Electronic Materials, Devices, and Signals in Electrochemical Sensors

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***Abstract*— The development of electrochemical sensors and biosensors have attracted great attentions in both fundamental studies and practical applications in various areas, including healthcare, the environment, and defense. One of the major challenges for the development of these sensors is the successful integration of electronic materials, device configurations, and signals on the devices. In recent years, there have been several a number of electronic materials, a few types of device configurations, and a few types of signal collection methods that have been used to construct electrochemical sensors. To successfully construct an electrochemical sensor for a target analyte, there are a few interesting questions: Which device configuration should be chosen? What electronic materials should be used to construct the device? How are the signals generated? Which type of signal should be used for the detection? This review will highlight the early attempts to address these questions and explore the fundamental and practical aspects for different types of electrochemical sensors.**

***Index Terms*— Biosensors, devices, electrochemical, electronic materials, sensors, signals.**

I. Introduction

AN ELECTROCHEMICAL sensor or biosensor is an analytical device used for the detection of a target analyte. Electrochemical sensors are widely studied because of their ability to detect a variety of target analytes in healthcare, the environment, and defense [1]–[5]. It has the advantage of being portable, sensitive, and rapid. One of the most successful electrochemical sensors is the glucose biosensor, which is based on the immobilization of glucose oxidase on a working electrode [6]; the worldwide market for these sensors is in the billions of dollars.

A traditional electrochemical sensor generally refers to an electrochemical system consisting of a working electrode, a reference electrode, and a counter electrode; and the sensing performance is characterized by a potentiostat. Though more and more new sensors and configurations are being developed, such as field-effect transistors that involve electrochemical reactions, this paper mainly focuses on the traditional electrochemical sensors that are based on working, reference, and counter electrodes. The counter electrode applies a current to the working electrode, the counter and working electrodes together construct a circuit; the current is then measured in the circuit. The potential of the counter electrode is adjusted to balance the reactions on the working electrode. The analyte causes electrochemical reactions on the working electrode. The reference electrode provides a stable and known electrode potential without a passing current. The potential of the working electrode is measured against the reference electrode. Some sensors are based on the above three-electrode configuration. For some sensors, the reference electrode and the counter electrode are combined into one electrode, and these sensors are based on a two-electrode configuration. The sensor can be used to detect electroactive substances without the immobilization of specific biorecepors, and the analytes are usually oxidized or reduced directly at certain potentials on the working electrodes. To enable the wide-range detection of a variety of other molecules, bioreceptors can be immobilized on the working electrodes for target analytes. The immobilized bioreceptors can be enzymes [7], [8], antibodies [9], [10], peptides [11], [12], deoxyribonucleic acids (DNAs) [13], [14], peptide nucleic acids [15], viruses [16], or cells [17], [18]. For the enzyme-based catalytic sensors, the target analyte can be catalyzed by enzymes to consume analytes and generates products. For the antibody, peptide, DNA, or virus-immobilized sensors, the detection is generally based on binding the analytes to the working electrode. For detecting toxic substances, heavy metals, or drugs, the biological sensors can be based on inhibition or molecular interaction. For cell-based sensors, the sensor could be a catalytic-based sensor or binding-based sensor. For these types of detection, in the presence of analyte, there is electron transfer because of oxidation or reduction, charge change, or impedance change on the working electrode surface. The electronic signals can be detected and correlated with the concentration of target analytes through calibration curves.

During the progress of the field of electrochemical sensors and biosensors, there are many electronic materials that have been studied for the construction of working, reference, and counter electrodes in electrochemical sensing systems. The devices can be constructed with different configurations and methods. To detect a target analyte, a specific bioreceptor for the analyte is immobilized on the working electrode. During the biological reactions between the analytes and the bioreceptors, depending on the mechanism of the reactions, catalytic or binding reactions, and whether there are potential-sensitive materials generated from the reactions, the electrical condition for the measurement can be determined—dc or ac.

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TABLE I
ELECTRONIC MATERIALS FOR WORKING ELECTRODES

Metal-based Main Electrode	Gold	[19-22]
	Platinum	[23-25]
	Silver	[26-29]
	ITO	[30,32-35]
	FTO	[31,36]
Carbon-based Main Electrode	Glassy carbon	[38-52]
	Diamond	[53-56]
	Carbon nanotube	[57-64]
	Pencil graphite	[65-76]
	Carbon paste	[77-86]
	Carbon ink	[87-93]
Polymer-based Main Electrode	PEDOT:PSS	[94,95]
	PEDOT:tosylate	[96]

This paper focuses on the recent progress of electrochemical sensors, including electronic materials for sensors, device configurations and manufacturing methods, and the signal generation and calculating methods. Electrochemical sensors are one of the most important types of sensors. There are a variety of review papers about the chemistry aspects of electrochemical sensors, such as the surface modification, nanomaterial synthesis, and applications for certain analytes. However, there are very limited reviews focusing on the electrical aspects of the electrochemical systems. This review explores the methodologies for these, which are necessary for the construction of sensors, and opens up exciting opportunities for the development of a variety of new electrochemical sensors for future applications.

II. ELECTRONIC MATERIALS

Electronic materials are used to construct the electrodes in electrochemical sensors, and the properties of electronic materials are important to the performances of these devices. The electrodes in an electrochemical sensor include a working electrode, a reference electrode, and a counter electrode; among them, the working electrode is rather important because the detection normally results from the change in the working electrode. There are a number of electronic materials that have been used for the construction of the working electrodes in the electrochemical systems, as shown in Table I. Metal, carbon, and polymers have been widely used as the main electrode materials for the working electrodes. Many other materials, including a variety of nanomaterials, polymers, and mediators, have been studied to functionalize the surfaces of main electrodes. This paper will describe the electronic materials for working electrodes in detail.

In terms of reference electrodes, silver and silver chloride (Ag/AgCl) electrodes and saturated calomel electrodes (SCE) have been studied as reference electrodes in electrochemical sensors. The SCE is based on elemental mercury and mercury (I) chloride. Currently, Ag/AgCl electrodes have widely replaced SCEs. Platinum has been widely used as a counter electrode in electrochemical sensors, such as platinum wire and platinum foil.

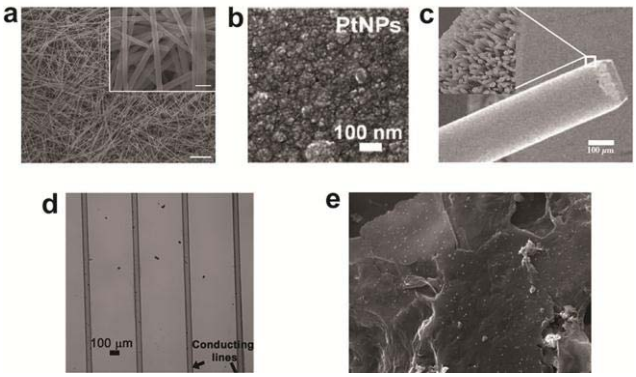

Fig. 1. Metal electrodes. (a) Silver nanowire modified gold electrode. Reprinted with permission from [19]. Copyright (2010) Springer. (b) Platinum nanoparticles on a platinum electrode. Reprinted with permission from [24]. Copyright (2016) Springer. (c) ZnO nanorods on Ag wire. Reprinted from [29]. Copyright (2010), with permission from Elsevier. (d) Micropatterned PEDOT: PSS on ITO. Reprinted from [35]. Copyright (2017), with permission from Elsevier. (e) Fe_2O_3/rGO/PEDOT/FTO-coated glass plate. Reprinted from [36]. Copyright (2017), with permission from Elsevier.

There are only several types of reference and counter electrodes that have been studied until now, while working electrodes with numerous new materials or new combinations of different materials have widely been studied. This section mainly focuses on the types of electronic materials for working electrodes, and it will not focus on device configurations.

A. Metal-Based Electrodes

Metals can oxidize or reduce electroactive substances effectively, and several types of metals and metal oxides have been studied to construct the working electrodes, including gold, platinum, silver solid amalgam, indium tin oxide (ITO), and fluorine doped tin oxide (FTO), as shown in Fig. 1. These materials are in the form of wire, disk, or film, and are used as the main electrodes of the working electrodes.

