tubules range in diameter from 2 to 25 nm with 0.34 nm between tubule sheets.[88,114] The mwCNTs behave as conductors and have electrical conductivities greater than metals, suggesting that their incorporation into sensing electrodes may be beneficial. Single-walled CNTs (swCNTs), which contain a single graphene sheet, are rolled seamlessly into individual cylinders (typically of 1–2 nm) with capped ends containing carbon atoms.[93] The swCNTs may be classified into three different types: armchair, zigzag, and chiral nanotubes, depending on how the two-dimensional (2D) graphene sheet is "rolled up."[9,42] Depending on the tube diameter and chirality, swCNTs can behave electronically as either metals or semiconductors, or small-band gap semiconductors, which can complicate their use in sensing electrodes.[41] However, CNT synthesis methods create a mixture that includes amorphous carbon, graphite particles, and CNTs, so that synthesis is typically followed by a difficult separation process.[72,75] The electrochemical behavior of CNTs varies considerably with the methods used for purification and preparation, including oxidation treatment.[46,118] For analytical applications, CNTs are most often used to modify other electrode materials, or as part of a composite electrode, because of difficulties in handling CNTs.[38]

Recently, graphene was demonstrated to be an attractive building block for nanoscale electronic devices, although little is known about its interfaces with biomolecules such as protein, cell, and tissue. Thus, further studies on graphene are expected to provide better insight into all carbon materials.[82] Graphene exhibits excellent electron transfer properties and excellent catalytic behavior toward small biomolecules.[99,100] Graphene is the basic building block for graphitic materials of all other dimensionalities (0D fullerene, 1D NT or NW, and 3D graphite)[12] and shows great promise in many biosensing applications because of its unique physiochemical properties: high surface area (theoretically 2630 m^2/g for single-layer graphene),[79,99] excellent thermal and electric conductivities, and high mechanical strength.[99] In comparison with CNTs, graphene is inexpensive, has a higher surface area, can be more easily processed, and is safer to use. Also, owing its higher purity (transition metals, Fe, Ni, etc. are absent in graphene because of graphene oxide reduction, not like CNTs), graphene provides a good platform to study the electrocatalytic effects of carbon materials.[12,73,144] In addition, graphene has excellent electron transfer properties with some enzymes, which makes graphene extremely attractive for enzyme-based biosensors.[51,99,107,128,144] In addition, the 2D-nanostructured interface of graphene can enhance cellular adhesion and activity, and thus have an intrinsic advantage for building interfaces with cells and tissue.[9,19]

Conducting Polymer Nanomaterials

Recently, many kinds of sensors using conducting polymer (CP) nanomaterials have been described for biological uses. CP nanomaterials are promising 1D-nanostructured materials due to their chemical-doping specificities, adjustable transport properties, high surface areas, structural diversity, low cost, and facile synthesis.[37,134] The oxidation level of CP materials can be easily influenced by their inherent reversible doping–dedoping (oxidation–reduction) mechanisms, which can cause variations in conductivity.[120,129] Also, the charge transport properties of CP materials are influenced by structural parameters, such as the diameter and aspect ratio.[133,134] Thus, CP materials are capable of exhibiting sensitive and rapid responses to specific chemical or biological species. Electrochemical synthesis, such as chemical polymerization, is frequently used to fabricate CP nanomaterials. The synthetic strategies can be classified into hard-template synthesis, soft-template synthesis, and template-free synthesis.[48,68,84,120] The most frequently used CPs for developing new types of biosensors are polypyrrole (PPy), polyaniline (PANI), and poly(3,4-ethylenedioxythiophene) (PEDOT).[37,48] PPy nanomaterials have been applied to various biosensors, such as DNA sensors[16] and aptamer sensors for thrombin, and vascular endothelial growth factor (VEGF) detection,[61,135] bioelectronic nose,[136] and anticancer agent detection,[60] because of their unique chemical and electrical properties, which originate from their conjugated π-electron system.[39,76] Also, recent reports have suggested that CP nanomaterials are excellent candidates for the development of high-performance biosensors.[16,129]

