Disposable biosensors for clinical diagnosis

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Disposable Biosensors for Clinical Diagnosis

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We present an overview on the use of disposable electrochemical biosensors for diagnostics, focusing on the applications of these devices as immunosensors and DNA sensors in the point-of-care diagnostics. Analytical biosensors have emerged as efficient alternatives for the detection of innumerable diseases, because of their high specificity and the convenience of detecting the electrochemical signals produced by the presence of an analyte using a portable equipment. This review highlights the recently developed strategies toward immobilization of different biological molecules on disposable electrodes such as carbon nanotubes and metal nanoparticles. In the course of the review, we first introduced the disposable biosensors, followed by an overview of the immunosensors, and discussed the applications of DNA sensors and disposable biosensors in point-of-care diagnostics. We also have evaluated the prospects and future applications of these devices in the field of biomedical research.

Keywords: Nanomedicine, Nanotoxicology, Electrochemical Biosensors, Disposable Electrodes, Biomedical Analysis, Screen-Printed Electrodes.

1. INTRODUCTION

Biosensors are analytical devices that find applications in different areas, including clinical analysis as well as food and environmental control. The benefits of using biosensors can be attributed to their simple preparation procedures, relatively low cost, versatility, and selectivity for quantification of various compounds of interest. Furthermore, the miniaturization of detection systems and development of disposable sensor units have also attracted considerable attention.1,2

A biosensor is a highly selective recognition device, wherein a transducer transforms the signal generated in the reaction of the analyte with a biological element such as an immobilized enzyme, antibody/antigen, plant or animal tissue, or a microorganism into an electronically measurable signal.3-15 Various analytes of environmental, medical, biological, pharmaceutical, or industrial interest can be monitored using an electrochemical biosensor, which provides a measure of the analyte concentration by detecting the current generated in the oxidation or reduction of the redox species.16 In electrochemical biosensors, the biological material is directly immobilized on a electrode by adsorption, covalent bonding, or encapsulation within a coating layer of a permeable conductive polymer or cross-linked reagent.17,18 These diverse strategies of immobilization lead to biosensors with different architectures such as monolayer,19,20 multilayers21 and thin films.22,23

Though a number of electrochemical biosensors have been developed using nondisposable electrodes like glassy carbon,24 boron doped diamond electrodes,9 or pyrolytic carbon25 as the transduction platform, disposable biosensors with screen-printed chip, indium tin oxide (ITO), and pencil graphite electrodes are extensively used in medical diagnoses.26 These devices are inexpensive, easy to fabricate, and can be effectively used for routine analysis. Development of new architectures for biosensors employing nanostructured materials including carbon nanotubes,27,28 metal nanoparticles,29-34 and graphene35 have attracted considerable interest.36 Immobilization of biological molecules on these electrode platforms reduces

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Review

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the overpotential by promoting electron transfer, resulting in an improved analytical response.

Recently, the applications of several disposable electrochemical biosensors such as DNA biosensors and immunosensors in medical analyses have been proposed.\(^{37-39}\) In this paper, we present a review of the disposable electrochemical biosensors for medical analyses based on the reported developments over the recent 5 years, with emphasis on their design, materials and methods for construction, as well as their applications in medical areas.

2. DISPOSABLE IMMUSENSORS

Immunosensors are a class of biosensors based on the transduction of signals generated in antigen-antibody interactions. Design and preparation of an ideal interface between the biomaterial and the detector is a fundamental requirement for the development of immunosensors.\(^{40}\) The antigen of interest is investigated by detecting the labeled antibodies and/or labeled antigens after the formation of antigen-antibody complexes.\(^{39}\) These devices are advantageous because of their fast response and simplicity of operation. Two types of voltammetric and amperometric immunosensors are known, which employ “sandwich”-type and competitive-type immunoassays, as shown in Figure 1. Impedimetric immunosensors also determine the concentration of the antigen of interest by detecting the impedance generated on the addition of antibodies.