These main electrodes can be used as the bare electrodes to construct the working electrodes or can be functionalized with other materials, such as nanomaterials, mediators, or polymers. These materials used for surface functionalization include graphene, silver nanomaterials, platinum nanoparticles, single-wall carbon nanotube, polypyrrole-nanotubes and gold nanoparticles nanocomposites, silver nanoparticles and carbon nanotubes, graphene oxide nanoparticle decorated polypyrrole layer, graphene oxide and PEDOT, and so on.

Among the different types of metal materials, gold and platinum are widely used as the major materials for constructing the working electrodes.

Gold electrodes or gold electrodes functionalized with other materials such as nanomaterials, polymers, or mediators, have been studied in electrochemical sensors. A variety of working electrodes based on modified gold electrodes have been constructed, including silver nanowires on a gold electrode [19], silver nanocubes on a gold electrode [20], silver nanoparticles (AgNPs)/carboxylated multiwalled carbon

nanotubes /polyaniline layer on the surface of gold electrode [21], and multiwalled carbon nanotube and conductive polymer, polyaniline on a gold electrode [22].

For the platinum working electrode, bare platinum electrodes can be used as the working electrodes. There are different surface modifications on the platinum electrodes to construct the sensors with. There are single-wall carbon nanotubes on a platinum electrode, microparticle-loaded polypyrrole film on a platinum electrode [23], and platinum nanoparticles on the surface of a platinum electrode [24]. In addition, composite metal has been studied, including platinum–iridium [25].

Silver has been studied as the major component in the working electrode. A mixer of silver epoxy hardener (40%), silver epoxy resin (40%), graphite powder (10%), and tetracyanoquinodimethane (5%) has been studied as the working electrode [26]. Silver solid amalgam electrodes have been used as the working electrode [27], [28]. ZnO nanorods grown on a thin Ag wire have been studied as the working electrode [29].

A few types of metal oxides have been studied as the working electrode, including ITO-coated glass [30] or FTO glass [31]. The metal oxides have been functionalized with other electronic nanomaterials to construct sensors, including polypyrrole-nanotubes and gold nanoparticles nanocomposites on ITO electrode [32], graphene oxide and polypyrrole on an ITO microelectrode [33], silver nanoparticles and carbon nanotubes on an ITO electrode [34], conducing polymer PEDOT: polystyrene sulfonate (PSS) on ITO [35], graphene oxide nanoparticle decorated polypyrrole layer on an FTO glass plate [31], and Fe_2O_3, graphene oxide and PEDOT on an FTO electrode [36].

Using metal or metal oxides as the main electrodes, either as bare electrodes or further functionalized with other materials, many electrochemical sensors have been successfully constructed to detect analytes. Though a large number of sensors have been developed, there are still a wide range of electrochemical sensors to be developed based on the different combination of metal materials and functionalized materials that can achieve high performance.

B. Carbon-Based Electrodes

There are several types of carbon materials that have been used to construct electrochemical sensors, including glassy carbon, diamond, carbon nanotube, pencil graphite, carbon paste, and carbon ink, as shown in Fig. 2. Among these, glassy carbon electrodes are traditionally used to construct electrochemical sensors, and printed carbon electrodes are widely used nowadays to construct screen-printed electrochemical sensors

Glassy carbon [37] has widely been used as an electrode material because of its attracting properties, such as high temperature resistance, low electrical resistance, and chemical resistance. Glassy carbon electrodes have been studied to construct a variety of electrochemical biosensors, and it is a traditional type of working electrode. A number of other materials have been studied to functionalize the surface of glassy carbon electrodes to construct new working electrodes for electrochemical sensors, including gold nanoparticles-decorated silver-bipyridine nanobelts [38], polypyrrole (PPy)-Nafion (Nf)-functionalized multiwalled carbon nanotubes nanocomposite [39], polypyrrole-coated palladium silver nanospherical [40], polypyrrole-pluronic F127-gold nanoparticles [41], decorated gold nanoparticles, silver nanowires [42], [43], silver nanocrystals and graphene oxide [44], silver nanoparticles and multiwalled carbon nanotubes [45], graphene oxide nanosheet [46], graphene oxide nanosheet and gold nanoparticles [47], Fe_3O_4 magnetic nanoparticles/graphene oxide nanosheets, single-wall carbon nanotubes [48], multiwalled carbon nanotubes [26], [48], PEDOT and carbon nanotubes [49], polyaniline hydrogels with platinum nanoparticles [50], manganese dioxide particles [51], and graphite nanoparticles [52].

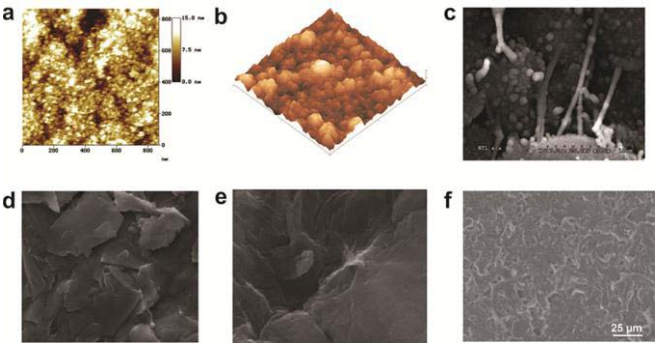

Fig. 2. Carbon-based working electrodes. (a) Glassy carbon. Reprinted with permission from [37]. Copyright (2002) American Chemical Society. (b) Diamond electrode. Reprinted from [53]. Copyright (2016) with permission from Elsevier. (c) Carbon nanotube paste electrode. Reprinted with permission from [59]. Copyright (2005) Wiley. (d) Pencil graphite electrode [76]. (e) Carbon paste electrode. Reprinted from [78]. Copyright (2016) with permission from Elsevier. (f) Carbon-ink-based screen-printed carbon electrode. Reprinted with permission from [88]. Copyright (2014) Wiley.

Boron-doped diamond films have been studied as possible working electrodes. Diamond has been functionalized with other materials as the working electrodes for electrochemical sensors, including carbon nanotubes [53], [54], platinum [55], and SnO_2 [56].

Carbon nanotubes have been printed as the working electrodes of screen-printed sensors [57], [58]. Multiwall carbon nanotube paste electrodes have widely been studied for their potential use in biosensors [59]–[63]. These electrodes can be constructed by mixing carbon nanotube powder with mineral oil, followed by being packed into a Teflon tube [60], [64].

Pencil graphite has been studied for its use in graphite-based working electrodes for electrochemical biosensors [65], [66], and graphite has been functionalized with a variety of other materials to construct hybrid materials as the working electrodes. These electrodes include ZnO nanorods/polypyrrole modified pencil graphite electrode [67], multiwalled carbon nanotubes/copper nanoparticles/polyaniline hybrid film electrodeposited on the surface of a pencil graphite electrode [68], graphene oxide nanoparticles-modified pencil graphite electrode [69], dioxide nanorods on graphite microfibers [70], carbon nanotubes on screen-printed graphite electrodes [71], [72], quantum dot ZnS–CdS modified pencil graphite electrode [73], Prussian blue modified graphite [74], poly(glycidyl

methacrylate-co-vinylferrocene)-film-coated pencil graphite electrode [75], and ionic liquid modified pencil graphite electrode [76].

Carbon paste electrodes are another type of electrodes that have been studied as a working electrode. One of the major components in carbon paste is graphite powder. Carbon paste electrodes have been functionalized with other materials to develop hybrid materials as the working electrodes in electrochemical sensors. These hybrid materials include rhodium, ruthenium, and iridium-dispersed carbon paste electrodes [77], magnetic bar carbon paste electrodes modified with nanomaterial of Fe_3O_4/reduced graphene oxide [78], nanoporous pseudocarbon paste electrode modified with gold nanoparticles [79], α-Fe_2O_3 nanocrystals incorporated carbon paste electrode [80], SnO_2NPs-modified carbon paste electrodes [81], carbon paste electrode modified with benzo[c]cinnoline and multiwalled carbon nanotubes [82], carbon nanotubes modified with carbon paste electrode [83]–[85], and zeolite-modified carbon paste electrode [86].

