Review Article

Application of Nanostructured Materials in Fabrication of Electrochemical Genosensors

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Abstract

A multi-walled carbon nanotube modified paste electrode (MWCN-PE) was used for determination of promethazine (PMZ) in drug formulations and blood plasma by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Results revealed that the MWCN-PE shows an electrocatalytic activity toward the anodic oxidation of PMZ by a marked enhancement in the current response in buffered solution at pH 5.0. At the MWCN/CPE, the anodic peak potential of PMZ was shifted by 28.0 mV to more negative potential in comparison with bare carbon paste electrode. The detection limit for this method was 0.025 µmol L-1. Results indicated that the modified electrode could be employed for the determination of promethazine hydrochloride in pharmaceutical formulations and plasma samples.

Abbreviations: BHT-Butylated hydroxytoluene; TEAC-trolox-equivalent antioxidant capacity; AUC-area under the curve

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Introduction

Electrochemical sensors/biosensors are powerful devices aimed at providing selective and sensitive determination of biologically active compounds (1-6). These devices can offer advantages such as easy miniaturization, low detection limit, wide linear response range, good stability and need to small analyte volumes.

DNA sequencing is an important area of molecular biology and clinical studies. The detection of specific gene sequences in human, viral and bacterial nucleic acids is important in some areas such as clinical diagnosis and identification of genetic mutations. In this context, electrochemical sensors/biosensors are useful.

DNA-based biosensors have attracted extensive attention in recent years. The importance of these biosensors is due to their wide range of applications, from diagnosis of many human diseases to determination of pathogenicity of diseases (7-11). Diagnosis of genetic diseases is very important in health preventive care (7, 8). Prevention and treatment of human disease requires realistic, effective and low-cost design of genomic sequence determination tools and this has many usages in gene expression monitoring, pharmacogenetic researches, drug discovery, clinical diagnosis, gene analysis, virus and bacteria determination, bioterrorism factors determination, biological wars and crime investigations (10). Genetic mutation diagnosis in the molecular level will provide reliable diagnosis of diseases before clinical signs manifestation. In addition, in relation to diagnosis of microbial pathogen agents, determination methods based on nucleic acids are more sensitive and specific than immune reaction-based methods (12). In addition, it is recommended to employ new direct and sensitive methods in bacterial diagnosis and explanation, instead of hard, harmful and time-consuming isolation and culture methods (13, 14). In order to achieve these broad goals, different methods of gene sequencing in live organisms and other complex samples were developed (9-11, 15-18). Molecular diagnosis based on gene sequence analysis have provided very sensitive and precise methods to diagnose pathogens and genetic changes. Conventional methods of analysis of certain gene sequence are based on direct sequencing or DNA hybridization.

DNA hybridization methods are simpler than direct sequencing methods. In DNA hybridization method, the target gene sequence is identified by a probe DNA that can complete the double stranded structure. This method is very efficient and specific and can recognize the target gene in a complex matrix containing different noncomplementary nucleic acids. Probe DNA is a single stranded DNA (ssDNA) containing a radioactive, light absorbing, light emitting or electrochemical active agent that can reveal the hybridization (19), because nucleic acids rarely produce distinguishable signals (9, 20). The signal transducer element will reveal the target DNA capture by the probe DNA via producing a recordable signal. One of the major research fields in DNA-based biosensors is developing these signal transducer elements. Although several DNA microarray technologies were developed, this way is going to be so dynamic by researches in order to save costs, simplify more, make more variety, and increase sensitivity and specificity.

There usually exists a problem that the amount of DNA to be recognized is less than the detection limit of current tools (at the femtomolar (fM) or attomolar (aM) level). Therefore, the transducer signal must be amplified. In some cases, PCR method is used beside these methods (14, 21, 22).

DNA detection methods based on hybridization can be divided into two groups of homogenous and heterogeneous methods. In the homogeneous method, both probe and target are in solution phase, while in the heterogeneous method, the probe DNA is immobilized on a solid surface. In homogenous method, different optical (23-26) or electrochemical (27, 28) methods are employed to reveal the hybridization. The disadvantage of homogenous method is impossibility of continuous monitoring and making the system miniaturized. Genosensors and DNA chips based on heterogeneous methods can solve these problems. Therefore, immobilization of probe DNA is one of the important stages in the fabrication of genosensors.

Immobilization of the probe DNA

The first step in genosensor development is the immobilization of probe DNA that is the most important stage of a genosensors fabrication. General methods of probe immobilization include surface adsorption, covalent immobilization and avidin (streptavidin)-biotin interaction (29).