Silicon Nanowire

SiNW, which is one of the best-characterized examples of semiconducting NWs, can be prepared as single-crystal structure with diameter as small as 2–3 nm.[21,22,127] Extensive investigations were carried out on the synthesis, physical properties, and device fabrication, and applications of SiNWs.[74,140] The physical properties including electrical, photoelectrical, and mechanical properties of SiNWs have been used as building blocks of nanodevices.[22] At present, most SiNWs used in nanodevices are synthesized by vapor–liquid–solid (VLS) and vapor–solid–solid (VSS) growth processes.[33,125,127] Usually, SiNWs fabricated using VLS or VSS techniques have well-defined surfaces and well-controlled diameters. These SiNWs-related FETs are quite sensitive because SiNWs have a high carrier mobility and high surface-to-volume ratio which ensures that mass carriers can be controlled easily by applying a weak electrical field on the gate.[20,25,142] Currently, highly sensitive and selective SiNW-based FET biosensors have been used for the detection of DNA hybridization,[35,69,80] proteins,[71,123,143] cell signaling,[86] and viruses.[87]

FUNCTIONALIZATION OF NANOMATERIALS FOR BIOSENSOR FABRICATION

A number of nanopatterning and functionalization technologies are being developed to control the location, distribution, amount, or conformation, and orientation of biomolecules on the surface of nanomaterial. Therefore, the interface between biological molecules and nanomaterials is critical to various applications. The two generalized approaches used to couple biological molecules and nanomaterials are the covalent and noncovalent modifications. Covalent functionalization is a chemical process in which a strong bond is formed between the nanomaterial and the biomolecule or its linker. In many cases, chemical modification of the surface is necessary to create active groups that can bind to biomolecules. The most commonly used method for covalent binding of

biomolecules to nanomaterial is the diimide-activated amidation of carboxylic acid-terminated nanomaterials.[121] In contrast to covalent functionalization, noncovalent functionalization can be used to immobilize biomolecules to the nanomaterial without destroying their geometric and electronic structures. Noncovalent interactions are of critical importance in many biological systems, including the complex tertiary structure of proteins. Hydrophobic and hydrophilic interactions are involved in the passive adsorption of biomolecules onto surface of the nanomaterial. The most prominent functionalization methods for the immobilization of biomolecules onto nanomaterials will be discussed in this section.

Carbon Nanotube

swCNTs are molecular wires that exhibit interesting structural, mechanical, electrical, and electromechanical properties. The swCNT is unique among solid-state materials in that every atom is on its surface. Surface chemistry can therefore be critical to the physical properties of swCNTs and their applications. The most commonly used method for covalent binding of proteins onto CNTs is diimide-activated amidation of carboxylic acid-functionalized CNTs.[121] Alternatively, it is possible to covalently functionalize amine-terminated nanomaterials with biomolecules. In terms of covalent attachment, the CNTs are oxidized to produce free carboxyl groups of their surface, which are then coupled to amino groups in biomolecules. While covalent modifications are often effective at introducing functionality, they can impair the desirable mechanical and electrical properties of swCNTs. For instance, Wong *et al.*[126] covalently modified swCNTs by creating carboxylic acid groups at the open ends of oxidized swCNT, which were then coupled to amines to create additional probes with hydrophobic functionality. On the other hand, noncovalent modifications constitute nondestructive processes, preserving the primary structures of the CNTs along with their unique mechanical and electronic properties. The most commonly used noncovalent functionalization method involves bifunctional molecules containing a pyrenyl moiety, which can allow the biomolecule to be irreversibly adsorbed onto the inherently hydrophobic surfaces of swCNTs *via* π-stacking. The pyrenyl moiety, which is highly aromatic in nature, is known to interact strongly with the basal plane of graphite *via* π-stacking, and also strongly interacts with the sidewalls of CNTs in a similar manner.[18] Chen *et al.* report a simple, general approach to noncovalently functionalize the sidewalls of swCNT with 1-pyrenebutanoic acid, and the used this approach to immobilize various biological molecules onto CNTs with a high degree of control and specificity.