Currently, carbon-ink-based screen-printed electrodes are widely used for the construction of electrochemical sensors and biosensors. Carbon inks can be prepared with different methods [87], and their composition can be complex. One of the major components is generally graphite [87]. Many different types of nanomaterials, mediators, or polymers have been studied to functionalize the surfaces of printed carbon electrodes, including zinc oxide nanoparticles [88], carbon ink containing cobalt phthalocyanine [89], tetrathiafulvalene and gold nanoparticles [90], polypyrrole/polyacrylonitrile and multiwall carbon nanotube [91], and single-wall carbon nanotubes [92], [93].

Carbon-based electrodes have widely been studied for their potential in making electrochemical sensors, such as screen-printed sensors. Generally, these electrodes have the advantage of low cost; however, for some carbon-based sensors, the sensing performance may not be as sensitive as metal-based sensors.

C. Polymer Electrodes

Recently, polymers and the potential for them in electrochemical sensors have been studied because of their low cost and easy processing properties, as shown in Fig. 3. Conducting polymer PEDOT: PSS has been studied as a working electrode for electrochemical sensors [94], [95]. Vapor-phase deposition to create PEDOT: tosylate films have been used to construct electrochemical sensors [96].

These polymer-based electrodes function as the main electrodes of the working electrodes. By using different types of nanomaterials to functionalize the electrode surface or by immobilizing different types of bioreceptors on the electrodes, a variety of electrochemical biosensors can be developed.

There have been a large number of studies on electronic materials for the sensors. Metal-, carbon-, or polymer-based electrodes are different alternatives for constructing the working electrodes for the sensors, and each has advantages and disadvantages. Many types of nanomaterials, mediators, and polymers can be used to functionalize the working electrodes, which generally enhance the sensing performance but increases the cost.

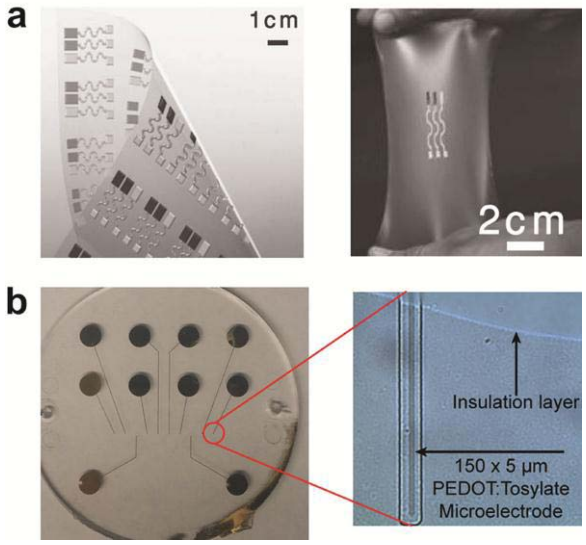

Fig. 3. Conducting polymer-based working electrodes. (a) PEDOT: PSS. Reprinted with permission from [94]. Copyright (2015) Wiley. (b) PEDOT: Tosylate. Reprinted with permission from [96]. Copyright (2016) American Chemical Society.

III. Device Configurations

A traditional electrochemical sensor is composed of a working electrode, a reference electrode, and a counter electrode. There are three-electrode systems and two-electrode systems in electrochemical sensors. In a three-electrode system, a working electrode, a reference, and a counter electrode are constructed independently to form three individual electrodes. In a two-electrode system, one electrode is a working electrode, and the other electrode functions as both a reference and counter electrode. Each electrode is connected to a lead from the instrument and inserted into the buffer solution. Electrochemical sensors can be constructed with different device configurations, including wire/disk, screen-printed electrodes, and microfabricated electrodes.

A. Wire/Disk-Based Sensor

In a traditional construction of an electrochemical sensor, each electrode of the working, reference, and counter electrodes is a cylinder, wire, or disk. The electrochemical systems can be either three-electrode systems or two-electrode systems. Each electrode can be a wire or a disk, which is inserted into the buffer solution. The diameters of the electrodes are generally in the range of millimeters. Platinum wires/disks [97], [98], gold wires, and glassy carbon electrodes [38], [39] have been studied as working electrodes. These electrodes can be used for the detection of different substrates or products by oxidation or reduction.

Fig. 4 shows a Clark-oxygen electrode [99] and a platinum disk-based working electrode [98]. Platinum cylinders can be fabricated from platinum wire. The bioreceptors, such as enzyme can be immobilized onto the metal surface. Platinum disks can be fabricated by cutting the wire transversely to

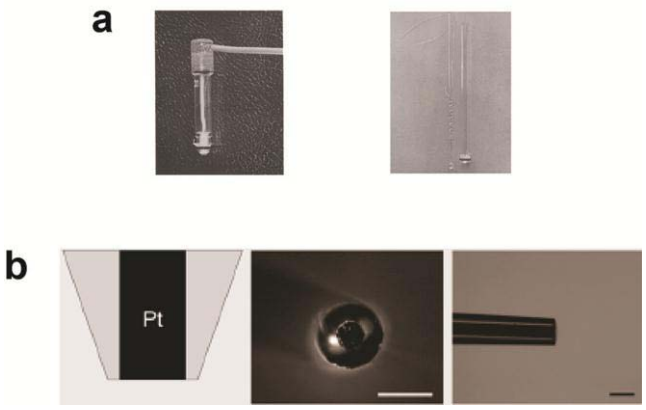

Fig. 4. Wire-based Sensor. (a) Clark-oxygen electrode-based wire sensor. Reprinted with permission from [99]. Copyright (1986) Springer. (b) Platinum disk-based working electrode. Reprinted with permission from [98]. Copyright (2014) American Chemical Society.

produce disks. A conventional three-electrode system can be employed with a platinum disk electrode as a working electrode, an SCE [97] or Ag/AgCl as the reference electrode [98], and a platinum wire as the counter electrode. The disk of platinum–iridium can be prepared by cutting Teflon-coated platinum–iridium (90/10%) wire [25]. ZnO nanorods grown on a thin Ag wire and an Ag/AgCl reference electrode are used as a two-electrode system for electrochemical biosensing [29].

The traditional sensors are generally wire-/disk-based sensors. Until today, many studies of electrochemical sensors, such as studying the effect of electrode surface modification, are still based on these configurations. However, these sensors are not cost effective, and the electrochemical cells occupy larger spaces. Thus, more cost-effective sensors and miniaturized sensors are desired.

B. Screen-Printed Sensor

Screen-printed sensors generally use screen-printing to print inks; these form the working, reference, and counter electrodes on a surface, and the thickness of the electrode is generally in the range of micrometers. These inks are generally in the form of thick films that are in the range of micrometers. Screen-printing processes use stencil masks, and squeegees move to print the ink layer on the substrate through the mask, followed by heating to solidify the ink. The electrodes prepared by screen-printing techniques are screen-printed electrodes and different parts of the screen require different stencil masks. The working electrode, reference electrode, and counter electrode use different masks during the printing process.

In screen-printed sensors, there are two-electrode sensing systems, and three-electrode sensing systems. In the two-electrode system, two inks are printed; one is for the working electrode, and the other is the reference/counter electrode. In the three-electrode system, the working electrode and counter electrode generally use the same type of ink, such as carbon-graphite ink, or gold ink, and the reference electrode is another type of ink, which is typically Ag/AgCl ink.

Several inks have been used as the working electrodes and counter electrodes, including carbon ink [100]–[102], platinum ink [103], gold ink [104], carbon-graphite ink, graphite ink,

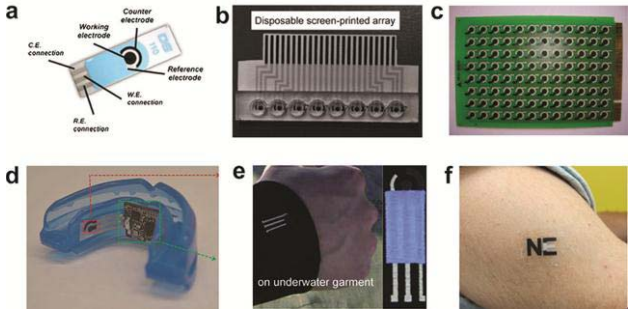

Fig. 5. Screen-printed electrochemical sensors. (a) Typical screen-printed sensor. Reprinted with permission from [106]. Copyright (2016) Springer. (b) Screen-printed sensor arrays. Reprinted with permission from [107]. Copyright (2010) Wiley. (c) Sensors on a 96 well. Reprinted with permission from [108]. Copyright (2007) with permission from Elsevier. (d) Mouth guard sensor on PET substrate. Reprinted with permission from [109]. Copyright (2015) with permission from Elsevier. (e) Sensor on an underwater garment. Reprinted with permission from [110]. Copyright (2011) with permission from Royal Society of Chemistry. (f) Sensor on a skin. Reprinted with permission from [111]. Copyright (2013) American Chemical Society.

carbon nanotube ink [57], [58], and PEDOT: PSS ink [94]. Ag/AgCl ink is generally used for the reference electrode. Carbon ink is the most widely used ink for printing the working electrode and counter electrode because of its low cost and high performance, and some metal oxides can be incorporated into carbon inks to develop hybrid materials for the screen-printed electrodes [105].