Surface adsorption

This route is one of the simplest ways of probe immobilization. This method relies on electrostatic interactions between the negatively charged DNA and positive charges on a surface. For example, chitosan was employed as a cationic biopolymer for DNA immobilization (30, 31). Similarly, a mixture of polyalkylamin and polystyrene sulfonate was used in DNA immobilization (32). In addition, physical adsorption of DNA on the surface of screen-printed carbon (33), pyrolytic graphite (34), gold (35), and glassy carbon (36) were reported to fabricate electrochemical genosensors.

Covalent immobilization

In one of covalent immobilization methods, the interaction between thiol functional group and the

gold metal is employed (37-40). A chemical reaction (covalent bond formation) between amine group of 5' end of probe DNA, and the carboxylic group via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide is also employed for probe immobilization (41-44). The probe can be immobilized on the surface of conducting polymers via this reaction (45, 46-48). Of course, the immobilization of DNA on the surface of conducting polymers is not performed only via this reaction; direct coupling between amine and carboxylic groups can be done (49-51). In this regard, polypyrrole (52-59), polyaniline, poly diaminobenzene and poly (3-4-methylenedioxy thiophen) (60, 61) were used. Also, self-assemble monolayer formation was used for covalent immobilization of DNA (62). Other methods of DNA covalent immobilization include immobilization by glutaraldehyde on the surface of atomic force microscopy (AFM) probe (63), immobilization on the surface of mercaptosilane-modified glass (64), and use of aniline derivatives (65-67).

Immobilization via avidin-biotin interaction

Avidin and streptavidin are tetrameric proteins that have four binding sites for biotin. In order to immobilize the probe DNA, avidin is attached to a surface and then biotinylated DNA is immobilized on the surface (68-72).

Some advantages and disadvantages of different immobilization methods are presented in Table 1.

Table 1. Advantages and disadvantage of immobilization methods.

Immobilization method	Advantages	Disadvantages
Surface adsorption	Simple, Cheap, Effective, Simple equipment and handling, Easily implement in industrial processes	Poor operation stability, Frequently reversible, Immobilization affected by different parameters (such as temperature, ionic strength and pH), The possibility of denaturation, Aggregation of biomolecules and reduces the activity, Desorption possibility
Covalent	Eextraordinary stable immobilization, Better control on the immobilization procedure, Over ligand density, Better biomolecules orientation, Spatial patterning, Providing accuracy and reproducibility	Rigidity of biomolecule, Low activity recovery
Avidin-biotin binding	Strong interaction, Possibility of in-situ immobilization, Easily renewable	Relatively expensive

Electrochemical genosensors

In electrochemical genosensors, hybridization can directly be converted to an electrical signal (45, 73, 74). Therefore, the complexities and difficulties of signal conversion are reduced, and signal detection with electrochemical devices will be easily possible. Electrochemical signal transducers are more sensitive and compatible with smaller sizes. These transducers can be enzymes, ferrocene and electroreactive species interacting with DNA. Also, some electrochemical genosensors act in the label-free fashion.

Enzymatic labels

In this route, the probe DNA or its complementary target is labeled with an enzyme. In a genosensor, a proxidase label was used that changed o-phenylenediamine to 2-2-di-aminobanzene (75). The later compound is a

chromophore and also an electroreactive species. In another study, a 26-mer sequence of ssDNA was detected using impedance spectroscopy (76). For this purpose, a thiolated oligonucleotide was immobilized on the surface of gold, and hybridization of a biotinylated target DNA provided a sandwich-like structure. Then, the obtained double strand structure reacted with a HRP-containing avidin. The resulting complex could catalyze the oxidation of 4-chloro-1-naphthol by hydrogen peroxide and resulted in an electron transfer interface. In other study, HRP-labeled liposome was used similarly (77). A nylon membrane-modified by target DNA was used in other works (78-81) and the target DNA was collected with a signal transducer on the surface of a graphite-epoxy composite. In all cases, the hybridization process was monitored by electrochemical methods through labeling with a HRP-streptavidin conjugate in the presence of hydrogen peroxide and hydroquinone. In another genosensor, cationic redox polymers containing osmium-bipyridine complex were coated on the electrode surfaces (47). The complex acted as an electron transfer mediator. After immobilization of the probe DNA, a HRP-labeled target DNA was hybridized, and the resulting complex could catalyze the reduction of hydrogen peroxide. These complexes would provide the electrical contact between peroxidase center and the electrode. This method was also used to detect the presence of a single base mismatch in an 18-mer oligonucleotide (82) so that a soy heat resistant peroxidase was used. Other copolymer of 4-mini pyridine and acrylamide with complexes of osmium bipyridine was also employed (83). Glucose oxidase was also used as an enzyme label. A redox cationic polymer containing osmium-bipyridine complex that can conjugate with glucose oxidase was synthesized (84). This redox polymer reacted with glucose oxidase after probe immobilization and hybridized with labeled target. In the presence of glucose, the generated current was measured. Other enzymes used to fabricate genosensors include alkaline phosphatase (30,31,85), glucose-6-phosphate dehydrogenase (32) and bilirubin oxidase (86).