In sandwich-type immunosensors,\(^{41}\) the primary antibodies first bind to the transducer material, followed by the addition of antigens, and finally, the enzyme-labeled antibodies react with the substrate to form antigen-antibody complexes. Competitive-type immunosensors, on the other hand, are based on the competitive interactions of labeled and unlabeled antigens with the substance of interest reacts with the substrate.\(^{42}\) In this case, binding of the primary antibodies to the transducer material is followed by the addition of enzyme-labeled antigens to form antigen-antibody complexes. The quantification is performed by the controlled addition of labeled and unlabeled antigens.

Several disposable electrochemical immunosensors using different electroactive materials such as conducting polymers,\(^{43}\) graphite,\(^{44}\) carbon nanotubes,\(^{45}\) or metals,\(^{46,47}\)
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Fig. 1. Immunosensors employing sandwich-type (A) and competitive-type (B) immunoassays. In (A), the primary antibodies first bind to the transducer material, followed by the addition of antigens, and finally, the enzyme-labeled antibodies react with the substrate to form antigen-antibody complexes. In (B), the immunosensors are based on the competitive interactions of labeled and unlabeled antigens.

have been reported in the literature. A number of disposable immunosensor arrays have been developed for the electrochemical determination of tumor markers. Recently, a disposable immunosensor array was fabricated using cellulose acetate membrane, thionine, and two types of antigens immobilized on carbon electrodes of a screen-printed chip. Several tumor markers were simultaneously investigated by a multianalyte test with a direct capture format, using horseradish peroxidase-labeled antibodies immobilized on screen-printed carbon electrodes modified with gold nanoparticles. This system can be applied in the accurate, point-of-care detection of carcinoma antigen 153, carcinoma antigen 125, carbohydrate antigen 199, and carcinoembryonic antigen in clinical serum samples with detection limits of 0.2 kU L\(^{-1}\), 0.5 kU L\(^{-1}\), 0.3 kU L\(^{-1}\), and 0.1 \(\mu\)g L\(^{-1}\), respectively.

Disposables sensors have been proposed by Ho et al. and Viswanathan et al. for the detection of the carcinoembryonic antigen, a tumor marker. In the former, the authors have proposed a sensitive electrochemical immunosensor based on a carbon nanoparticle-modified screen-printed graphite electrode covered with specific antibodies, using Cds nanocrystals as bioracers as a strategy for signal amplification. The system has shown promise for home-care self-diagnostic tool, with a detection limit of 32 pg mL\(^{-1}\). In the latter paper, the authors used ferrocene liposomes and multiwalled carbon nanotubes screen-printed electrode to detect carcinoembryonic antigens. They have employed square wave voltammetry to study the redox response of the released ferrocene carboxylic acid from the immunoconjugated liposomes present on the electrode surface. A detection limit of 1.0 pg mL\(^{-1}\) was reported.

Lin et al. have been reported a competitive electrochemical immunosensor to detect polychlorinated biphenyls (labeled with horseradish peroxidase), which performed a sensitive immunoassay based on disposable screen-printed electrodes modified magnetic beads with a detection limit of 10 pg mL\(^{-1}\). A new sensitive electrochemical immunosensor has been proposed by Huang et al. to detect the carcinoembryonic antigen using a composite electrode containing Au nanoparticles, multi-walled carbon nanotubes and chitosan. The electrode presented a detection limit of 0.01 ng mL\(^{-1}\), which can be applied in the diagnosis and in the monitoring of this type of carcinoma.

A modern and simple electrochemical immunosensor based on paper sensors with an addressable electrode array has been proposed by Ge et al. The authors designed a label-free disposable electrochemical biosensor modified with gold nanoparticles for the detection of interleukin-12, an important analyte for diagnosis of multiple sclerosis. Lai et al. designed an electrochemical immunosensor (Fig. 2) using glucose oxidase-functionalized antibodies immobilized on screen-printed carbon electrodes modified with Prussian blue and gold nanoparticles attached to carbon nanotubes, for the sensitive simultaneous multiplexed detection of tumor markers. Another immunosensor was developed by Lai et al. for the multiplexed detection of tumor markers, using covalent immobilization of antibodies on chitosan-modified screen-printed carbon electrodes containing silver and gold nanoparticles.