The substrates for screen-printed sensors have been studied, and they range from traditional ceramics to flexible substrates. The screen-printed sensors have widely been constructed on ceramic substrate and have been commercially available for decades [106]. The sensor arrays have been studied for their use in multiplex detection [107]. Sensor arrays are studied in the construction of a plastic plate for "ELISA"-like assay [108]. Recently, sensors have been studied on a variety of flexible substrates, including flexible PET film [109], underwater garments [110], and skin [111] (Fig. 5).

Screen-printed sensors are widely used in today's market for the construction of a variety of electrochemical biosensors. The electrode preparation processes are simple, and the overall cost of the sensors is relatively low. Screen-printed sensors based on both conventional hard substrates and flexible/wearable substrates have been developed.

C. Microfabricated Sensor

Microfabrication techniques are traditionally used to fabricate electronic chips and circuits. Recently, microfabrication techniques have been studied for fabricating components in electrochemical sensors. The electronic components are generally in the form of thin films that have thickness in the range of nanometers. Thin films can be metal thin films, metal oxide thin films, or polymer thin films. Microfabrication techniques can fabricate electrodes with smaller dimensions and more complicated shapes which providing more choices of electronic materials.

Several methods have been studied to generate the thin films, including evaporating metals and organic monomers,

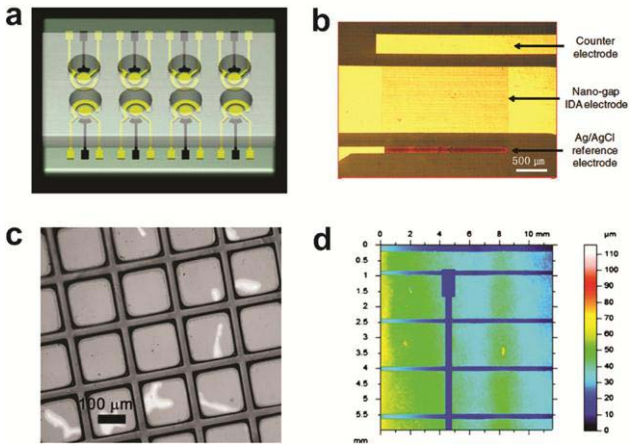

Fig. 6. Microfabricated electrochemical sensors. (a) Electrodes with conventional configuration. Reprinted with permission from [114]. Copyright (2005) American Chemical Society. (b) Interdigital electrodes. Reprinted with permission from [112]. Copyright (2013) IOP Publishing. (c) PEDOT: PSS patterns on ITO. Reprinted from [35]. Copyright (2017), with permission from Elsevier. (d) Patterns of conductive photoresist. Reprinted from [115]. Copyright (2012), with permission from Elsevier.

sputtering of metal oxides, and spin coating conductive polymers. To form certain patterns, photolithography is used to generate patterns in photoresist. The patterned photoresist can be used in combination with metal evaporation and lift off processes to generate patterns in metal, or in combination with etching processes to generate patterns in metal or conducting polymers. Metal evaporation can generate thin films of metals, by thermal evaporation or e-beam evaporation, and the metals can be gold [112]–[114], and platinum. Sputtering can generate thin films of metal oxides, such as ITO [35] and FTO [31]. Spin coating has been widely used for the deposition of conducting polymer, such as PEDOT: PSS [95]. Hotplates and petri dishes have been used to evaporate the monomer to synthesize a conducting polymer film of PEDOT: Tosylate [96]. SU-8 added with graphite carbon fibers as conductive photoresist enables the patterning of electrodes with only a photolithography process [115] (Fig. 6).

Microfabrication can generate thin film electrodes in metal with controlled geometry, and the thickness of the electrodes can be in nanometers, and the dimension can be as small as low micrometers. Compared to screen printing, microfabricate can more precisely generate controlled thinner electrodes with smaller dimensions. The cost, though, is generally higher.

IV. Signal Generations

Sensing using electrochemical biosensors is based the detection of electrical signals, and the signals are generated from biological reactions on the surfaces of electrodes. From the absence of a target analyte to the presence of a target analyte, there is a change in the electrical signal. The detected signal can be current, voltage, impedance, and so on. Both dc and ac signals have been used to measure analytes with electrochemical biosensors. Several types of signal collections have been studied and developed for the construction of different types of electrochemical sensors, including amperometric sensors, potentiometric sensors, voltammetric sensors, capacitive sensors, and impedimetric sensors.

To detect a target analyte, choosing a specific biological receptor for the target analyte and immobilizing the bioreceptor on the electrode's surface is crucial for the generation of specific electrical signals. There are mainly two types of biological interactions; one is based on catalytic reaction, and the other is binding- or hybridization-based reaction. Catalytic reactions are generally based on enzymes or cells, which consume substrates and generate products during the biological reactions. The co-substrates or products in the reactions can be electrical-active substances and generate changes in their electrical signals. The analytes for enzymatic reaction are generally small molecules. Binding- or hybridization-based reactions can be antibody, peptide, DNA, virus, or cell-based reactions. During the binding reactions, the analytes can be captured on the electrode surfaces, and the electrical signals can be changed because of the presence of analytes. The analytes for the binding-based reactions can be proteins, DNAs, viruses, or cells.

A. DC Signals

DC signals are used in a variety of electrochemical biosensors. There are many methods based on dc signals, including amperometry, potentiometry, linear voltammetry, cyclic voltammetry, current pulse, and potential pulse.

Amperometry is using a constant voltage between the working electrode and reference electrode and measuring the current changes because of the presence of analytes. Amperometric sensors are widely used for the construction of sensors [49], [116]. The plot of current versus time is generally studied, and the concentration of the analyte is related to the current change. Glucose meters, which have total sales in the billions of dollars, are based on amperometric measurements.

Potentiometry is using a constant current at the working electrode and measuring the voltage change because of the presece of analytes [117]–[119]. The concentration of analyte is related to the potential change.

Voltammetry is based on varying the potential of a working electrode and measuring the current to determine the status of the electrode's surface [120], [121]. The plot of the current as a y-axis versus the voltage as an x-axis is usually studied. Cyclic voltammetry is a typical method in voltammetry, and it is carried out by varying the potential and measuring the current in cycles [122]. Cyclic voltammetry is generally used to study the sensing properties of the sensor to a target analyte or the functionalization of the sensor's surfaces. The direct binding of the analyte to the bioreceptors immobilized on the electrode's surface can be characterized by cyclic voltammetry. Differential pulse voltammetry has been studied for the detection of analytes as well [123] (Fig. 7).

A few other methods have been explored, including current pulse and potential pulse [124]–[126]. When a pulse is applied, a transient response is obtained. These methods have been used to analyze the relationship between the capacitance change and the concentration of analytes to determine the analyte concentration.