Conducting Polymer Nanomaterials

Biological functionalization of CP nanomaterials is vital for the use in sensor applications. Suitable surface functionalization of the CP nanomaterials can lead to a significant improvement in properties relevant to their sensor applications. For this purpose, functional groups such as amino, carboxyl, and alkyl group can be incorporated within the CP matrix by using polymers with specific properties. In terms of covalent approaches, CP nanomaterials can be easily modified by grafting functional groups on the polymer backbone or by employing inherently functionalized monomers during polymerization. For instance, carboxylated PPy nanotubes were synthesized *via* the polymerization of an intrinsically functionalized monomer (P3CA) and their functionality was further modified by covalently coupling the surface carboxyl groups with biomolecules.[136] In addition, covalent approaches allow for control over surface functionality by adjusting the molar ratio of the functionalized monomer to nonfunctionalized monomer during polymerization. On the other hand, the noncovalent approaches take advantage of incorporation of the appropriate counter ions into the polymer during synthesis and the electrostatic adsorption of guest molecules on the CP nanomaterial surface.

Silicon Nanowire

The configuration of SiNW has practical advantages because the interaction between biomolecules and the surface of SiNWs are rapidly translated to electrical outputs. The most commonly used method for the modification of the SiNW surface is silanization. Before the SiNWs are modified, the surface of the SiNWs is treated with water-vapor plasma to generate a hydroxyl-terminating silicon oxide surface, which is highly hydrophilic. The hydroxide layer is then activated with an organosilane, which introduces reactive groups. The functionalized siloxane on the SiNWs can then be used to assemble biomaterials.[24] For example, the surface of SiNWs can be functionalized with 3-aminopropyltriethoxysilane (ATPES) to provide a surface that can undergo protonation and deprotonation, where changes in the surface charge can chemically gate the SiNW.

APPLICATIONS OF NANOMATERIAL-BASED BIOSENSOR IN BIOMEDICAL FIELDS

A wide range of biomolecules, such as nucleic acids, enzymes, antibodies, receptors, and whole cells, have been extensively explored for the development of

sensitive and selective biosensors. However, the low sensitivity of conventional biosensors has limited their application.[10,62,92,95] Thus, the integration of biomaterials into electrical device with nanomaterials offers significant advantages over conventional sensing methods. Recently, various nanomaterial-based biosensor platforms, as listed in Table 1, have been developed by combining nanomaterials with biomolecules and these platforms hold great promise for use in physics, chemistry, biology, medicine, material science, and interdisciplinary fields. Several examples of nanobiosensors will be discussed in this section, including some general mechanisms and conductivity measurements based on biological interactions.

Nucleotide-Based Nanobiosensor

The development of nanobiosensor to detect specific DNA has the potential to impact basic biological research as well as genetic screening associated with diseases. The method of detection using DNA biosensors typically relies on the immobilization of single-stranded DNA (ssDNA) on the nanomaterial surface of a sensor, which allows the hybridization of sequence-specific DNA, and the recognition of the complementary target DNA.[53,69,105] Conventional methods for detection of sequence-specific DNA are the PCR technique and fluorescent assays. However, these methods are expensive, labor intensive, and time consuming. On the other hand, nanomaterial-based sensor platforms are expected to offer simple, label-free and real-time methods for DNA detection.[23,35,69]

Staii *et al.*[105] developed a DNA-decorated swCNT-based nanobiosensor for detecting gas odors. ssDNA is known to have high affinity for swCNT due to a favorable π–π stacking interaction[141] and can be directly immobilized onto swCNT surfaces. The selective interaction between gas odors and ssDNA oligomers

TABLE 1. Comparison of the performance of diverse nanomaterial-based biosensors for detection of biomolecules.

