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Review Article

DNA Electrochemical Nanobiosensors for the Detection of Biological Agents

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Abstract

In this study, we explained a nanobiosensor for DNA sequence detection, featuring sequence specificity, cost efficiency, speed, and ease of use. Without the need for labels or indicators, it may be ideal for the detection of biological agents. This review describes recent advances in electrochemical impedance spectroscopy (EIS) with an emphasis on using nanoparticles and nanotechnology. A powerful biosensor system requires a high-performance biosensor component as well l as a user-friendly instrumental setup. However, biosensor setups have to be adapted to specific applications. Rapid, selective and sensitive detection technologies for biological agents are critical in clinical diagnosis, environmental monitoring and food safety. Recent developments in nanomaterial create many opportunities to advance DNA sensing and gene detection. The fact that gold nanoparticles are able to provide a stable immobilization of biomolecules that retain their bioactivity is a major advantage for the preparation of biosensors. Although, there are a lot of researches reporting electrode modification by different nanomaterial to improve the DNA biosensor performance, the preparation of nanomaterial or the electrode modification strategy is often relatively complex. Furthermore, some DNA biosensors based on nanomaterial modification are still very limited for the improvement of DNA biosensor performance. Thus, the construction of nanostructure modified electrode by a simple strategy to improve the DNA detection sensitivity is highly desirable.

Keywords: DNA Hybridization, Electrochemical Impedance Spectroscopy, Nanobiosensor

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Introduction

In the late 1990s, the term ‘‘biosensor’’ has become more popular with analytical chemistry, molecular biology and biochemistry. Biosensor is an analytical device that converts biological reactions into measurable physiochemical signals which is proportional to analyte concentration. Biosensors can be used for the detection of various substances like metabolites, pollutants, microbial load, control parameters etc. The first biosensor was invented by Leland C Clark, known as the father of the biosensor concept. In 1956, Clark published his definitive paper on the oxygen electrode, a schematic of which is shown in Figure 1. The concept was illustrated by an experiment in which glucose oxidase was entrapped at a Clark oxygen electrode using dialysis membrane [1-3].

However, since late 20th century the concept of biosensors existed but their uses were limited only in laboratories and with the advent of sciences several modern biosensors were designed. Novel biochemical elements, e.g. aptamers and materials with molecular imprints, have been introduced in the biosensors assemblies. The family of DNA sensors have been added to the traditional biosensors with their own specific goals, like the detection of oligonucleotides, proteins and the low molecular compounds able to bind to DNA [5-7]. Overall, there are three generations of biosensors; the first generation in which the normal product of the reaction diffuses to the transducer and causes the electrical response, the second generation involves specific “mediators” between the reaction and the transducer in order to generate improved response, and the third generation in which the reaction itself causes the response and no product or mediator diffusion is directly involved [8-10]. A summary of some of the key events in the evolution of biosensors is shown in Table 1.

In order to have reliable diagnostic tools for the rapid detection and identification of biological agents, new methods allowing label-free and real time measurement of simultaneous interactions (as harmful agent/receptor or DNA recognition) should be developed. Biosensing devices, fabricated with nanotechnologies are powerful devices which can fulfill these requirements and have the added draw of being portable to perform “point-of-care” analysis. These devices can also have the multiplex capability in a biodetection platform to perform the identification of biological agents in a faster way than routine clinical analysis [11, 12].

With the progression in sciences, nanobiosensors with superbly dedicated miniature sensors with highly miniaturization were designed and developed in 21st century based on the ideas of nanotechnology. Recently, researchers have used an integrated approach by combining nanosciences, electronics, computers and biology to create biosensors with extraordinary sensing capabilities that show unprecedented spatial and temporal resolution and reliability. Nanosensors with immobilized

bioreceptor probes that are selective for target analyte molecules are called nanobiosensors. A nanobiosensor is usually built on the nanoscale to obtain process and analyze the data at the level of atomic scale. Nanobiosensors open up new opportunities for basic research and provide tools for real bioanalytical applications, which was impossible in the past [31-33]. This review will outline the developments in nonobiosensors for detecting the biological agents.