The dc signals can be used for binding-based assays for indirect detection of target analytes. During the binding or hybridization procedures, the analytes are captured on

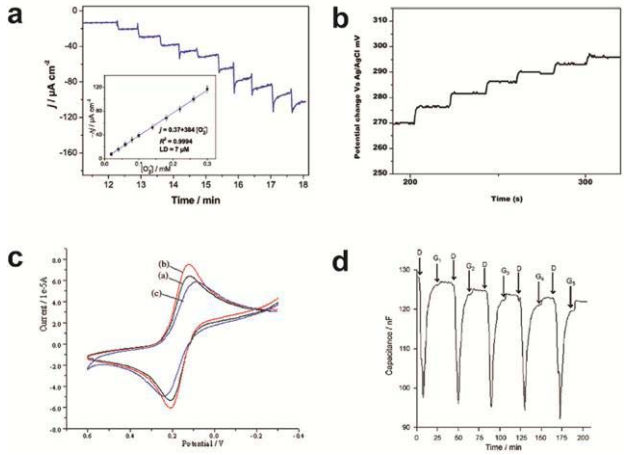

Fig. 7. DC signal-based methods for detection of analytes. (a) Amperometry. Reprinted from [49]. Copyright (2016), with permission from Elsevier. (b) Potentiometry. Reprinted with permission from [119]. Copyright (2014) Royal Society of Chemistry. (c) Cyclic voltammetry. Reprinted with permission from [122]. Copyright (2013) IEEE. (d) Potential pulse. Reprinted from [126]. Copyright (2010), with permission from Elsevier.

the working electrodes. Applying dc signals to the sensors is not enough to generate enough signal changes because of the direct binding of analytes. Thus, the analytes can further capture other substances that are used as labels on the electrodes with specific binding or hybridization. During these processes, both the analytes and the analyte-associated labels are captured on the electrodes, and the dc signals are used for the oxidation or reduction of the labels.

B. AC Signals

AC signals are generally used for the measurement of surface modification of the working electrodes in electrochemical sensors. Binding- or hybridization-based assays are based on capturing the analytes on the working electrodes, such as antigens or DNAs, which are additions of new materials on the surface. Some enzymes can result in the cleavage of immobilized bioreceptors, such as peptides, which remove existing materials on the surface. These changes in the surface materials can result in changes of electrical properties.

To detect the binding-based analytes, the analytes need to generate enough signals so that they can be differentiated by the instrument. However, applying dc signals to the sensors for the detection of these analytes is not surfficient to generate enough signal changes to identify the presence of analytes. Therefore, ac signals are used for the direct detection of binding-based analytes.

Electrochemical impedance spectroscopy (EIS) is widely used for the ac measurement of the electrochemical systems in the detection of binding-based analytes. EIS measures the impedances of the electrochemical system over a range of frequencies [127]–[130]. A Nyquist plot or a bode plot is usually used to study the binding behavior.

In the Nyquist plot, the frequency is swept, which results in different impedances. The real part of the impedance is plotted on the x-axis, and the imaginary part is plotted on the y-axis, as shown in Fig. 8(a). The Nyquist plot is always used to

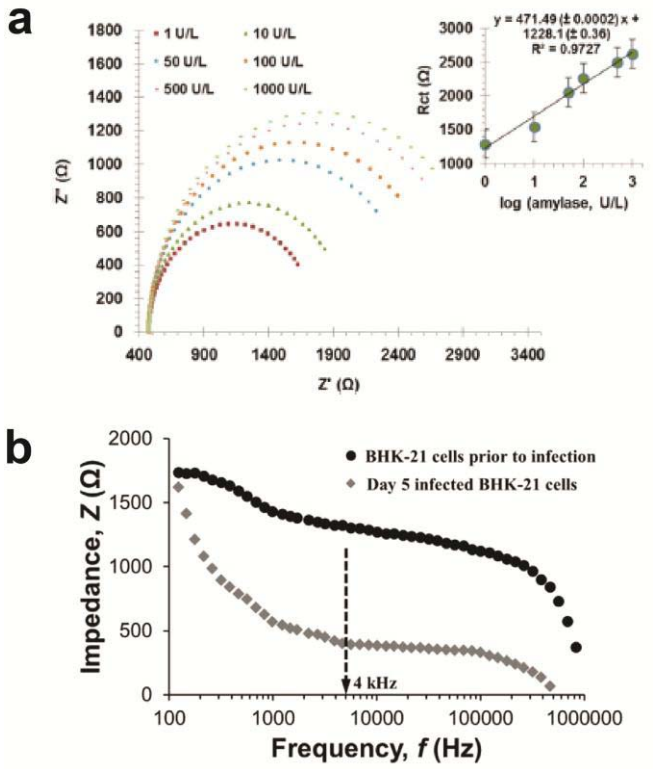

Fig. 8. EIS for detection of analytes. (a) Nyquist plot. Reprinted from [129]. Copyright (2016), with permission from Elsevier. (b) Bode plot. Reprinted from [130]. Copyright (2015), with permission from Elsevier.

analyze the detection process, and there are a semicircle part and a linear part in the curve. Randle's equivalent circuit is used to fit the physico-chemical process on the working electrode, which includes double layer capacitance (Cdl), electrolyte solution resistance (Re), charge-transfer resistance (Rct), and the Warburg diffusion element (W). The semicircle is related to the charge-transfer process, and the intercept values in the x-axis are Re and Rct. EIS has widely been studied for its use in the detection of a variety of analytes that are based on binding or hybridization. The surfaces of working electrodes are immobilized with specific bioreceptors, and in the presence of target analytes, the analytes bind to the bioreceptors on the electrode's surfaces. The binding processes affect the capacitance and resistance of the electrochemical sensor cell, which are related to the concentration of the analyte. The calibration curve can be plotted between logarithm Rct and the logarithm of the analyte concentration.

Similarly, a Bode plot is usually magnitude versus frequency curve or phase versus frequency curve, as shown in Fig. 8(b). The calibration curve can be plotted between the logarithm of the capacitance change and the logarithm of the analyte concentration.

For small molecules, many can be involved in enzymatic-based biological reactions. For large molecules, some can be involved in enzymatic reactions, and almost all of them can be involved in antibody, peptide, or DNA-based binding. Even the bioreceptors may not exist for the target analytes, and they can be screened and identified from biological libraries in the future. Because of the biological reactions between the

analytes and the immobilized specific bioreceptors, the analytes can generate surface changes in the working electrodes, such as electron transfer or impedance changes. Both dc and ac signals have been widely used to study these surface changes.

V. Conclusion and Outlook

Electrochemical sensors based on working, reference and counter electrodes, are fascinating, miniaturized sensing devices that have attracted a great amount of attention for various fundamental studies and practical applications. These sensors can significantly shorten detection time and simplify detection procedures. The field has generated a wide range of opportunities for the detection of target analytes for a variety of applications in healthcare, environment, food safety, and defense purposes. The ongoing challenges of the field are the sensitivity, accuracy, reproducibility, and stability of the sensing performance. Attempts at addressing the challenges have been exploring new electrode materials, device configurations, surface properties of the sensing electrodes, biological molecules immobilized on the electrodes, and electronic signal generation and detection methods. Though these explorations have improved the fundamental understanding of the field and opened new opportunities for practical applications, the problems have not been fully solved and some of the challenge remains. It is anticipated that the electronic aspects for the sensors, including electronic materials, device configurations, and signal generations, may be a way to solve the problems, and further efforts need to be made to achieve advances; this can be done by exploring alternative materials, device configurations, and signal generations of electrochemical sensors.

References

[1] C. Ho and H. Z. Yu, "Aptamer-based electrochemical biosensors for the detection of small molecules and plasma proteins," *Neuromethods*, vol. 80, pp. 319–346, Mar. 2013.

[2] L. Karadurmus, S. Kurbanoglu, B. Uslu, and S. A. Ozkan, "Electrochemical DNA biosensors in drug analysis," *Current Pharm. Anal.*, vol. 12, p. 2, Sep. 2016.

[3] N. Plumeré, J. Henig, and W. H. Campbell, "Enzyme-catalyzed O_2 removal system for electrochemical analysis under ambient air: Application in an amperometric nitrate biosensor," *Anal. Chem.*, vol. 84, no. 5, pp. 2141–2146, 2012.

[4] K. Cizek *et al.*, "Integrated explosive preconcentrator and electrochemical detection system for 2,4,6-trinitrotoluene (TNT) vapor," *Anal. Chim. Acta*, vol. 661, no. 1, pp. 117–121, 2010.

[5] Y. Zeng, Z. Zhu, D. Du, and Y. Lin, "Nanomaterial-based electrochemical biosensors for food safety," *J. Electroanal. Chem*, vol. 781, pp. 147–154, Nov. 2016.

[6] J. Wang, "Electrochemical glucose biosensors," *Chem. Rev.*, vol. 108, no. 2, pp. 814–825, 2008.