An inexpensive and efficient immunosensor was designed for detection of cancer biomarker proteins using the gold-covered side of compact discs, on which the authors prepared a immunoarray comprising 8 electrodes into a microfluidic device. The authors carried out a sandwich immunoassay for interleukin 6 detection that exhibited a detection limit of 10 fg mL\(^{-1}\). Ho et al. have developed an electrochemical sandwich immunosensor for the detection of the antigen \(\alpha\)-enolase-associated with human lung cancer-based upon the adsorption of anti-\(\alpha\)-enolase monoclonal antibody on polyethylene glycol-modified disposable screen-printed electrodes (Fig. 3).
The detection system exhibited a detection limit of 2.38 pg mL\(^{-1}\). Disposable arrays have been also developed for the detection of prostate-cancer-specific antigens, using magnetic-microparticle-modified antibodies coupled to multiplexed electrochemical screen-printed electrodes.\(^{60}\) Arrays containing 8 electrodes have been prepared using antibody-modified magnetic microparticles to attain a sensitive immunosensor with a detection limit of 1.4 ng mL\(^{-1}\).

Screen-printed carbon electrodes have several advantages because of their reasonable price and usability in a wide range of potentials as well as in several media. The applications of screen-printed carbon electrodes in the detection of a variety of proteins such as phosphorylated acetyl cholinesterase,\(^{61}\) Shigella flexneri,\(^{62}\) urine albumin,\(^{63}\) mycotoxins ochratoxin A,\(^{64}\) and in the determination of Escherichia coli\(^{65}\) have been reported. Using a similar strategy, Zhang et al. have proposed an electrochemical immunosensor for the determination of microcystin-LR using a double-sided microporous gold electrode in cartridge-type cells.\(^{66}\) Presenting a detection limit of 100 pg mL\(^{-1}\), the biosensor was applied in the determination of relevant analytes in environmental samples.

An impedimetric electrochemical immunosensor based upon printed circuit electrodes\(^{67}\) was developed and applied in the determination of interleukin-12. The immunosensors could detect interleukin-12 at physiological levels. Konstantinov et al. have been designed a portable electrochemical immunosensor for the rapid detection of anti-chromatin autoantibodies in systemic lupus erythematosus in human serum.\(^{68}\) Nonspecific immunoglobulin was detected showing promising applicability of this simple and low-cost sensor. Chen et al. have been fabricated an immunosensor to detect \(\alpha\)-fetoprotein, using screen-printed carbon electrodes, anti-\(\alpha\)-fetoprotein antibodies and gold nanoparticles conjugated to primary amine functionalized polypyrrole (PDA).

**Figure 2.** Schematic representation of a carbon nanotube-based immunosensor, as proposed by Lai et al. Reprinted with permission from [56], G. S. Lai, et al., Anal. Chem. 81, 9730 (2009). © 2009, American Chemical Society.

**Figure 3.** Operation of an electrochemical immunosensor for detection of Human Lung Cancer-Associated Antigen, \(\alpha\)-enolase. Reprinted with permission from [50], J. A. A. Ho, et al., Anal. Chem. 82, 5944 (2010). © 2010, American Chemical Society.
antibody, and glass fiber membranes conjugated with ferrocene monocarboxylic acid-labeled α-fetoprotein in order to develop a robust sensor for point-of-care testing. Detection limits as low as 6.0 ng mL$^{-1}$ were achieved.

Disposable electrochemical immunosensors can also be used in the detection of bacteria. For example, a very sensitive immunosensor based on the electrochemical enzymatic immunoassay using thiolated antibodies immobilized on a gold surface has been proposed for the detection of *Staphylococcus enterotoxin B.* The authors studied and compared some crosslinking agents for immobilization of the capture antibody onto the gold electrode. The immusensor was very specific and under the optimum parameters, it exhibited a detection limit of 1 ng mL$^{-1}$. Immunosensors have also been developed for the detection of *Staphylococcus aureus* in milk samples. In this case, a detection limit of 1.4 × 10$^7$ cells mL$^{-1}$ was reported.