Labels interacting with DNA

- Groove binders

One of these labels is bipyridine-ruthenium complex that oxidizes guanine through a catalytic reaction. Therefore, this label reveals the presence of guanine (33,87). Similar complexes of cobalt with bipyridine or phenanthroline can be used as labels which also bind to double stranded DNA (dsDNA) selectively and reversibly. These complexes bind to the minor groove of dsDNA. Also, complexes of cadmium with pyridine-benzamide derivatives were used to study to bind to DNA (34). Hoechst (Figure 1) is a compound that binds to the minor groove of DNA which is rich in adenine/thymine. This binds to DNA and its oxidation current is relevant to the hybridization degree (35,36). This compound was used in 32-channel array (88-90) and DNA chips (91) fabrication.

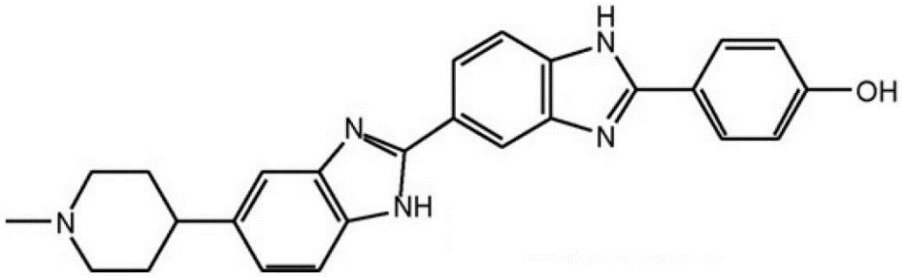

Figure 1: Chemical structure of Hoechst.

- *DNA intercalators*

Anthracycline antibiotics like daunomycine and doxorubicin are currently used in cancer treatment. Details of daunomycine interaction with DNA were studied (92-95). This drug intercalates into the dsDNA and has more affinity to guanine/cytosine base pairs and is one of the best compounds to develop genosensors. The probe DNA can be attached to the surface of carbon electrode and its hybridization with target DNA can be followed by different electrochemical methods (96). Also, the probe can be thiolated and adsorbed at the surface of gold and hybridization can be similarly followed (97). Detection limit of this method was 10^{-11} M. Doxorubicin is also a special intercalator (98, 99). This drug was also used to detect DNA with 27 base pair (100). Probe DNA immobilization with avidin-biotin interaction and hybridization detection by doxorubicin lead to electrochemical detection of mutation in neuroblastoma.

The electrochemical behavior of other interchalators, such as azo dyes, acridine, ethidium bromide, anthracyclinas, tetracyclines and bis-benzamides was also studied (4-101). Anthraquinone derivatives, such as 2,6-anthraquinone disulfonic acid, were also used and electron transfer to these labels performed through a long pathway (102). Ethidium bromide, as a redox intercalator, can detect DNA at 10^{-10} M level (103). Extensive studies were performed on the methylene blue-DNA interaction (104, 105, 112, 113). This compound has more affinity to intercalate to ssDNA, than dsDNA. This is due to its high affinity to bind with guanine base. Therefore, upon ssDNA hybridization, the current decreases in the voltammograms of methylene blue (106, 107). In the study of intercalation of labels to DNA, carbon nanotubes (41), glassy carbon (4) and gold (108-112) electrodes were employed. Using methylene blue, the DNA of hepatitis type B virus was detected (107). Proflavine (3,6-diaminoacridine) is also a positive-charge intercalator with no electroreactivity (114). However, after hybridization and binding to DNA, the ssDNA negative is neutralized. Therefore, the hybridization can be followed using an electrochemical active species with a negative charge such as ferrocyanide ions. When ssDNA exists on an electrode surface, ferrocyanide ions show weak currents due to repulsive forces. After hybridization, proflavine intercalates into dsDNA and neutralizes its negative charge. Now ferrocyanide ions can approach the surface and generate current proportional to hybridization degree. This method could detect DNA up to 10^{-14} M (114).