[7] Y. Fang, Y. Umasankar, and R. P. Ramasamy, "A novel bi-enzyme electrochemical biosensor for selective and sensitive determination of methyl salicylate," *Biosens. Bioelectron.*, vol. 81, pp. 39–45, Jul. 2016.

[8] M. Moreno-Guzmán, L. Garcí-Carmona, Á. Molinero-Fernández, F. Cava, M. Á. L. Gil, and A. Escarpa, "Bi-enzymatic biosensor for on-site, fast and reliable electrochemical detection of relevant D-amino acids in bacterial samples," *Sens. Actuators B, Chem.*, vol. 242, pp. 95–101, Apr. 2017.

[9] W. Saleem, C. Salinas, B. Watkins, G. Garvey, A. C. Sharma, and R. Ghosh, "Antibody functionalized graphene biosensor for label-free electrochemical immunosensing of fibrinogen, an indicator of trauma induced coagulopathy," *Biosens. Bioelectron.*, vol. 86, pp. 522–529, Dec. 2016.

[10] R. Sharma *et al.*, "Label-free electrochemical impedance biosensor to detect human interleukin-8 in serum with sub-pg/ml sensitivity," *Biosens. Bioelectron.*, vol. 80, pp. 607–613, Jun. 2016.

[11] H. J. Hwang *et al.*, "High sensitive and selective electrochemical biosensor: Label-free detection of human norovirus using affinity peptide as molecular binder," *Biosens. Bioelectron.*, vol. 87, pp. 164–170, Mar. 2017.

[12] F. Meng, W. Liang, H. Sun, L. Wu, X. Gong, and P. Miao, "A peptide-based electrochemical biosensor for facile measurement of whole-blood heparin," *ChemElectroChem*, vol. 4, no. 3, pp. 472–475, 2017.

[13] M. Chen, C. Hou, D. Huo, J. Bao, H. Fa, and C. Shen, "An electrochemical DNA biosensor based on nitrogen-doped graphene/Au nanoparticles for human multidrug resistance gene detection," *Biosens. Bioelectron.*, vol. 85, pp. 684–691, Nov. 2016.

[14] W. Wang *et al.*, "Ultrasensitive electrochemical DNA biosensor based on functionalized gold clusters/graphene nanohybrids coupling with exonuclease III-aided cascade target recycling," *Biosens. Bioelectron.*, vol. 91, pp. 183–189, Dec. 2017.

[15] P. Teengam, W. Siangproh, A. Tuantranont, C. S. Henry, T. Vilaivan, and O. Chailapakul, "Electrochemical paper-based peptide nucleic acid biosensor for detecting human papillomavirus," *Anal. Chim. Acta*, vol. 952, pp. 32–40, Feb. 2017.

[16] Y. Zhou and R. P. Ramasamy, "Phage-based electrochemical biosensors for detection of pathogenic bacteria," *ECS Trans.*, vol. 69, pp. 1–8, Mar. 2015.

[17] S. P. Trosok, B. T. Driscoll, and J. H. T. Luong, "Mediated microbial biosensor using a novel yeast strain for wastewater BOD measurement," *Appl. Microbiol. Biotechnol.*, vol. 56, nos. 3–4, pp. 550–554, 2001.

[18] D. Chen, Y. Cao, B. Liu, and J. Kong, "A BOD biosensor based on a microorganism immobilized on an Al_2O_3 sol-gel matrix," *Anal. Bioanal. Chem.*, vol. 372, nos. 5–6, pp. 737–739, 2002.

[19] M. J. Song, S. W. Hwang, and D. Whang, "Amperometric hydrogen peroxide biosensor based on a modified gold electrode with silver nanowires," *J. Appl. Electrochem.*, vol. 40, no. 12, pp. 2099–2105, 2010.

[20] P. Yang, L. Wang, Q. Wu, Z. Chen, and X. Lin, "A method for determination of glucose by an amperometric bienzyme biosensor based on silver nanocubes modified Au electrode," *Sens. Actuator B, Chem.*, vol. 194, pp. 71–78, Jun. 2014.

[21] R. Rawal, S. Chawla, and C. S. Pundir, "Polyphenol biosensor based on laccase immobilized onto silver nanoparticles/multiwalled carbon nanotube/polyaniline gold electrode," *Anal. Biochem.*, vol. 419, no. 2, pp. 196–204, 2011.

[22] E. Bayram and E. Akyilmaz, "Development of a new microbial biosensor based on conductive polymer/multiwalled carbon nanotube and its application to paracetamol determination," *Sens. Actuator B, Chem.*, vol. 233, pp. 409–418, Mar. 2016.

[23] S. Arjsiriwat, M. Tanticharoen, K. Kirtikara, K. Aoki, and M. Somasundrum, "Metal-dispersed conducting polymer-coated electrode used for oxidase-based biosensors," *Electrochem. Commun.*, vol. 2, no. 6, pp. 441–444, 2000.

[24] S. B. Adeloju and S. Hussain, "Potentiometric sulfite biosensor based on entrapment of sulfite oxidase in a polypyrrole film on a platinum electrode modified with platinum nanoparticles," *Microchim. Acta*, vol. 183, no. 4, pp. 1341–1350, 2016.

[25] Z. M. Zain, R. D. O'Neill, J. P. Lowry, K. W. Pierce, M. Tricklebank, A. Dewa, and S. A. Ghani, "Development of an implantable d-serine biosensor for *in vivo* monitoring using mammalian d-amino acid oxidase on a poly (o-phenylenediamine) and Nafion-modified platinum-iridium disk electrode," *Biosens. Bioelectron.*, vol. 25, no. 6, pp. 1454–1459, 2010.

[26] R. F. Dutra, G. D. Coelho, V. L. Silva, W. M. Ledingham, and J. L. L. Filho, "A reusable amperometric biosensor based on a novel silver-epoxy electrode for immunoglobulin detection," *Biotechnol. Lett.*, vol. 22, pp. 579–583, 2000.

[27] B. Josypčuk, J. Barek, and O. Josypčuk, "Flow electrochemical biosensors based on enzymatic porous reactor and tubular detector of silver solid amalgam," *Anal. Chim. Acta*, vol. 778, pp. 24–30, Jun. 2013.

[28] O. Josypčuk, J. Barek, and B. Josypčuk, "Construction and application of flow enzymatic biosensor based of silver solid amalgam electrode for determination of sarcosine," *Electroanalysis*, vol. 27, no. 11, pp. 2559–2566, 2015.

[29] M. Q. Israr, J. R. Sadaf, M. H. Asif, O. Nur, M. Willander, and B. Danielsson, "Potentiometric cholesterol biosensor based on ZnO nanorods chemically grown on Ag wire," *Thin Solid Films*, vol. 519, no. 3, pp. 1106–1109, 2010.

[30] E. Zor, I. Hatay Patir, H. Bingol, and M. Ersoz, "An electrochemical biosensor based on human serum albumin/graphene oxide/3-aminopropyltriethoxysilane modified ITO electrode for the enantioselective discrimination of d- and l-tryptophan," *Biosens. Bioelectron.*, vol. 42, no. 1, pp. 321–325, 2013.

[31] N. Chauhan, R. Rawal, V. Hooda, and U. Jain, "Electrochemical biosensor with graphene oxide nanoparticles and polypyrrole interface for the detection of bilirubin," *RSC Adv.*, vol. 6, no. 68, pp. 63624–63633, 2016.

[32] A. Sharma and A. Kumar, "Study of structural and electro-catalytic behaviour of amperometric biosensor based on chitosan/polypyrrole nanotubes-gold nanoparticles nanocomposites," *Synth. Met.*, vol. 220, pp. 551–559, Oct. 2016.

[33] Y. Li *et al.*, "Construction of a cell impedance biosensor based on graphene oxide/polypyrrole-indium tin oxide micro-electrode for detecting cell adhesion and proliferation," *Chin. J. Sens. Actuators*, vol. 29, no. 6, pp. 787–796, 2016.

[34] J. Lin, C. He, Y. Zhao, and S. Zhang, "One-step synthesis of silver nanoparticles/carbon nanotubes/chitosan film and its application in glucose biosensor," *Sens. Actuators B, Chem.*, vol. 137, no. 2, pp. 768–773, 2009.

[35] R. K. Pal, S. C. Kundu, and V. K. Yadavalli, "Biosensing using photolithographically micropatterned electrodes of PEDOT:PSS on ITO substrates," *Sens. Actuators B, Chem.*, vol. 242, pp. 140–147, Mar. 2017.