Biomolecule type	Biological recognition component	Nanomaterials (transducer part)	Target molecules	Detection limit	Ref
DNA	ssDNA	swCNT	DMMP	25 ppm	105
	ssDNA	Graphene	DNA	10 pM	27
	ssDNA	SiNW	DNA	25 pM	69
	PNA	SiNW	DNA	10 fM	35
	Aptamer	swCNT	Thrombin	10 nM	101
	Aptamer	CPPy NT	Thrombin	50 nM	135
Enzyme	Glucose oxidase	swCNT	Glucose	100 nM	11
	Glucose oxidase	Graphene–chitosan nanocomposite	Glucose	20 nM	51
	Acetylcholinesterase	swCNT	Acetylcholine	10 nM	130
	Cholesterol oxidase	swCNT	Cholesterol	10 μM	14
	GLDH	mwCNT/thionine composite film	Glutamate	15.9 nM	94
	LDH	mwCNT–chitosan nanocomposite	Lactate	760 nM	115
	Alcohol dehydrogenase	mwCNT–chitosan composite	Ethanol	520 nM	67
Antibody	Anti-PSA Ab	swCNT/In_2O_3 NW	PSA	1.4/0.14 nM	70
	Au NP–anti-IgG	Graphene	IgG	2 ng/mL	78
	Rotavirus Ab	Graphene	Rotavirus	10^3 pfu/mL	50
	Anti-CA 125 Ab	PPy	CA 125	1 U/mL	6
	Anti-PSA Ab, anti-ACT–PSA Ab, anti-CEA Ab, anti-mucin-1 Ab	SiNW	PSA, PSA–ACT, CEA, mucin-1	0.09 pg/mL	143
	Anti-influenza type A Ab	SiNW	Influenza A	50 viral particles/μL	87
	Anti-hapten Ab	GMR sensor	DNA	4 pM	57
Sensory receptor	OR	swCNT	Odorant	100 fM	54
	OR	CPPy	Odorant	10 fM	136
	Bitter taste receptor	swCNT	Bitter tastant	100 fM	55
Cell	Neuron	SiNW	Cellular potential	–	86
	Cardiac tissue	SiNW	Cellular potential	–	113
	Cardiomyocyte	Graphene/SiNW	Cellular potential	–	19

DMMP: dimethyl methylphosphonate; ppm: parts per million; PNA: peptide nucleic acid; CPPy NT: carboxylated polypyrrole nanotube; NP: nanoparticle; Ab: antibody; PSA: prostate specific antigen; ACT–PSA: PSA–*a*1-antichymotrypsin; CEA; carcinoembryonic antigen; IgG: Immunoglobulin G; GMR: giant magnetoresistance; GLDH: glutamate dehydrogenase; LDH: lactate dehydrogenase; OR: olfactory receptor.

produced minor perturbations in the swCNT, and dimethyl methylphosphonate (DMMP; a stimulant for the nerve agent sarin[45]) was detected at a concentration of 25 parts per million (ppm) by this device. In addition, the ssDNA chemical recognition layer was reusable through at least 50 cycles without requiring regeneration. Also, Johnson *et al.*[49] developed a DNA-coated swCNT-based nanobiosensor for breath analysis. The analysis of breath and body odors can provide valuable information relevant to disease detection, diagnosis, and treatment. Thus, this type of nanosensor may prove highly important to the clinical analysis of human breath.

Also, a highly sensitive and sequence-specific DNA sensor was using SiNWs and covalently immobilized ssDNA probes.[69] The SiNWs were surface functionalized with 3-mercaptopropyltrimethoxy-salian (MPTMS) with free thiols, and then the ssDNA probe was immobilized onto SiNWs. The covalent anchoring of DNA on the SiNW surface provided better stability and less nonspecific hybridization for DNA sensing when compared with noncovalent attachment methods.[43] A target DNA concentration of 25 pM could be detected using this system with excellent discrimination against single-base mismatches. When the target DNA attached to probe DNA on the SiNW surfaces, the increase in negative charges introduced by hybridization enhanced the carrier concentrations in the *p*-type SiNWs, resulting in the observed changes in the SiNW conductance.