- *DNA labeling with metal nanoparticles*

In most genosensors made with metal nanoparticles, gold (115-117) and silver (118) nanoparticles were used. DNA was immobilized on a carbon surface and then hybridized with gold nanoparticles-labeled target DNA (119). This genosensor was used to detect mutation in factor V of leiden. The electrochemical signal of gold nanoparticles can be measured after their dissolution (120). These nanoparticles can be dissolved through reaction with bromine/bromhidric acid mixture and converted to gold ions. These ions were then concentrated through electrochemical reduction and their amounts were measured (121). In order to increase sensitivity, gold nanoparticles can be covered with silver after DNA hybridization. This process was performed through chemical or electrochemical reductions. After that, silver was measured (68-71,122-125) or hybridization was assessed by measuring the electrical conduction (72).

Gold nanoparticles were used to label polymeric beads. In these cases, target DNA labeled with gold nanoparticles were hybridized with the probe DNA immobilized on the surface of magnetic beads (126). After that, gold nanoparticles can be quantified.

Iron-containing nanoparticles were employed to detect DNA hybridization (127). In a method, the core/shell nanoparticles containing iron core and gold shell were used. The biotinylated target hybridized with a probe labeled with iron-gold nanoparticles on the surface of polystyrene beads covered with streptavidin. In another method, commercial magnetic beads containing iron were employed. The immobilized probe DNA hybridized with magnetic beads-labeled target DNA. Then, the iron-containing nanoparticles were dissolved and the amount of iron was measured.

In Table 2, Application of some DNA labels and their electrochemical measurement techniques are presented.

Table 2. Some DNA labels and their electrochemical measurement techniques. HRP: Horseradish peroxidase, DPV: Differential pulse voltammetry, IS: Impedance spectroscopy, Amp: Amperometry, CV: Cyclic voltammetry, LSV: Linear sweep voltammetry, ASV: Anodic stripping voltammetry

DNA label	Techniques	Applications	Reference
HRP	DPV	Detection of human cytomegalovirus genome	75
HRP	IS	Detection of Tay-Sachs genetic disorder	76, 77
HRP	Amp	Detection of DNA hybridization	78
HRP	IS	Detection of staphylococcus aureus genome	79
Glucose oxidase	Amp	Detection of Escherichia coli genome	84
Alkaline phosphatase	DPV	Food-contaminating pathogenic bacteria genomes	85
Glucose-6-phosphate dehydrogenase	Amp	InvA gene of Salmonella typhimurium	32
Bilirubin oxidase	DPV	Detection of DNA hybridization	86
Bipyridine-ruthenium complex	CV	Detection of the 3 Ras genes in humans (HRAS, KRAS and NRAS)	33, 87
Hoechst	LSV	Detection of v-myc oncogene	35
Daunomycine	CV	Detection of yAL3 gene	97
2,6-Anthraquinone disulfonic acid	DPV, CV	Detection of single-base mismatch	102
Ethidium bromide	CV	Detection of 24-mer deoxyribonucleic acid	103
Methylene blue	CV, DPV	Detection of hepatitis B virus genome	107
Silver nanoparticles	ASV, IS	Detection of DNA hybridization	118
Gold nanoparticles	CV, DPV	Detection of Factor V Leiden mutation	119

- *Labeling of probe or target DNA and detection of hybridization*

The methods that use labels have some drawbacks such as plenty of noise in the background. This is due to the non-specific binding of redox labels to the electrode or DNA. In order to overcome this problem, the redox label can be attached to a DNA sequence (signal producing DNA). Of course, the disadvantage of this method is the risk of DNA demolition or its mixing with the label. The target DNA acts as a molecular bridge between probe DNA (that is attached to the surface of an electrode), and the signal producing DNA (128-132). Therefore, two processes of

hybridization occur during the function of this type of genosensor. A schematic design of this type of genosensor is shown in Figure 2. In order to fabricate such a genosensor, a ssDNA modified electrode is made and 6-mercapto 1-hexanol molecule is immobilized between the probe strands to prevent non-specific adsorption of probe DNA. Then, the target is hybridized with the probe and then this complex is hybridized with signal producing DNA. The label attached to the signal producing DNA can be an enzyme or a nanoparticle.