[36] N. Chauhan, S. Chawla, C. S. Pundir, and U. Jain, "An electrochemical sensor for detection of neurotransmitter-acetylcholine using metal nanoparticles, 2D material and conducting polymer modified electrode," *Biosens. Bioelectron.*, vol. 89, pp. 377–383, Mar. 2017.

[37] K. Shi and K. K. Shiu, "Scanning tunneling microscopic and voltammetric studies of the surface structures of an electrochemically activated glassy carbon electrode," *Anal. Chem.*, vol. 74, no. 4, pp. 879–885, 2002.

[38] A. Boujakhrout, P. Díez, A. Sánchez, P. Martínez-Ruíz, J. M. Pingarrón, and R. Villalonga, "Gold nanoparticles-decorated silver-bipyridine nanobelts for the construction of mediatorless hydrogen peroxide biosensor," *J. Colloid Interf. Sci.*, vol. 482, pp. 105–111, Nov. 2016.

[39] B. K. Shrestha *et al.*, "High-performance glucose biosensor based on chitosan-glucose oxidase immobilized polypyrrole/Nafion/functionalized multi-walled carbon nanotubes bio-nanohybrid film," *J. Colloid Interf. Sci.*, vol. 482, pp. 39–47, Nov. 2016.

[40] M. R. Mahmoudian, W. J. Basirun, and Y. Binti Alias, "Sensitive dopamine biosensor based on polypyrrole-coated palladium silver nanospherical composites," *Ind. Eng. Chem. Res.*, vol. 55, no. 25, pp. 6943–6951, 2016.

[41] C. Sun *et al.*, "Biocompatible polypyrrole-block copolymer-gold nanoparticles platform for determination of inosine monophosphate with bi-enzyme biosensor," *Sens. Actuators B, Chem.*, vol. 230, pp. 521–527, Dec. 2016.

[42] L. Wang, X. Gao, L. Jin, Q. Wu, Z. Chen, and X. Lin, "Amperometric glucose biosensor based on silver nanowires and glucose oxidase," *Sens. Actuators B, Chem.*, vol. 176, pp. 9–14, Jan. 2013.

[43] M. Ahmad, H. Sun, M. Hussain, S. Karim, A. Nisar, and M. Khan, "Development of silver nanowires based highly sensitive amperometric glucose biosensor," *Electroanalysis*, vol. 27, no. 6, pp. 1498–1506, 2015.

[44] L. Zhong *et al.*, "Electrochemically controlled growth of silver nanocrystals on graphene thin film and applications for efficient nonenzymatic H_2O_2 biosensor," *Electrochim. Acta*, vol. 89, pp. 222–228, Mar. 2013.

[45] Y. Li, Y. Li, and Y. Yang, "A new amperometric H_2O_2 biosensor based on nanocomposite films of chitosan-MWNTs, hemoglobin, and silver nanoparticles," *J. Solid State Electr.*, vol. 16, no. 3, pp. 1133–1140, 2012.

[46] K. Liu, J. Zhang, G. Yang, C. Wang, and J. J. Zhu, "Direct electrochemistry and electrocatalysis of hemoglobin based on poly(diallyldimethylammonium chloride) functionalized graphene sheets/room temperature ionic liquid composite film," *Electrochem. Commun.*, vol. 12, no. 3, pp. 402–405, 2010.

[47] Y. Fang, S. Guo, C. Zhu, Y. Zhai, and E. Wang, "Self-assembly of cationic polyelectrolyte-functionalized graphene nanosheets and gold nanoparticles: A two-dimensional heterostructure for hydrogen peroxide sensing," *Langmuir*, vol. 26, no. 13, pp. 11277–11282, 2010.

[48] J. Wang, M. Musameh, and Y. Lin, "Solubilization of carbon nanotubes by Nafion toward the preparation of amperometric biosensors," *J. Amer. Chem. Soc.*, vol. 125, no. 9, pp. 2408–2409, 2003.

[49] M. Braik, M. M. Barsan, C. Dridi, M. Ben Ali, and C. M. A. Brett, "Highly sensitive amperometric enzyme biosensor for detection of superoxide based on conducting polymer/CNT modified electrodes and superoxide dismutase," *Sens. Actuators B, Chem.*, vol. 236, pp. 574–582, Mar. 2016.

[50] L. Li *et al.*, "A nanostructured conductive hydrogels-based biosensor platform for human metabolite detection," *Nano Lett.*, vol. 15, no. 2, pp. 1146–1151, 2015.

[51] M. Aigner *et al.*, "Amperometric biosensor for total monoamines using a glassy carbon paste electrode modified with human monoamine oxidase B and manganese dioxide particles," *Microchim. Acta*, vol. 182, nos. 5–6, pp. 925–931, 2015.

[52] Y. Piao, D. J. Han, and T. S. Seo, "Highly conductive graphite nanoparticle based enzyme biosensor for electrochemical glucose detection," *Sens. Actuators B, Chem.*, vol. 194, pp. 454–459, Jun. 2014.

[53] N. Zehani *et al.*, "Highly sensitive electrochemical biosensor for bisphenol A detection based on a diazonium-functionalized boron-doped diamond electrode modified with a multi-walled carbon nanotube-tyrosinase hybrid film," *Biosens. Bioelectron.*, vol. 74, pp. 830–835, Dec. 2015, doi: 10.1016/j.bios.2015.07.051.

[54] S. K. Lee, M. J. Song, J. H. Kim, Y. K. Lim, Y. S. Chun, and D. S. Lim, "Selective growth of carbon nanotubes on boron-doped diamond for electrochemical biosensor application," *RSC Adv.*, vol. 5, no. 30, pp. 23395–23400, 2015.

[55] M. J. Song, J. H. Kim, S. K. Lee, D. S. Lim, S. W. Hwang, and D. Whang, "Analytical characteristics of electrochemical biosensor using pt-dispersed graphene on boron doped diamond electrode," *Electroanalysis*, vol. 23, no. 10, pp. 2408–2414, 2011.

[56] R. Geng, G. Zhao, M. Liu, and M. Li, "A sandwich structured SiO_2/cytochrome c/SiO_2 on a boron-doped diamond film electrode as an electrochemical nitrite biosensor," *Biomaterials*, vol. 29, no. 18, pp. 2794–2801, 2008.

[57] R. Khan, W. Gorski, and C. D. Garcia, "Nanomolar detection of glutamate at a biosensor based on screen-printed electrodes modified with carbon nanotubes," *Electroanalysis*, vol. 23, no. 10, pp. 2357–2363, 2011.

[58] F. Rezazadeh, M. Mohamadi, D. Afzali, T. Shamspur, and A. Mostafavi, "A quercetin biosensor based on chitosan-entrapped carbon nanotube paste electrode coated with DNA," *J. AOAC Int.*, vol. 98, no. 5, pp. 1375–1381, 2015.

[59] M. Chicharro, E. Bermejo, M. Moreno, A. Sánchez, A. Zapardiel, and G. Rivas, "Adsorptive stripping voltammetric determination of amitrole at a multi-wall carbon nanotubes paste electdrode," *Electroanalysis*, vol. 17, nos. 5–6, pp. 476–482, 2005.

[60] M. D. Rubianes and G. A. Rivas, "Carbon nanotubes paste electrode," *Electrochem. Commun.*, vol. 5, no. 8, pp. 689–694, 2003.

[61] T. M. B. F. Oliveira *et al.*, "Biosensor based on multi-walled carbon nanotubes paste electrode modified with laccase for pirimicarb pesticide quantification," *Talanta*, vol. 106, pp. 137–143, Mar. 2013.

[62] J. P. Marco, K. B. Borges, C. R. T. Tarley, E. S. Ribeiro, and A. C. Pereira, "Development of a simple, rapid and validated square wave voltametric method for determination of promethazine in raw material and pharmaceutical formulation using DNA modified multiwall carbon nanotube paste electrode," *Sens. Actuators B, Chem.*, vol. 177, pp. 251–259, Jun. 2013.

[63] P. K. Brahman, R. A. Dar, and K. S. Pitre, "DNA-functionalized electrochemical biosensor for detection of vitamin B1 using electrochemically treated multiwalled carbon nanotube paste electrode by voltammetric methods," *Sens. Actuators B, Chem.*, vol. 177, no. 2, pp. 807–812, 2013.