One of the problems with biological attacks is actually determining whether an attack has occurred. The solution to this detection problem is to employ molecular techniques, which can identify chemical markers from known biological agents. In the case of the bacterial species and viruses, nucleic acid techniques can be used to identify the organisms or affinity molecule can be used to detect surface sites on these organisms.

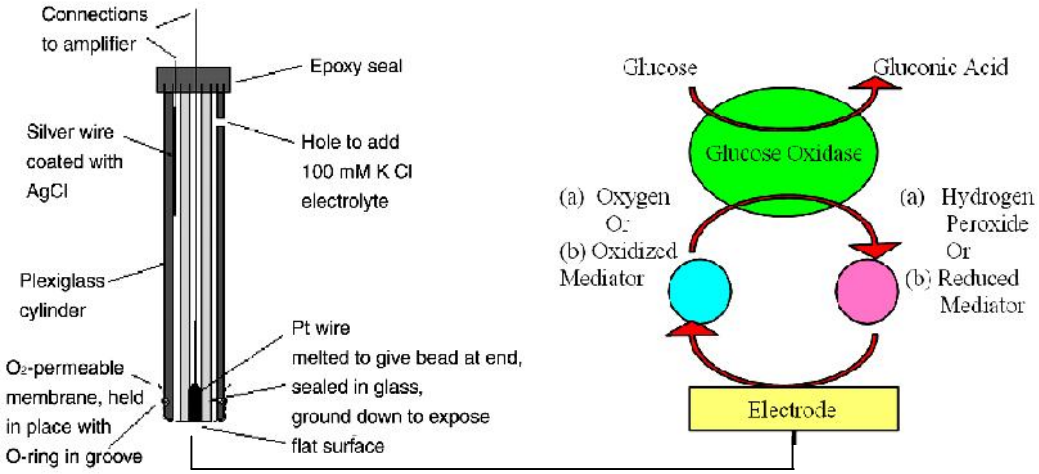

Figure 1. Schematic of Clark oxygen electrode [4].

Table 1. Events in the history of biosensor development.

No.	Event	Year	Ref.
1	First report on the immobilization of proteins: adsorption of invertase on activated charcoal	1916	13
2	First glass pH electrode	1922	14
3	Invention of the oxygen electrode	1956	1
4	First description of a biosensor: an amperometric enzyme electrode for glucose	1962	2
5	First potentiometric enzyme sensor: urease immobilized on an ammonia electrode to detect urea	1969	15
6	Invention of the ion-selective field-effect transistor (ISFET)	1970	16
7	The use of thermal transducers for biosensors was proposed	1974	17
8	First microbe-based biosensor First immunosensor on a potentiometric transducer: ovalbumin on a platinum wire Invention of the pO_2/pCO_2 optode (fluorescence signal & gaspermeable membrane usage)	1975	18-19
9	First bedside artificial pancreas (Miles)	1976	2
10	Karl Camman coined theterm biosensor	1977	21
11	First fiber optic pH sensor for in vivo blood gases	1980	4
12	First needle-type enyme electrode was described	1982	22
13	First surface plasmon resonance (SPR) immunosensor	1983	23
14	First mediated amperometric biosensor: ferrocene used with glucose oxidase for the detection of glucose	1984	24
15	Launch of the MediSense ExacTech blood glucose biosensor	1987	25
16	Launch of the Pharmacia BIACore SPR-based biosensor system	1990	4
17	i-STAT launches hand-held blood analyzer	1992	26
18	Glucocard launched	1996	27
19	Abbott acquires MediSense for $867 million	1996	4
20	Launch of Life Scan Fast Take blood glucose biosensor and Merger of Roche and Boehringer Mannheim to form Roche Diagnostics	1998	27
21	Use of electrodeposition paints (EDPs) as immobilization matrices for biosensors	2002	28
22	An enzymatic glucose/O_2 fuel cell was implanted in a living plant	2003	29
23	An implanted glucose bilsensor operated for five days	2007	30
24	Nanobiosensors: BioNMES, Quantum dots, Nanoparticles, Nanocantilever, Nanowire and Nano-tube	To now	

Constituents of biosensors

A typical biosensor comprises 3 components; biologically sensitized elements (biorecognition molecules), transducer, and detector as described in Figure 2 [34]:

1) Functional biomaterials with high affinity and high specificity include receptors, enzymes, antibodies, nucleic acids, molecular imprints, lectins, tissue, whole cells, microorganisms, organelles etc., which are either a biologically derived material or bio-mimic component that receives signals from the analytes (sample) of interest and transmits it to transducer. The fundamental and key feature of a biosensor is the construction of the biorecognition element for the interaction with the targets. Initially, biosensor recognition elements were isolated from living systems. However, many biosensor recognition elements now available are not naturally occurring but have been synthesized in the laboratory. The sensing of targets (analytes of interest) is already being influenced by the emergence of engineered binding proteins. Employing the techniques of modern biotechnology, it is now possible to construct DNA polynucleotides at will, thus opening new paths for generation of biosensor recognition elements arising from paths not taken by nature. With the advent of nanostructures and new interface materials, these recognition elements will be major players in future biosensor development [35].

2) The transducer acts as an interface, measuring the physical change that occurs with the reaction at the bioreceptor/sensitive biological element then transforming that energy into measurable electrical output. Depending on the mode of action transducers may be classified into following categories and discussed in details. The key part of a biosensor is the transducer which makes use of a physical change accompanying the reaction. This physical change may be the heat output by the reaction, changes in the distribution of charges causing an electrical potential to be produced, movement of electrons produced in a redox reaction, light output during the reaction or a light absorbance difference between the reactants and products, or effects due to the mass of the reactants or products.

3) The detector element traps the signals from the transducer, which are then passed to a microprocessor where they are amplified and analyzed; the data is then transferred to user friendly output and displayed/stored [36].

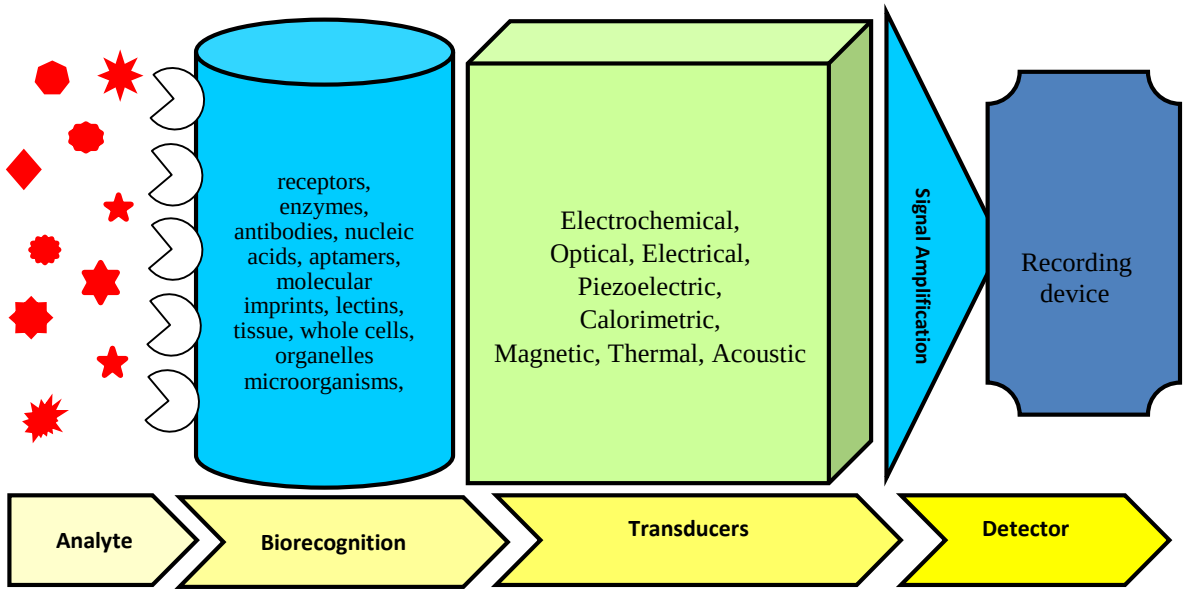

Figure 2. Elements and selected components of a typical biosensor.

The main challenges associated with these devices are the sensitivity and the specificity of the receptor and the transducer. These problems are often addressed by the immobilization of specific sensing element in relatively high concentration and the choice of signal transduction mechanism. A high concentration sensing element near the transducer can serve as a kind of preconcentrating analyte before detection. Transduction mechanisms to detect DNA hybridization include electrochemical and optical systems.