Yang *et al.*,[131] reported a giant magnetoimpedance (GMI)-based microchannel system for genotyping of human papilloma virus (HPV) 16/18. HPV infection is an important carcinogenic factor in the uterine cervix.[40] HPV genotyping can be determined by the changes in GMI ratio in the microchannel after hybridization with pre-capture probes conjugated with superparamagnetic particles under a magnetic field.

In recent years, peptide nucleic acid (PNA) has been used instead of DNA as the primary transducer.[15,35,137,139] The reason for using PNA as capture probes is to produce ultralow background electric charges.[139] Moreover, PNA has a greater affinity and stability than their DNA counterparts at low ionic strength where a low background signal is observed, again because of their neutral character, which eliminates electrostatic repulsion between the two hybridized strands.[137] For instance, SiNWs-FET modified with PNA as a real-time DNA sensor was able to selectively detect complementary target DNA at concentrations as low as tens of femtomolars.[35] In this report, PNA was used as a receptor, which can distinguish the ΔF508 mutation site in the cystic fibrosis transmembrane receptor (CFTR). The detection of the presence or the absence of the ΔF508 mutation serves as an indicator for cystic fibrosis disease. Conductance measurements enabled identification of fully complementary vs. mismatched DNA samples.

Aptamers, which are synthetic DNA or RNA oligonucleotide probes, hold great promise as alternatives to antibodies in biological applications because of their ability to bind to a wide variety of entities (e.g., metal ions, small organic molecules, proteins, and cells) with high specificity and affinity.[104] The aptamers can be discovered from combinatorial nucleic acid libraries using *in vitro* selection methods called the systematic evolution of ligands by exponential enrichment (SELEX), which is initiated using a random library of nucleotides.[116] Synthesizing aptamers are relatively inexpensive, and can be engineered easily for immobilization and the development of nanobiosensors.[97] Furthermore, aptamers are capable of reversible denaturation, meaning that the biosensors can be reused continuously. Lee and co-workers[101] suggested the use of aptamers as an alternative to protein-based sensing for recognition of biomolecules in a swCNT-FET biosensor. Yoon *et al.*[135] utilized thrombin aptamer-conjugated carboxylic acid-functionalized polypyrrole (CPPy) nanotubes for label-free electrochemical protein detection. The functional carboxyl groups were effectively incorporated into the polymer backbone during the polymerization. The thrombin aptamers were attached to the NT surface *via* covalent linkages as the molecular recognition element. The specificity of thrombin aptamers combined with the charge transport property of CPPy nanotubes enabled the direct electrical detection of thrombin proteins.

Enzyme-Based Nanobiosensor

Enzymes can be used as recognition elements of biosensors because of their unique recognition and catalytic properties.[99] The biocatalytic activity of enzymes allows the analytes to undergo specific biochemical reactions through catalytic reaction cycles. The binding of the analyte to the enzyme temporarily changes its charge state, and conformational changes occur in the enzyme, which can be detected using NT-FET devices.[11,99,128] Also, the concentration changes in the substrate or ionic changes during the enzymatic reaction with the substrate can be detected by the underlying sensor platform. Therefore, these enzyme-based biosensors hold great promise for detecting interfacing biological recognition events with electric signal transduction and can be used to design new bioelectronic devices with high sensitivity and stability.

Most studies on enzyme–based biosensors have focused on glucose sensing using glucose oxidase (GOx) because of the importance of diagnosing and managing diabetes.[11,34,51,128] Many sensitive and selective