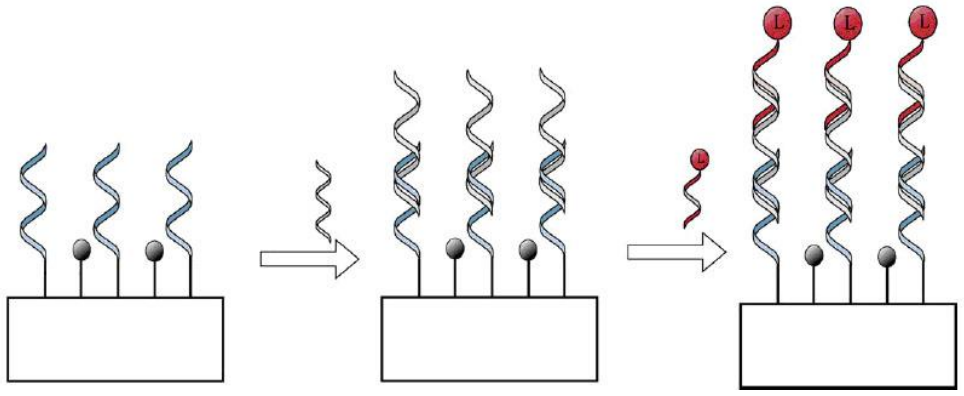

Figure 2. Conjugation of a redox label with target DNA.

In a research, immobilization of probe DNA was performed by an avidin bridge, and a HRP labeled-signal producing DNA was employed. The detection limit was 10^{-13} M (128). In another study (130), gold nanoparticles modified with streptavidin was used as a label and then attached to a probe DNA through biotin. Then, gold nanoparticles were dissolved and their concentration was determined.

Changes in DNA structure during hybridization and double strand formation can be used to fabricate genosensors. In this case, one end of probe DNA is attached to a label and the probe goes away from electrode surface after hybridization. This process is shown in Figure 3 (131,132). Using this method, DNA at 10^{-9} M in blood serum was detected (133).

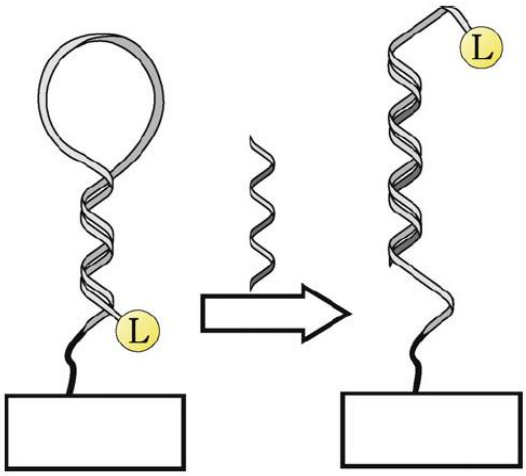

Figure 3. Changes in DNA structure followed by hybridization and formation of double stranded structure

Design of unlabeled genosensors Field effect genosensors

Because DNA is naturally charged, it can be used in field effect transistors. Attachment of DNA to an interface will change the potential of semi-conductive gap. This change is a result of charge variation at the interface or redistribution of charges in the inter-molecular space due to the DNA adsorption (134). Different interfaces, such as silicon nitride (135), diamond (52) and carbon nanotubes (129) were used to fabricate these genosensors.

Electrochemical genosensor-Electrochemical inherent properties of DNA-Direct oxidation of DNA bases

Early studies on electrochemistry of DNA were based on guanine oxidation on the surface of mercury drop electrode (53), and the first method of DNA detection was its direct oxidation at the surface of mercury drop electrode (53). During the oxidation process, guanine and rarely adenine are oxidized and this is closely relevant to DNA spiral structure. Advantages of this method are simplicity and no need to any modification or manipulation of DNA (54). One of the disadvantages of this method is irreversible oxidation of DNA bases that leads to longevity decrement of the genosensor. In most of these genosensors, oxidation current of guanine continuously increases because the total surface concentration of guanine is increased upon hybridization. Therefore, this is a major drawback of this method. To overcome this problem, inosine was replaced with guanine in the probe DNA (55, 56). Inosin forms a base pair with cytosine and any generated signal will be a result of guanine oxidation directly from hybridization (57-60). In another method, guanine oxidation process was performed via an indirect catalytic reaction through transition metal complexes (not directly at the electrode surface) (61,136,137).