Zhao et al. proposed a new electrochemical immunosensor based on horseradish peroxidase-labeled antibodies for detection of *Shigella flexneri*. The very low detection limit exhibited by the sensors, viz.: 3.1 × 10$^3$ cfu mL$^{-1}$, revealed the great potential of these systems for technological applications.

Also important to note is that a number of immunosensor devices have also been used effectively in the determination of various hormones in human samples, such as cortisol (Fig. 4), testosterone, human growth hormone (hGH), adrenocorticotropic, prolactin, and estradiol. Regarding the field of cardiology, immunosensor devices have been employed for predicting the risk of fatal cardiovascular diseases by sensing the nonspecific biomarker C-reactive protein. Silva et al. have designed an immunosensor to detect cardiac troponin T, a specific biomarker for acute myocardial infarction. In the latter study, the surface of screen-printed carbon electrodes was modified with streptavidin polystyrene microspheres to enhance sensitivity. The sensors presented a detection limit of 0.2 ng mL$^{-1}$ with potential for applications in point-of-care diagnostics. An immunosensor for the detection of protein P-selectin, associated with cardiac diseases, has also been reported by Ho et al. In this case, the authors developed a simple, disposable electrochemical sandwich immunosensor using a screen-printed carbon electrode modified with carbon nanotubes and gold nanobones. Detections limits of 0.85 pg mL$^{-1}$ have been reported.

### 3. DISPOSABLE DEVICES FOR DNA DETECTION

The rapid progress of nanomedicine, especially in the field of medical diagnostics, has motivated the development of new devices and/or arrays which can be combined with biological materials for specific medical applications. Genosensors based on electrochemical detection have been a promising development in this area. These analytical devices exhibit high specificity and sensitivity and can be applied for the early detection of many genetic diseases, in pathology, food safety and in various other fields. Disposable genosensors have gained considerable importance in analytical chemistry since they employ DNA hybridization for the early diagnoses of diseases by detecting the oxidation signal of guanine/adenine or by using the intercalation of dsDNA and ssDNA in a variety of complexes containing aromatic condensed rings (antibiotics like clonbuterol or metal coordination complexes such as [Co(phen)$_3$]$^{2+}$ or [Ru(bpy)$_3$]$^{2+}$).

The DNA bases, linked via hydrogen bonds, are located on the inner side of the double helix and, hence, are inaccessible for reaction. The intercalators are used to overcome the hindrance of electron transfer from the interior of the double-stranded DNA to the electrode surface. An increasing number and variety of disposable DNA sensors have been developed in the recent years using new materials as transducers for specific applications such as gene polymorphism analysis. Owing to the high sensitivity and compatibility with microfabrication chip technology, carbon-based nanomaterials, including graphite, carbon nanotubes or graphene have been used to produce disposable genosensors. In most of these devices, carbon electrodes were coupled with an electrochemical impedance spectroscopy (EIS) system and DNA molecules to improve the detected signal. EIS generates electric signals in the frequency domain, from the interactions of the immobilized biomolecules, thereby improving the sensitivity of detection. The characterization and hybridization of single- or double-stranded DNA layers can be performed by measuring the current passing through an electrochemical cell after the application of an AC potential. Faradaic and non-Faradaic impedance spectroscopy analyses are used to correlate the impedance signals to different diseases.

Disposable genosensors are used to detect pesticides, microbiological diseases, toxic algae, and untreated raw biological samples like serum and urine. DNA sensors based on guanine oxidation have been reported.
to detect pesticides such as malathion and chlorpyri-fos by monitoring changes in the DNA redox properties or by employing an electro-active analyte intercalated on a DNA layer.96 Pencil graphite,96, 101, 102 carbon nanotubes modified with graphite,103–105 and screen-printed graphite electrodes106, 107 were employed to monitor DNA hybridization in specific sequences of Hepatitis B and C virus for early diagnoses of the infectious agents.108