electrochemical biosensors have been developed to monitor blood glucose levels, which involve immobilization of GOx onto different nanomaterials. Strano and co-workers[7] developed a CNT-based nanobiosensor for long-term glucose sensing. They proposed a design for *in vivo* applications. GOx-coated swCNT-FET has been studied by Besteman *et al.*[11] Controlled attachment of the GOx to the swCNT sidewall was achieved through a linking molecule that contained 1-pyrenebutanoic acid. The GOx-coated swCNT acts as a pH sensor with reversible changes in conductance upon changes in pH. In the catalytic reaction where glucose ($C_6H_{12}O_6$) was converted to gluconolactone ($C_6H_{10}O_6$), GOx changed its charge state, and charged groups on the GOx become more negative with increasing pH, which resulted in a change in the conductance of the swCNT. Similar to GOx-coated swCNT FET as mentioned above, electrochemical glucose detection is based on enzymatic glucose oxidation and subsequent hydrogen peroxide detection on the CNT electrodes. Other enzyme-functionalized nanobiosensor platforms, which have been demonstrated recently, were reported to accurately monitor the biocatalytic activities of enzymes. For example, glutamate dehydrogenase (GLDH),[94] acetylcholinesterase,[130] lactate dehydrogenase (LDH),[89,115] cholesterol oxidase,[14] and alcohol dehydrogenase[67] were successfully immobilized onto nanomaterial-based sensors for the efficient detection of each analyte.

Antibody-Based Nanobiosensor

Antibodies are proteins produced by the immune system of humans and other mammals. Antibodies recognize and bind with large organic molecules such as antigens or viruses; thus, they are particularly well suited for biosensors. Recent studies have indicated that nanobiosensors are capable of sensitive and selective real-time detection of antigens that are associated with diseases when antigens bind to their corresponding antibodies functionalized on the surface of the device. Therefore, antibody-functionalized nanobiosensors hold great promise for use in identifying and distinguishing antigens for cancer diagnosis or clinical management and discovering novel biomarkers for cancer.

Recently, various cancer-specific biomarkers have been discovered, such as prostate-specific antigen (PSA), VEGF, α-fetoprotein (AFP), and carcinoembryonic antigen (CEA).[59,90] Li *et al.*[70] studied the complementary detection of PSA using indium oxide (In_2O_3) NW and a network of swCNT as a FET sensor. PSA is an oncological marker for the presence of prostate cancer, which is the most frequently diagnosed cancer in men. Anti-PSA antibody was covalently attached to In_2O_3 NW surfaces *via* functionalization with 3-phosphonopropionic acid and anchoring to the functionalized CNT surface with 1-pyrenebutanoic acid through π–π stacking method. Using this sensor, PSA was detected at concentrations as low as 5 ng/mL, which is low enough for clinical diagnosis of prostate cancer. Bangar *et al.*[6] reported a single conducting polymer NW-based biosensor for the detection of cancer biomarker protein—cancer antigen, CA 125—where it is used for ovarian cancer screening of women.[8] The single PPy nanowire was assembled using ac dielectrophoretic alignment between pair electrodes and further functionalized with antibodies against CA 125 by covalent surface modification with *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodimide hydrochloride (EDC). The developed biosensor was able to reliably detect CA 125 in human blood sample with no sample pretreatment.

As another example, Lieber and co-workers[143] achieved highly sensitive and multiplexed detection of cancer marker antigens using SiNWs. Modification of the arrays with cancer marker antibodies allowed for real-time multiplexed detection of PSA, PSA–α-antichymotrypsin (PSA–ACT) complex, CEA, and mucin-1, including detection of concentrations as low 0.9 pg/mL in undiluted serum samples. The multiplexed electric signals in nanobiosensors can detected based on interactions between the antibodies and antigens.

Mao *et al.*[78] reported specific protein detection using thermally reduced graphene oxide (TRGO) sheets decorated with AuNP-antibody conjugates. The response by immunoglobulin (IgG) and anti-IgG interactions increased with an increase in protein concentration, which saturated at 20 ng/mL. The target protein was selectively detected using the TRGO FET sensor at low concentrations in the presence of mismatched/nonspecific proteins, such as IgM and horseradish peroxidase (HRP). Vo-Dinh *et al.*[119] report in situ intracellular measurements of single cells using an antibody-conjugated nanoprobe, which was composed of silica fibers. The nanoprobe employs an anti-benzopyrene tetrol (BPT) antibody targeted to BPT, a metabolite of the carcinogen benzo[a]pyrene (BaP), and the BaP-–DNA adduct. Detection of BPT is of great biomedical interest, since this species can serve as a biomarker for monitoring DNA damage because of BaP exposure. Nanoprobes were inserted into individual cells, and BPT was detected at a concentration of 9.6×10^{-11} M in the individual cells. Lieber's group was also able to demonstrate the detection of single viruses using SiNWs.[87] Particularly, they used bifunctional molecules to attach antibodies specific to influenza A for electrochemical detection using SiNWs device.