Electrochemical genosensors based on conducting polymer transduction-Impedance measurement

Electronically conducting polymers are organic polymers that have electrical properties like metals. These polymers have special electronic structures, electrical conduction, low ionizing potential and high electronegativity. The most prominent and famous of these polymers are polyaniline, poly(phenyl vinylene), polypyrrole and polythiophene (Figure 4).

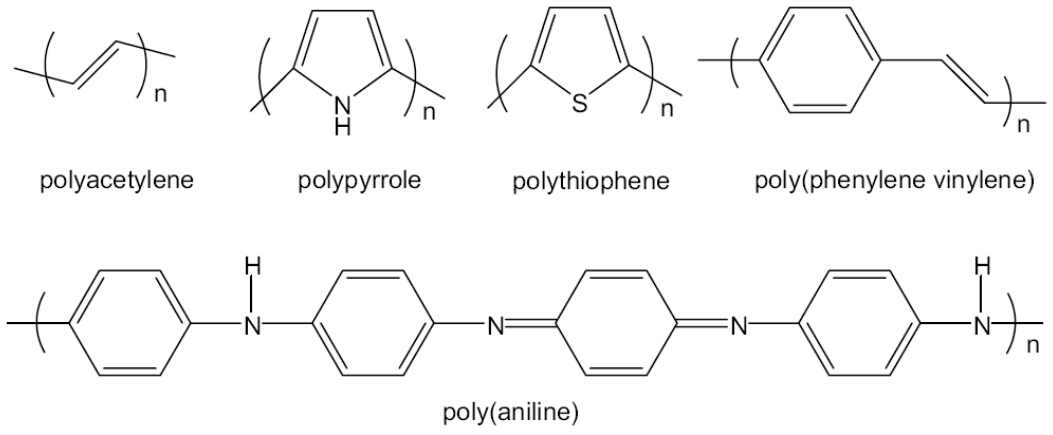

Figure 4. The chemical structure of some conductive polymers.

These polymers can be dopped by ions and have self-concentrating motivations (polarons, bipolarons and solitons) that cause their electrical conduction. Immobilization of probe DNA on the surface of polymer is performed through entrapping, covalent binding or electrostatic/hydrophobic attractions.

For entrapment, the polymer is synthesized from its monomer in the presence of probe DNA. This also causes the probe DNA (as an anion) to be doped in the polymer. In covalent immobilization, the probe

DNA is usually functionalized by amine or acid functional groups and then attached to the polymer surface. Avidin-biotin conjugation can also be used. Since the electrical properties of conductive polymers depend on its structure, dopping level and its surface interactions, electrical measurements on these polymers can produce relevant signal upon hybridization. Therefore, electrochemical impedance spectroscopy will be the most common tool in hybridization detection and genosensors response recording based on conductive polymers.

A study showed that the impedance spectra of oligonucleotide-functionalized polypyrrole had a significant difference before and after hybridization with complementary oligonucleotide (45). Similar differences were observed in copolymer of poly(1,3-acetic acid pyrrole) and poly(3-N-hydroxyphthalimide pyrrole) (138). A genosensor based on polyaniline was presented (139) and its function is illustrated in Figure 5. To fabricate this genosensor, avidin was attached to the surface of polyaniline. Then, probe DNA that was attached to biotin at its 5' end, was immobilized at the surface of polymer. In order to obtain hybridization signal, direct oxidation of guanine and methylene blue were used. Using this genosensor, a detection concentration at 2 fM was obtained.