Understanding the binding interactions of DNA with the drug molecules is an important area of research in pharmaceutical development.109 Erdem et al. developed a sensitive sepiolite-carbon nanotube device for the electrochemical detection of DNA and mitomycin C, an anti-cancer antibiotic drug used in clinical chemotherapy. The sepiolite mineral and carbon nanotubes improved the electroactive surface area of the sensor electrodes and thereby emerged as good candidates for clinical applications.110 Likewise, graphene oxide was integrated in an electrochemical device,111 which resulted in a decrease in the guanine signal intensity due to the interaction of mitomycin C with DNA molecules, catalyzed by graphene. Particular attention has been paid to the development of disposable sensors capable of monitoring the interaction between DNA and quinazoline molecules. These devices have been used in various clinical applications as antimalarial agents and in cancer treatments.91, 112

Screen-printed carbon electrodes decorated with nano-structures were used in the detection of bacterial pathologies. The use of gold nanoparticles as the immobilization and transduction surface enhanced the sensitivity and detection limit of the genosensor developed by Martinez-Paredes, for the detection of bacteria associated with respiratory tract infections such as Mycoplasma pneumonai e, Legionella pneumophila, Chlamydophila pneumonai e, and Streptococcus pneumonia.113 Oligonucleotide molecules were adsorbed onto the screen-printed electrodes using mercaptohexanol as a spacer in the detection of enterobacteriacea e lac Z, which is an indicator of faecal contamination.114 A disposable magnetic genosensor was also proposed by the same group of researchers for the analysis of Escherichia coli DNA fragments by enzyme-amplified coupling of streptavidin-peroxidase to immobilized biotinylated lacZ gene target sequences.115 Loaiza et al. modified the above architecture using a magnetic field on the surface of a home-made carbon screen-printed electrode, as shown in Figure 5.116 Escherichia

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**Figure 5.** Illustration of the magnetic genosensor developed by Loaiza, et al.: (1) formation of magnetic Fe@Au nanoparticles; (2) modification of Fe@Au with the thiolated probe; (3) hybridization with the biotinylated target; (4) enzymatic labeling with streptavidin-HRP; (5) hybrid-modified magnetic bead deposition on the SPEs; (6) voltammetric detection of the mediated reduction of hydrogen peroxide with hydroquinone. Reprinted with permission from [116], O. A. Loaiza, et al., Biosens. Bioelectron. 26, 2194 (2011). © 2011, Elsevier B.V.
coli pathogen was used to develop a zirconium-modified screen-printed genosensor. Methylene blue molecule was employed as the redox indicator to analyze DNA immobilization and hybridization.\textsuperscript{117}

One of the most interesting disposable DNA sensors was developed by Ahmed et al. by combining isothermal amplification with an electrochemical system to detect a modified \textit{cry9c} gene in a corn line\textsuperscript{118} (Maize line CBH 351 trade name StarLink\textsuperscript{TM}), banned for human consumption. The products of loop-mediated isothermal amplification were combined with a redox active molecule, Hoechst 33258, and analyzed by a DNA stick integrated within an electrochemical device as demonstrated in Figure 6. DNA binding induced by Hoechst 33258 molecule, along with changes in the anodic current peak, were used to detect the modified \textit{cry9c} gene variety with high efficiency.\textsuperscript{118}

Disposable sensors were developed to detect the genetic polymorphism for the purpose of diagnosis prior to the onset of the disease symptoms; however, the sensibility and selectivity of these devices still pose a challenge. Kara et al. developed a genosensor capable of the allele-specific electrical detection of toll-like receptor-2 gene \textit{arg753gln} polymorphism.\textsuperscript{119} The DNA probe was covalently immobilized on a disposable graphite electrode surface. The biosensor was able to detect the complementary sequence and single nucleotide polymorphism in PCR amplified real samples, using Meldola blue molecule as the hybridization label. Development of analytical tools for the real time detection of undesirable molecules in foodstuff is of utmost importance in food industry. In order to investigate the presence of hazelnut in foodstuffs, an electrochemical DNA array combined with polymerase chain reaction (PCR) has been designed using a gold screen-printed electrode. The results were compared with those obtained from enzyme-linked immunosorbent assay (ELISA) tests, which indicated the applicability of the device for semi-quantitative analysis.\textsuperscript{120}