The validity of the approach depends on the biochemical materials to be detected (Table 2). For example, amperometric transducers are particularly suitable for redox enzyme systems, because recognition is through an enzymatic reaction that generates or consumes redox-active species to be detected. However, they are inappropriate for the detection of the hybridization of a DNA single strand with its complementary strand since their selective recognition properties are based on affinity. DNA biosensors have been based on electrochemical and optical methods, whereby the local hybridization event is converted into an electrical or optical signal and measured.The difference lies in the probe design scheme, the binding method between capture DNA and the probe, and the signal readout technique [37]. Electrochemical methods have been extensively studied since the electroactivity of nucleic acids was discovered. Most of them are indirect, using radiochemical, enzymatic, fluorescent, or electro-chemiluminescent indicators. However, these techniques are inconvenient due to their need for labels as indicators and their excessive pretreatments, which may increase the risk of contamination and preclude in situ monitoring.

Table 2. Correct selection of the bioreceptor and transducer

Bioreceptror / Transducer	Enzymes	Tissues	Microorganisms /Cells	Antibodies (Immunoagent)	Nucleic acids /DNA
Electrochemical	✓	✓	✓	✓	✓
Optical	✓	✓	✓	✓	✓
Piezo-electric	✓	✖	✖	✓	✖
Heat (Thermometric)	✓	✖	✖	✓	✖
Mass	✓	✖	✖	✓	✖

DNA as biorecognition elements

DNA is especially well suited to biosensing applications due to its tremendous molecular recognition potential [38]. DNA biosensors exploit the preferential binding of complementary single stranded nucleic acid sequences. DNA biosensors for the detection of nucleic acid sequences have attracted ever increasing interests in connection with highly demanding research efforts directed to gene analysis, clinical disease diagnosis, or even forensic applications [39, 40].

DNA hybridization, single-nucleotide mismatches, and DNA's interactions with other molecules remain popular subject comprehensive review on electrochemical DNA hybridization sensors. DNA analysis generally include: DNA's hybridization on the electrode leads to a much more crowded surface than that covered by single-stranded DNA. Also, highly populated negative charges that arise from phosphates linking DNA bases of hybridized double-stranded DNA render the interface hostile to approaching anionic-probe ion pairs, namely $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$.

Such ion pairs modulate the electrode's capability to transfer electrons to the redox probe, and charge transfer resistance (R_{ct}) values increase significantly at double-stranded DNA–covered electrodes compared to single-stranded DNA–covered electrodes [41].

The detection of DNA sequences is of great importance in a broad range of areas including genetics, pathology, criminology, pharmacogenomics, public health, food safety, civil defense, and environmental monitoring. Classical methods are often inconvenient because of their time-consuming process and the requirement for trained personnel and additional reagent preparation. As a result, significant research has been devoted to the development of DNA sensors that are sensitive, selective and relatively simple to use [42, 43].

A capture probe is an element used to recognize and bind to the target DNA. Usually, capture probes are immobilized onto a solid substrate, such as the electrode surface. However, they can also be immobilized on nanomaterials or other biomolecules. A reporter probe is a molecule that generates the signal in response to electrochemical reactions. Additional components, such as electrode coatings and intermediate molecular linkers, are also commonly integrated for improved sensor performance.

Common molecules utilized as probes include single-stranded oligonucleotides, aptamers, peptides and DNA-related proteins [44]. These compounds have been utilized for several clinical related diagnostic applications, such as the detection of cystic fibrosis, pathogenic Escherischia coli bacteria, mycobacterium tuberculosis, hepatitis B virus, the human immunodeficiency virus (HIV), and familial dysbetalipoproteinemia [45-47].