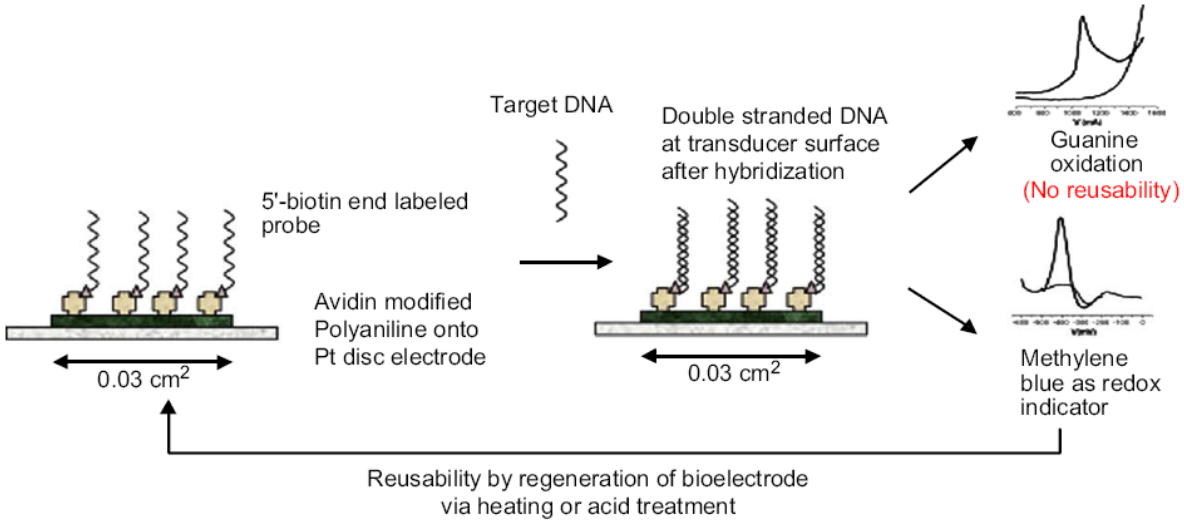

Figure 5. A scheme for an ultrasensitive genoensor based on polyaniline.

Electrochemical genosensor based on electrochemical properties of nanostructured materials

Unique physicochemical properties of nanostructured materials have led to widespread applications of these materials in genosensor fabrication.

Polymeric nanoparticles

Some genosensors were fabricated from conducting polymeric nanoparticles possessing high electrical conductivity, good processability, low ionizing potential and great stability (140). The most important factors influencing electrochemical properties of these genosensors are signal transduction, thickness and shape, which are well controlled through polymer sizing. Poly (indole-6-carboxylic acid) nanostructure was synthesized for a non-labeled genosensor with picomolar (pM) level detection-limit (141). Another genosensor was designed based on polyaniline nanostructure (142) that can detect neisseria gonorrhoea in the presence of neisseria meningitides and E.coli. An arrangement of polyaniline nanotubes provided electron transfer channels to DNA active sites and provided a detection limit of fM (143).

Metallic nanomaterials

Metallic nanomaterials show size, surface and catalytic effects related to their small dimensions. These nanomaterials were used in genosensor fabrication as immobilizing surface for probe DNA, signal amplification or as labels. Noble metal nanoparticles such as palladium, platinum, gold and silver were more used in this area (68, 72, 130, 144). For example, platinum nanoparticles deposited on the surface of glassy carbon were

used to detect soy DNA (145). Other nanomaterials were also employed together with metal nanomaterials. For example, platinum nanoparticles and carbon nanotubes, placed in Nafion polymer, were used to fabricate genosensors and can detect 10^{-11} M DNA (146). A similar study reported using palladium nanoparticles (147). Among metals, gold nanoparticles the most use in genosensors fabrication. Different strategies for using gold nanoparticles in genosensor fabrication are illustrated in Figure 6. These strategies include: a) the use of gold nanoparticles as a label, and then their dissolution and determination of the resultant gold ions, b) direct determination of the amount of gold nanoparticles, c) using silver accompanied by conductometric detection, d) improving gold nanoparticles surface with silver, e) using gold nanoparticles as carriers of other nanoparticles, or f) other electrochemical labels.