Another approach to detect amplified DNA with a GMR sensor using superparamagnetic particles was described by Koets *et al.*[57] Amplification of an *E. coli*-specific DNA was performed by PCR and then the amplified target DNA was functionalized with superparamagnetic particles and hapten. The haptens are low molecular weight molecules, which do not elicit an immune response and hence can be used as an immunogenic carrier of conjugated DNA or protein.[13] The amplified DNA can be detected by the interaction between the immobilized anti-hapten antibodies at the GMR sensor surface and hapten labeling of target DNA. A magnetic actuation was applied to concentrate the target DNA–particle complexes at the sensor surface, and the amplicons could be measured at target DNA concentrations ranging from 4 to 250 pM of target DNA.

Sensory Receptor-Based Nanobiosensor

Natural sensory systems, such as olfaction or taste sensing, in humans and animals exhibit remarkable functional properties. These systems can discriminate between a large number of structurally diverse odorants and tastants, and perceive extremely low concentrations.[1,3,44,77] Thus, sensory receptor proteins can be used as primary transducers to detect various chemical species. In particular, olfactory receptors (ORs), which initiate a neuronal response that leads to the perception of smell in the nose,[30] can be used as primary detectors for detecting versatile chemical compounds. Recently, various types of "bioelectronic noses" have been fabricated by combining natural olfactory functional components with diverse secondary transducers, such as quartz crystal microbalance,[56,108] surface plasmon resonance,[65,66] microelectrode array,[63,64] swCNT-FET,[54] and CPNT-FET.[136] A bioelectronic nose based on swCNT conjugated with human olfactory receptor (hOR) was described by Kim *et al.* The hOR protein, expressed from *E. coli* and partially purified, was immobilized onto the swCNT-FET. Yoon *et al.* reported the integration of the hOR protein and CP nanotubes into a FET platform. The field-induced sensitivity derived from odorant recognition was observed even at a concentration of 10 fM. In addition, an artificial taste sensor utilizing the human taste receptor protein was reported to monitor the specificity of human taste receptor using a swCNT-FET transistor, which allowed for the development of a "bioelectronic taster" that could recognize bitter tastants with human tongue-like selectivity and high sensitivity.[55] Since these sensory receptor proteins provide human-like selectivity, these artificial sensing devices are expected to mimic the natural sensory system more closely. Also, highly sensitive and selective nanobiosensors could be successfully used for biomedical applications, such as disease diagnosis and detection of bacterial infection, or deleterious chemical compounds.

Cell-Based Nanobiosensor

Recently, nanomaterial-based biosensors have also been used for the detection of cellular signaling.[86,113] Cell-based sensing systems employ whole cells as primary transducers for signal generation and then the generated signals are converted by a secondary transducer for detection, which is mostly an electrical signal. Typically, the interactions between cells and analytes are initiated by the binding of foreign agents to cellular receptors. For example, cell membrane receptors might interact with other proteins or ligands and induce downstream intracellular signaling pathways as a secondary response.[5] In addition, assessing hazard-induced physiological responses, such as gene expression, membrane damage, apoptosis, and oncosis of living organisms can provide insight into the basis of toxicity for a particular hazard. To date, electrophysiological measurements made using micropipette electrode such as the patch-clamp technique can record the intracellular and extracellular potential *in vitro* and *in vivo* with good spatial resolution,[28,36,98] but it is difficult to multiplex using this approach. However, nanofabricated structures like FET arrays, have the potential for being used to form tight cell–substrate junctions and large-scale multiplexing in parallel and have enabled measurements at the level of individual axons and dendrites in neural networks.[58,86]