Immobilization of DNA

The self-assembled monolayers (SAM) provide a simple and well-studied method of immobilizing gold nanoparticles onto electrodes, allowing a high degree control of the composition and the thickness of transducer surface [48]. Colloidal gold-modified electrodes can be prepared by covalently tethering the gold nanoparticles with surface functional groups (–CN, $–NH_2$, or –SH) of SAMs-modified electrode surface. Short-chain molecules such as 3-mercaptoproprionic acid can be self-assembled on the modified electrode for further nanoparticle binding [49]. These molecules also provide the functional groups necessary for covalent immobilization of the enzyme. One suitable model system for fundamental studies consists of thiol-containing probes that are immobilized through self-assembly to gold surfaces, as recently employed by several investigators [50]. This system usually relies on the immobilization of a single stranded DNA (ssDNA) probe

on a surface to recognize its complementary DNA target sequence by hybridization. This original biosensor could be applied for free label detection of DNA hybridization, and offers a promising approach for rapid, sensitive and real-time solution monitoring of biological agents. The critical process in fabricating the DNA biosensor is the specific single strand probe immobilization, while the immobilization efficiency depends on the chemical functionalization in the nanoparticles.

The process of immobilization of biological molecules is one of the most important steps in the construction of a biosensor. A key issue faced with any DNA hybridization biosensor is the immobilization amount and accessibility of probe DNA for hybridization recognition [51, 52]. Increasing the immobilization amount and controlling over the molecular orientation of probe DNA would markedly improve the performance properties of DNA biosensor. It has been well elaborated that the immobilization amount and the molecular orientation of probe single-stranded DNA could remarkably influence the operational performance of DNA electrochemical biosensor [53, 54]. Therefore, numerous different immobilization strategies have been proposed and employed aimed at improving the link stability between DNA and transducer surface [55], or increasing the amount of immobilized DNA [56], and sometimes simplifying the immobilization procedure [57]. In addition to the ssDNA capture probe, a mixed monolayer comprising mercaptohexanol (MCH) and mercaptopropionic acid (MPA) is suggested as an interface design which allows a high efficiency of the DNA hybridization with a reliable modulation of the charge-transfer resistance of the electrode upon hybridization. Upon dilution of the MCH monolayer with MPA, the average thickness of the self-assembled monolayer (SAM) gets shorter. The monolayer has a less hydrophobic character and the Vander Waals interactions are abated, which leads altogether to an increase in the dielectric constant of the SAM. Moreover, molecules with an ionic character are introduced into the monolayer, which should have a pronounced impact on the potential drop due to the stronger attraction of counter ions from the solution. These changes lead to a faster drop of the potential across the binary SAM a phenomenon that becomes more pronounced if the concentration of MPA is increased with respect to MCH. If a thiol-tethered ssDNA is immobilized at a gold electrode, a negatively charged monolayer is formed which repels the negatively charged $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, causing a modulation of its diffusional access to the modified surface. It results in attenuating the redox conversion of redox reporter in dependence on the AC perturbation frequency, which is seen by EIS as an increase of the R_{ct}. Upon hybridization, the negative charge at the interface is increased leading to an even higher R_{ct} (Fig. 3) [58, 59].

Electrochemical DNA sensors

A typical electrochemical DNA sensor consists of an electrode, a capture probe and a reporter probe. A capture probe is an element used to recognize and bind to the target DNA and is usually immobilized onto a solid substrate, such as the electrode surface. However, they can also be immobilized on nanomaterials or other biomolecules. A current electrochemical sensor for DNA diagnostics are based on hybridization DNA detection uses nucleotides as the probe and targets elements. The performance of electrochemical sensors based on this method is highly dependent on the affinity between the probe and target molecules, which can be tuned by the probe design, environmental conditions, and additional amplification processing [60].