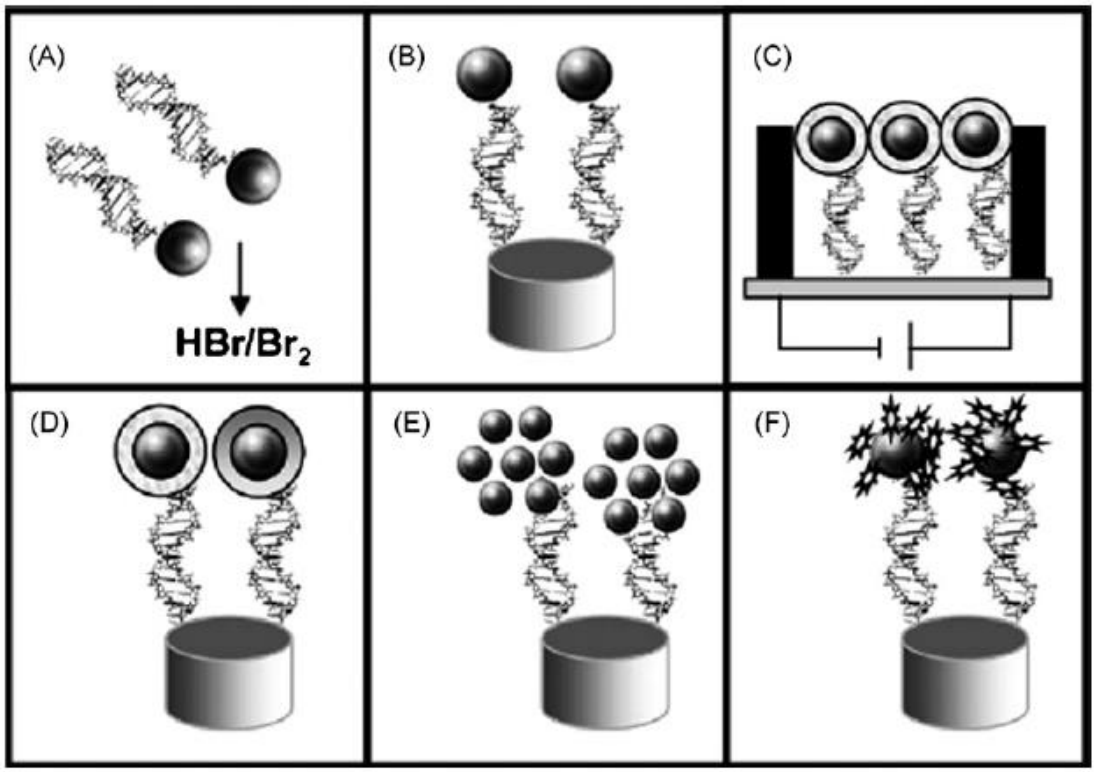

Figure 6. Strategies for using gold nanoparticles in genosensor fabrication.

In a study, gold nanoparticles were labeled on DNA and employed to fabricate a genosensor with a detection limit at aM level (148). In another study, a co-polymer of 2,6 pyridine and carboxylic acid was firstly synthesized on the surface of carbon and then, a layer of gold nanoparticles was covered on the polymer (149). The probe DNA was then fixed on the surface of gold, and methylene blue was employed as the indicator of hybridization. Catalytic effect of gold nanoparticles and biological compatibility of the polymer are both effective in performance of this genosensor. In another work, gold nanoparticles were formed at the surface of carbon electrode and then a mixture of polycystamine/polyglutamic acid was covered on it

(150). The probe DNA was immobilized on the resultant surface.

Other methods of probe DNA immobilization consisted of layer-by-layer covalent binding of gold nanoparticles and thiol-functionalized carbon nanotubes on the surface of gold electrode (151). In this genosensor, carbon nanotubes provided electron-conducting matrix, and gold nanoparticles had the role of a catalyst. A gold nanoparticles surface was covered with polyamidoamine and 3-mercaptopropionic acid for the DNA immobilization (152). Rutenium-amin complex was used as the hybridization indicator and 10^{-14} M DNA concentration was detected.

Metal oxide nanomaterials

Metal oxide semiconductors have been used in designing and developing biosensors as a new class of advanced materials (153-158). Cerium oxide/chitosan nanocomposite was employed for probe DNA immobilization and further detection of colon cancer gene (159). Due to the presence of chitosan, the nanocomposite had a high biocompatibility. Methylene blue was used in this genosensor and a detection limit at 10^{-11} M level was obtained. Cerium oxide was also used in fabrication of other genosensors with carbon nanotubes and the ionic liquid of (1-butyl 3-methylimidazolium hexafluoro phosphate). This genosensor was used in detecting pyruvate carboxylase gene (160). Zirconia was applied as a thin film on the surface of gold to attach a probe DNA through its end phosphate group (161). Methylene blue was the hybridization indicator. Zirconia (together with carbon nanotubes and chitosan) was the main component of another genosensor (161). Probe DNA was also immobilized on the surface of zinc oxide with a detection limit at 10^{-11} M level (162). In order to immobilize zinc oxide, biopolymers (162) or sol-gel method (163) were employed. Hollow microspheres of copper (I) oxide was employed to immobilize a probe DNA to detect hepatitis B virus (164).

Conclusion and outlook

This review described the utilization of nanostructured materials for fabrication of electrochemical genosensors. The electrochemical genosensors, as powerful detection devices, have the potential to significantly impact the diagnosis of diseases, such as cancers and genetic disorders. Nanostructured materials became important components in genosensing devices since they clearly improve the performance of these instruments. Furthermore, the application of different nanomaterials to increase the efficiency of electrochemical genosensors is a well-accepted strategy. Although many improvements are required in reproducibility and sensitivity, there is no doubt that a growing number of nanomaterial-based electrochemical genosensors will soon be offered for the diagnosis of diseases and monitoring following therapy.

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