Low et al.\textsuperscript{121} developed a disposable horseradish peroxidase based genosensor for the chronoamperometric detection of single-stranded asymmetric \textit{lolB} gene from PCR amplicon (118 bp in length), present in the food-borne pathogen, \textit{Vibrio cholera} causing the diarrheal disease cholera. The analytical evaluation of the assay using 19 bacterial strains showed 100% specificity and a detection

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**Figure 6.** Detection of modified \textit{cry9c} gene using an electrochemical sensor: (a) isothermal amplification solution (LAMP) is placed into the amplification part of the chip along with the target DNA; (b) chip detection site where the Hoechst 33258 molecule and buffer mixture are added; (2) LAMP solution is placed on the heat block at 65 °C for DNA amplification; (3) valve is distorted to facilitate the mixing of fresh amplicon and Hoechst 33258 in buffer solution; (a) mixing of amplicon, Hoechst 33258 molecule, and buffer by mild shaking with hand; (4b) opening of the valve in the chip; (5) inversion of the chip for electrochemical detection; (6a) connection of the chip to a potentiostat with the help of a connector; (6b) schematic diagram exhibiting the interaction of DNA with Hoechst 33258, on the DEP chip surface made of polypropylene. Reprinted with permission from [118]. M. U. Ahmed, et al., \textit{Analyst} 134, 966 (2009). © 2009, Royal Society of Chemistry.
Genosensor devices have been implemented in the detection of polymorphism of real samples, for example, in the detection of functional polymorphism in the catechol-O-methyl transferase (COMT) gene from PCR amplicons. Single nucleotide polymorphism (Val108/158Met) in the COMT gene leads to various psychiatric disorders such as schizophrenia, alcoholism, bipolar disorder, and obsessive-compulsive disorder. The Val108/158Met COMT genotype detection was performed on real samples containing healthy controls, and polymorphic and PCR products. The detection limit of the biosensor was 2.4 pmol. A single-stranded oligonucleotide probe was immobilized to detect the mutation in the COL4A5 gene causing Alport syndrome, which is a progressive renal disease affecting cochlear and ocular organs.

Komarova et al. developed a disposable multispecific array based on the electrochemical impedance measurements, using eight gold electrodes connected with a built-in counter electrode and a reference electrode holder, as demonstrated in Figure 7. The array allowed the incorporation of multiple negative controls in the course of a single binding experiment, and parallel experiments were performed to improve the reliability of detection. These features extend the application of these disposable point-of-care sensors in biomedical research.

Yamanaka et al. demonstrated the use of a continuous-flow microfluidic RT-PCR chip connected with an electrochemical device for the rapid amplification and sensing of influenza (AH1pdm) virus. As observed in Figure 8, the RT-PCR chip consisted of four distinct zones: RT reaction zone, initial denaturing zone, thermal cycle zone, and pressurizing-channel zone. Methylene blue molecules were used as DNA intercalators for electrochemical measurements. These devices were integrated into a microfluidic system and directly loaded onto the DEP chips.

The development of these disposables led to significant progress in the design of genosensors for the detection of specific nucleotide sequences in nucleic acids of clinical, food, and environmental interest. However, the need for robustness and miniaturization of the devices poses a challenge in the development of these disposable devices.

4. POINT-OF-CARE DIAGNOSTICS

Point-of-care testing (POCT) involves rapid diagnostic tests carried out at or near the site of patient care to obtain immediate results. This type of diagnostic tool is highly beneficial in health management because of the rapid disease diagnosis or dysfunctions. Disposable electrochemical sensors and biosensors have significant applications as diagnostic tools in the point-of-care testing. The use of several disposable biosensors in the point-of-care assessment of acetylcholinesterase, bacteria, cancer biomarkers, and DNA targets has been reported in the literature. A portable sensor system comprising sixteen electrodes for DNA detection is illustrated in Figure 9.