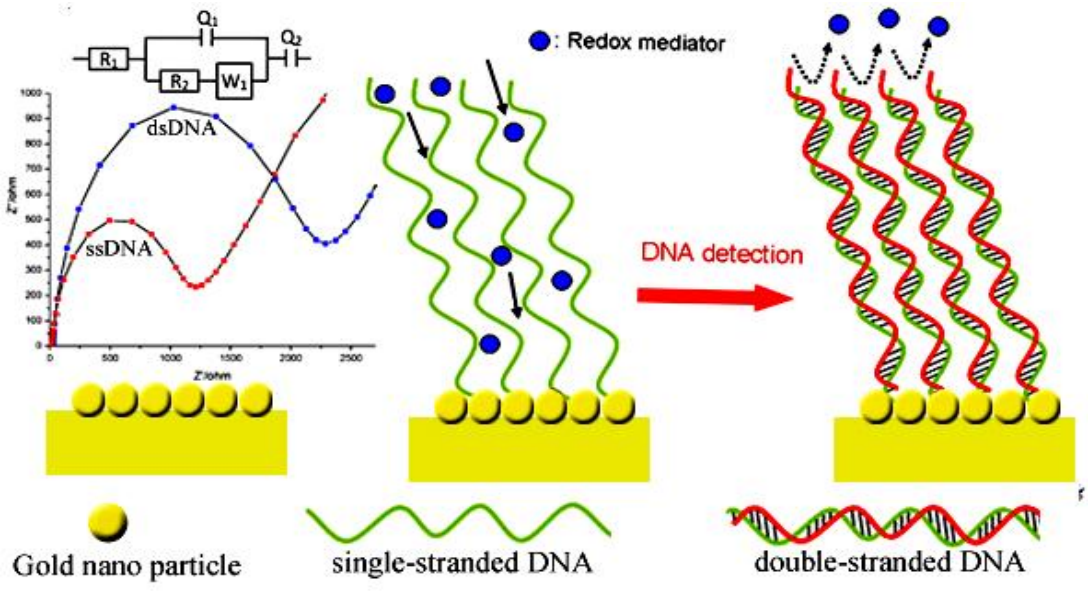

Figure 3. Schematic illustration of the fabrication steps of the electrochemical DNA nanobiosensor and modified Randle's equivalent circuit.

Electrochemical DNA sensors largely rely on nucleotide hybridization during the detection process, which involves specific electrostatic charge distributions and strong hydrogen bonding. Since the backbone of a nucleotide is composed of phosphoric acids and base units, the entire molecule is heavily charged with a negative potential. Therefore, hybridization between nucleotides needs to overcome the strong repulsion force between each other. An advantage of electrochemical sensors is their simplicity in manipulating molecules within the sample fluid, which is carried out via electrical fields generated by the electrode. Specifically, the speed of these procedures is limited by chemical reaction times and heat/mass transfer processes within the solution. In contrast, electrochemical sensors can generate high electrical fields within a very short time [61]. Additionally, thin dielectric double layers generated in high intensity electric fields can be utilized to enhance mixing and sample manipulation.

In addition, the strong hydrogen bonding within DNA makes precise control over hybridization/denaturation quite difficult [62].Various techniques including optical, electrochemistry, surface plasmon resonance spectroscopy, and quartz crystal microbalance, etc have been well developed for DNA detection [63]. Among them, electrochemistry method offers great advantages such as simple, rapid, low-cost and high sensitivity [64]. Among the various biosensors that have been employed for DNA detection, electrochemical sensors show great promise since they are capable of precise DNA recognition and efficient signal transduction. Recently, an impressive number of inventive designs for DNA-based electrochemical sensing have appeared. These sensors combine nucleic acid layers with electrochemical transducers to produce a biosensor and promise to provide a simple, accurate and inexpensive platform for patient diagnosis (Table 3).

Table 3. Comparison of biosensors for DNA electrochemical sensing.

Type of sensor	Advantages	Disadvantages	Ref.
Direct DNA electrochemistry	Highly sensitive (femtomoles of target); requires no labeling step; amenable to a range of electrodes	High background signals; cannot be multiplexed; destroys the sample	65, 66
Indirect DNA electrochemistry	Highly sensitive (attomoles of target); usually requires no labeling step; multiple-target detection at same electrode	Probe substrate can be difficult to prepare; destroys the sample	67, 68
DNA-specific redox in director detection	Moderate to high sensitivity (femtomoles of target); well suited to multiple-target detection; samples remain unaltered	Chemical labeling step required unless 'sandwich' method used; sequence variations can be problematic	69, 70
DNA-mediated charge transport	Highly sensitive (femtomole range) and simple assay; requires no labeling; uniquely well suited to mismatch detection; sequence independent; amenable to multiplexing; applicable to DNA-protein sensing step	Biochemical preparation of target sample required	71, 72
Nanoparticle-based electroche-mistry amplification	Extremely sensitive (femtomole to zeptomole target, 10^{-15} to 10^{-21} moles); well suited to multiple-target detection with different nanoparticles	Many development steps in assay; reliability and robustness of surface structures problematic; sample usually de-stroyed	73, 74, 75