Cellular adhesion and guidance can be enhanced by unique interactions between the nanotopographic surface and cell membrane. Nanomaterials ensure the appropriate size compatibility and biocompatibility between the nanomaterials and cells.[19] For example, nanostructured surfaces, formed by CNTs, graphene, and SiNW, promote cellular adhesion, spreading, and direct axonal growth, even in the absence of adhesion molecules, such as poly lysine, laminin, fibronectine, and so on.[81,85,86,96,102] Electrical recording from the NW-FET arrays have been reported from various cells such as neurons, cardiomyocytes, and so on.[86] For instance, Patolsky *et al.*[86] reported that SiNW-FET arrays integrated with individual axons, and dendrites of live neurons can be used to record changes in the extracellular field in a highly sensitive manner with stimulation and inhibition of neuronal signal propagation as shown in Fig. 3. The result shows the behavior of SiNW-FET array, which exhibits good temporal correlation between intracellular potentials and the signal recorded by the interfaced SiNW-FET. In addition, SiNW–axon junction arrays were tested for signal propagation of neuron by local electrical

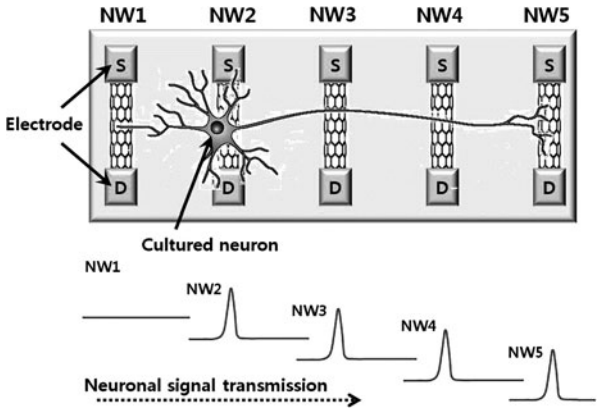

FIGURE 3. Extracellular potential recording of neuronal axon signals in the NW-FET array sensor. The heterogeneous patterning can be utilized to enhance neuronal adhesion and growth across many NW devices, while preventing unwanted adsorption on other regions of the device. Thus, time-correlated signal from axon can be measured using a NW-FET array.

stimuli at a level of at least 50 synapses per neuron. Whole tissue, as another tissue/nanomaterial interface, could be used to detect electrophysiological signals to better understand tissue dysfunction. The 2D single layer graphene-FET devices interfaced with cardiomyocytes also yield well-defined extracellular signals when compared with SiNW-FETs.[19] Moreover, recording cellular responses with cardiac tissue in NW-FET arrays on flexible polymeric substrates was demonstrated by Timko *et al.*[113] More interestingly, NW-FET arrays fabricated on increasingly flexible plastic and/or biopolymer substrates have the potential to become unique tools for electrical recording from other tissue/organ samples or as powerful implants.[113] However, cell-based nanobiosensors have several issues that might limit the extent of their application, including specificity, reliability, long-term stability, and scalability with low price. In addition, Chen *et al.*[17] used the GMI-based biosensing system for the detection of gastric cancer cells. Nanoparticles, which were used as magnetic tags in GMI sensors, were functionalized with the RGD peptides that can recognize α_v integrin in gastric carcinomas[52] and the functionalized-nanoparticles were then added to gastric cancer cells. When cancer cell–nanoparticle complexes on the GMI sensors were exposed to external magnetic fields, GMI responses can be measured. In this case, the presence or the absence of the nanoparticle can alter the sensor's magnetoimpedance, providing a detection signal.

CONCLUSION

In this review, recent advances in nanobiosensors based on nanomaterials modified with biomolecules, including their fabrications, characteristics, functionalizations, and current biosensing applications, were discussed. These nanobiosensors have a number of key features, including label-free, and real-time electrical signal measurement with ultrahigh sensitivity and exquisite selectivity. The nanobiosensor, which is a powerful detection platform, has the potential to significantly impact diagnosis of diseases, such as cancers and metabolic disorders, genetic disorders screening, and new drug discovery. Also, nanobiosensors can allow for personalized medicine, such as regular health checkup and detection of hazardous substances causing health problems in the near future.

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