Rowe et al. have designed a ribonucleic acid-based biosensor to detect gentamicin, an aminoglycosic antibiotic. The reported electrochemical biosensors comprised an array of 32 gold electrodes that exhibited rapid responses for point-of-care diagnostics in blood serum samples. An electrochemical immunoassay for detection of hippuric acid, one of major metabolites in toluene-exposed humans, has been proposed by Yoo et al. The authors used a portable microfluidic chip with integrated microelectrodes made by screen-printing hydrophilic carbon ink with the possibility of application as point-of-care systems.
Different strategies have been proposed for the detection of cardiac biomarkers under the concept of point-of-care diagnostics\textsuperscript{129–135}. For example, Jagadeesan et al. have designed a low-cost and rapid method for the modification of paper electrodes with conducting polymers for the amperometric detection of troponin\textsuperscript{138} for point-of-care diagnosis. Polyaniline was deposited on the patterned screen printed paper electrodes by electrodeposition, followed by immobilization of anti-cardiac troponin-I using 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride and N-hydroxysuccinimide coupling. A sensitivity of 5.5 \( \mu \text{A/ng mL}^{-1} \text{cm}^{-2} \) was achieved in a physiological range of 1–100 ng/mL.

Suprun et al. have been proposed an electrochemical biosensor to quantify the myocardial infarction biomarker myoglobin in human plasma\textsuperscript{139}. The method was based on the direct electron transfer between Fe(III)-heme and the transducer surface. The system comprised a screen-printed graphite electrode modified with gold nanoparticles, didodecyl[3-dimethylaminopropyl]carbodiimide hydrochloride and N-hydroxysuccinimide coupling. A sensitivity of 5.5 \( \mu \text{A/ng mL}^{-1} \text{cm}^{-2} \) was achieved in a physiological range of 1–100 ng/mL.

As described by Wu et al. aptamers are artificial, short, single-stranded DNA/RNA oligonucleotides isolated from random-sequence nucleic acid libraries by an \textit{in vitro} evolution process called \textit{systematic evolution of ligands by exponential enrichment} (SELEX).\textsuperscript{141} These oligonucleotides have attracted considerable attention in the recent years as they can recognize different targets with specific properties and hence can be used as an economic, alternative detection tool in place of the traditional protein antibodies. Indeed, McMullan et al. proposed the application of an aptasensor as a highly sensitive, point-of-care diagnostic tool for the detection of the blood clotting enzyme thrombin, using covalently attached aptamers on a gold electrode.\textsuperscript{142} An electrochemical aptasensor based on graphene-3,4,9,10-perylenetetracarboxylic dianhydride and functionalized hollow PtCo nanochains as enhancers was developed for the ultrasensitive detection of thrombin.
in clinical screening and point-of-care diagnostics. In order to detect the influenza virus H1N1, a virus affecting population across the world, an electric immunoassay using immunochip modified with carbon nanotubes was designed by Lee et al. A detection limit of 180 TCID$_{50}$/ml of swine influenza virus (SIV) was achieved.

5. FINAL REMARKS AND PERSPECTIVES

Disposable electrochemical sensors are of considerable interest because of their efficient application in obtaining specific information in a faster, simpler, and cost-effective manner, as opposed to the traditional analytical methods. Although significant progress has been attained in the development of disposable electrochemical devices for medical application in recent years, this field of research is still at an early stage because of the challenges with regard to stabilization of the biological molecules at the platform and the miniaturization of the disposables without loss of specificity and selectivity. Recent research has focused on the development of miniaturized devices capable of mismatch discrimination and the use of transducers for improving sensitivity and selectivity by signal amplification. To conclude, considerable attention should be directed toward the development of immobilization procedures and the use of nanomaterials as graphene and nanoparticles as transducers for signal amplification. Integration of multiple devices on a single disposable platform should lead to significant advantages in terms of cost and speed of the detection of a specific disease. It is expected that the increased application of nanomaterials in the electrochemical field will lead to the development of new disposables for the detection of different types of cancers, along with other diseases like malaria, leishmaniasis and dengue. Future research should emphasize on the development and application of simple analytical devices for early diagnosis in medical practice.

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