A convenient electrochemical technique is electrochemical impedance spectroscopy (EIS), which is widely applied in DNA detection. Li et al. were first reported the impedance label-free detection of DNA hybridization [76]. In recent years, the number of studies involving electrochemical impedance spectroscopy (EIS) in bioanalysis has increased significantly. EIS belongs to the most sensitive tools for the study of interfacial phenomena upon surface modifications [77, 78]. According to previous studies, EIS has been successfully applied for the detection of DNA conductivity [79], DNA hybridization [80, 81], single-nucleotide mismatches in double-stranded DNA (dsDNA) [82], in situ hybridization of PNA/DNA, DNA and RNA replication [83, 84] and the interaction of metal ions with DNA [85, 86].

Electrochemical DNA Nanobiosensors

The nanobiosensors offer several advantages including limited hands-on time, high-throughput screening, improved detectability, real-time analysis and label-free detection methods and devices over existing techniques.

The application of nanotechnology for the advancement of biosensor leads to an efficient nanobiosensor with miniature structure as compared to conventional biosensors. Nanobiosensors can be effectively used for sensing a wide variety of biological agents [87]. Nanomaterial are important elements in impedance-based DNA sensors, especially those that poses semiconductor properties [88, 89]. Such materials enhance the sensor performance through increasing the electrode surface area, electrical conductivity and connectivity, chemical accessibility and electrocatalysis (Fig. 3).

The most widely used nanomaterial in impedance sensors are Au nanoparticles and carbon nanotubes (CNTs), which amplify the impedance signals by forming nano-particle-biomolecule conjugates in a solution phase.

A wide variety of nanomaterial can be applied, where the most common include metal nanoparticles, cadmium sulfide nanoparticles, carbon nanotubes and silicon nanowires.

An extensive and detailed review on the applications of nanomaterials for DNA biosensors can be found in literature [90, 91]. Nanoparticles play most important roles in the development of nanobiosensor because:

1) Nanoparticles exhibit higher ratios of surface area to volume than their bulk counterparts, so nanoparticle modified electrochemical interfaces will provide larger electrochemically active areas and therefore probably lead to higher detection sensitivity for target molecules;

2) Some novel nanoparticles, particularly metal nanoparticles, can easily act as enhancing agents for effective acceleration of electron transfer between electrode and detection molecules, so leading to more rapid current response for target molecules;

3) Nanoparticles can act as a supramolecular assembling unit with advanced functional properties for constructing a variety of architectures on the surface of electrodes and further tailoring of an electrochemical-sensing interface;

4) Nanoparticles can be conjugated with some important biomolecules and also act as nanoconnectors that activate redox enzymes or electrical labels for biorecognition events; and, 5) nanoparticle-modified electrochemical interfaces behave as nanoelectrode ensembles [92]. Inspired by these important features, literature already shows numerous examples of incorporating nanoparticles into biosensors. So far, there are nanobiosensors for the specific detection of biologically-relevant molecules (e.g., nucleic acids [93], proteins [94] and enzymes [95] and for the detection of infectious agents [96].

Characteristics for Ideal Nanobiosensors [97]

- Highly specific for the purpose of the analyses *i.e*. a sensor must be able to distinguish between analyte and any "other" material.
- Stable under normal storage conditions.
- Specific interaction between analytes should be independent of any physical parameters such as stirring, pH and temperature.
- Reaction time should be minimal.
- The responses obtained should be accurate, precise, reproducible and linear over the useful analytical range and also be free from electrical noise.
- The nanobiosensor must be tiny, biocompatible, non-toxic and non-antigenic.
- Should be cheap, portable and capable of being used by semi-skilled operators.

Advantages of Nanobiosensors over Conventional Biosensors

- These sensors are ultra-sensitive and can detect even ultra-low concentrations of a substance that could be potentially harmful.
- Nanobiosensors works at atomic scale with highest efficiency.
- Nanobiosensors also have increased surface to volume ratio.

The nanobiosensors also have disadvantages such as:

- Nanobiosensors are very sensitive and error prone.
- Nanobiosensors are still under infancy stage and in the study and research.

Advancements in nanotechnologies, specifically fabrication techniques and new nanomaterial, have enabled for the development of highly sensitive, highly specific nanobiosensors which makes them attractive for the detection of small sequence variations and biological agents.

Future Perspectives

Biosensors are a prospective alternative to traditional analytical methods and probably one of the most promising ways to solve some problems concerning simple, fast, repetitive, and cheap detection of biological agents. Electrochemical biosensors, in particular amperometric ones that maintain a superior sensitivity and better linear range than the potentiometric devices, have an important position among biosensors as a class of the most widespread, numerous, and successfully commercialized devices based on biomolecular electronics. Furthermore, they have some advantages over optical devices, such as low cost, simple operation, small size; they are disposable and incorporate multiple sensing elements in a single chip like device. The introduction of nanomaterials into electrochemical sensors and biosensors certainly brings advantages such as: decrease over potentials of many analytically important electrochemical reactions, ensure the reversibility of some redox reactions, which are irreversible at unmodified electrodes or bring novel labelling opportunities including multidetection capabilities [98]. The immobilization of DNA to the surface of the working electrode is a significant step in the fabrication of the DNA biosensor [99]. Electrochemical Impedance Spectroscopy (EIS) could provide further information on the impedance changes of the electrode surface during the modification process. In EIS, the semicircle diameter could represent the charge transfer resistance, R_{ct}, which dominates the electron transfer kinetics of the redox probe at the electrode interface [100].

Even though there are a lot of research works reported for electrode modification by different nanomaterials to improve the DNA biosensor performance, the preparation of nanomaterials or the electrode modification strategy is often relatively complex. Sometimes, the introduction of some organic molecules for example surfactant or polymer matrix in the preparation or assembly of nanomaterials often causes some uncertain effects in DNA detection. Furthermore, some DNA biosensors based on nanomaterial modification are still very limited for the improvement of DNA biosensor performance. Thus, the construction of nanostructure modified electrode by a simple strategy to improve the DNA detection sensitivity is highly desirable. Among all kinds of nanomaterials, gold-based nanomaterials were the mostly used ones for the fabrication of electrochemical biosensor owing to its easy and rich surface function strategies and good biocompatibility [101, 102].

Recently nanobiosensors, implanted biosensors and integrated biosensors are in current research and development. In the past 40 years various biosensors have been researched and developed encompassing a wide range of applications but the number of commercially available biosensors is limited. Hence Biosensor technology is an upcoming field and with continued progress, we can expect that biosensors can become simpler and more widely available commercially. With the abundant

literature about the DNA biosensors, it seems clear that electrochemical biosensors for selective DNA sequence detection have a very promising future.

Conclusion

Conventional identification methods for biological agents are the culture and colony counting, immunological techniques and polymerase chain reaction (PCR). In addition to culture based methods, techniques such as immunoassays, enzyme assays, and nucleic acid probe assays have been developed for the identification of biological agents. Unfortunately, despite these approaches being powerful and error-proof, most of them are laborious, complex and time consuming, and they do not afford the necessary detectability and specificity towards the target. As alternative to the conventional methods, nanobiosensor techniques have triggered strong interests for its simpler nucleic acid assays and faster, cheaper process [103].

The nanobiosensors provide reliable, rapid, quantitative, cheap and high throughout detection of biomolecules and have been extensively used in different fields. Highly sensitive biosensing methods are urgently needed for measuring different markers present at ultra-low levels. Small, fast and high-throughput devices are also highly desired for replacing time-consuming laboratory-analyses to realize the screening of large population. The emergence of nanotechnology opens new horizons for the development of highly efficient biosensing strategies, nanoprobes, nanosensors and nanosystems suitable for fast, selective, sensitive and convenient detection of biomolecules. Functional nanomaterials offer improved biocompatibility, additional binding sites and high signal intensity. Hence, electrochemical DNA is being gradually applied to nanobiosensors for markedly enhancing the sensitivity and specificity of biological agent detection. According to the results obtained is determined which to study the hybridization process of DNA/DNA, the method of electrochemical impedance spectroscopy is an appropriate sensitive and efficient technique. The use of nanoparticles for the fabrication of nanostructured gold electrode modified with DNA is a simple strategy for improving the detection sensitivity. With further development and integration of emerging technologies, electrochemical DNA sensors will become more prominent biological agent detection. The increasing advancement of miniaturization and nanomaterials research has stimulated the application of these materials for sensing several key pathways and regulatory events.

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