[64] M. D. Rubianes and G. A. Rivas, "Enzymatic biosensors based on carbon nanotubes paste electrodes," *Electroanalysis*, vol. 17, no. 1, pp. 73–78, Oct. 2005.

[65] S. Donmez, F. Arslan, and H. Arslan, "Sequence-specific label-free nucleic acid biosensor for the detection of the hepatitis C virus genotype 1a using a disposable pencil graphite electrode," *Artif. Cells Nanomed. Biotechnol.*, vol. 44, no. 3, pp. 912–917, 2016.

[66] S. Tajik, M. A. Taher, H. Beitollahi, and M. Torkzadeh-Mahani, "Electrochemical determination of the anticancer drug taxol at a ds-DNA modified pencil-graphite electrode and its application as a label-free electrochemical biosensor," *Talanta*, vol. 134, pp. 60–64, Mar. 2015.

[67] B. Batra, M. Yadav, and C. S. Pundir, "L-Glutamate biosensor based on l-glutamate oxidase immobilized onto ZnO nanorods/polypyrrole modified pencil graphite electrode," *Biochem. Eng. J.*, vol. 105, pp. 428–436, Jun. 2016.

[68] K. Dagar and C. S. Pundir, "An improved amperometric L-lactate biosensor based on covalent immobilization of microbial lactate oxidase onto carboxylated multiwalled carbon nanotubes/copper nanoparticles/polyaniline modified pencil graphite electrode," *Enzyme Microb. Tech.*, vol. 96, pp. 177–186, Jul. 2017.

[69] B. Batra, V. Narwal, and C. S. Pundir, "An amperometric lactate biosensor based on lactate dehydrogenase immobilized onto graphene oxide nanoparticles-modified pencil graphite electrode," *Eng. Life Sci.*, vol. 16, no. 8, pp. 786–794, 2016.

[70] J. Zhang *et al.*, "Construction of titanium dioxide nanorod/graphite microfiber hybrid electrodes for a high performance electrochemical glucose biosensor," *Nanoscale*, vol. 8, no. 17, pp. 9382–9389, 2016.

[71] N. Hernández-Ibáñez, L. Garcí-Cruz, V. Montiel, C. W. Foster, C. E. Banks, and J. Iniesta, "Electrochemical lactate biosensor based upon chitosan/carbon nanotubes modified screen-printed graphite electrodes for the determination of lactate in embryonic cell cultures," *Biosens. Bioelectron.*, vol. 77, pp. 1168–1174, Mar. 2016.

[72] E. P. Medyantseva, D. V. Brusnitsyn, R. M. Varlamova, M. A. Beshevets, H. C. Budnikov, and A. N. Fattakhova, "Capabilities of amperometric monoamine oxidase biosensors based on screen-printed graphite electrodes modified with multiwall carbon nanotubes in the determination of some antidepressants," *J. Anal. Chem.*, vol. 70, no. 5, pp. 535–539, 2015.

[73] B. Ertek, C. Akgül, and Y. Dilgin, "Photoelectrochemical glucose biosensor based on a dehydrogenase enzyme and NAD+/NADH redox couple using a quantum dot modified pencil graphite electrode," *RSC Adv.*, vol. 6, no. 24, pp. 20058–20066, 2016.

[74] S. H. Lee, J. H. Chung, H. K. Park, and G. J. Lee, "A simple and facile glucose biosensor based on Prussian blue modified graphite string," *J. Sensor.*, vol. 2016, Dec. 2016, Art. no. 1859292.

[75] M. Dervisevic, E. Çevik, and M. Şenel, "Development of glucose biosensor based on reconstitution of glucose oxidase onto polymeric redox mediator coated pencil graphite electrodes," *Enzyme Microb. Tech.*, vol. 68, pp. 69–76, Oct. 2015.

[76] C. Sengiz, G. Congur, and A. Erdem, "Development of ionic liquid modified disposable graphite electrodes for label-free electrochemical detection of DNA hybridization related to Microcystis SPP," *Sensors*, vol. 15, no. 9, pp. 22737–22749, 2015.

[77] J. Wang, G. Rivas, and M. Chicharro, "Iridium-dispersed carbon paste enzyme electrodes," *Electroanalysis*, vol. 8, no. 5, pp. 434–437, 1996.

[78] S. Jahanbani and A. Benvidi, "A novel electrochemical DNA biosensor based on a modified magnetic bar carbon paste electrode with Fe_3O_4NPs-reduced graphene oxide/PANHS nanocomposite," *Mater. Sci. Eng. C*, vol. 68, pp. 1–8, Jul. 2016.

[79] Y. Liu *et al.*, "Acetylcholinesterase biosensor for carbaryl detection based on nano-porous pseudo carbon paste electrode," *Nanosci. Nanotechnol. Lett.*, vol. 8, no. 9, pp. 785–790, 2016.

[80] C. Sarika, M. S. Shivakumar, C. Shivakumara, G. Krishnamurthy, B. Narasimha Murthy, and I. C. Lekshmi, "A novel amperometric catechol biosensor based on α-Fe_3O_4 nanocrystals-modified carbon paste electrode," *Artif. Cells Nanomed. Biotechnol.*, vol. 45, no. 3, pp. 1–10, 2016.

[81] G. A. Tlğ, D. K. Zeybek, and S. Pekyardlmcl, "Fabrication of amperometric cholesterol biosensor based on SnO2nanoparticles and Nafion-modified carbon paste electrode," *Chem. Paper*, vol. 70, no. 4, pp. 1–11, 2016.

[82] A. C. Çelik *et al.*, "Amperometric lactate biosensor based on carbon paste electrode modified with benzo[c]cinnoline and multiwalled carbon nanotubes," *Electroanalysis*, vol. 27, no. 12, pp. 2820–2828, 2015.

[83] Ü. Anik, and S. Çevik, "Double-walled carbon nanotube based carbon paste electrode as xanthine biosensor," *Microchim. Acta*, vol. 166, nos. 3–4, pp. 209–213, 2009.

[84] Ü. Anik and M. Çubukçu, "Examination of the electroanalytic performance of carbon nanotube (CNT) modified carbon paste electrodes as xanthine biosensor transducers," *Turk. J. Chem.*, vol. 32, no. 6, pp. 711–719, 2008.

[85] Y. Pang, G. M. Zeng, L. Tang, Y. Zhang, Z. Li, and L. J. Chen, "Laccase biosensor using magnetic multiwalled carbon nanotubes and chitosan/silica hybrid membrane modified magnetic carbon paste electrode," *J. Center South Univ. T.*, vol. 18, no. 6, pp. 1849–1856, 2011.

[86] K. Balal, H. Mohammad, B. Bahareh, B. Ali, H. Maryam, and Z. Mozhgan, "Zeolite nanoparticle modified carbon paste electrode as a biosensor for simultaneous determination of dopamine and tryptophan," *J. Chin. Chem. Soc-TAIP*, vol. 56, no. 4, pp. 789–796, 2009.

[87] V. K. Rao, M. K. Sharma, P. Pandey, and K. Sekhar, "Comparison of different carbon ink based screen-printed electrodes towards amperometric immunosensing," *World J. Microb. Biot.*, vol. 22, no. 11, pp. 1135–1143, 2006.

[88] S. Palanisamy, C. Karuppiah, S. M. Chen, and P. Periakaruppan, "A highly sensitive and selective enzymatic biosensor based on direct electrochemistry of hemoglobin at zinc oxide nanoparticles modified activated screen printed carbon electrode," *Electroanalysis*, vol. 26, no. 9, pp. 1984–1993, 2014.

[89] T. Shimomura, T. Sumiya, M. Ono, T. Ito, and T. A. Hanaoka, "Amperometric l-lactate biosensor based on screen-printed carbon electrode containing cobalt phthalocyanine, coated with lactate oxidase-mesoporous silica conjugate layer," *Anal. Chim. Acta*, vol. 714, pp. 114–120, Dec. 2012.

[90] B. Molinero-Abad, M. A. Alonso-Lomillo, O. Domínguez-Renedo, and M. J. Arcos-Martínez, "Malate quinone oxidoreductase biosensors based on tetrathiafulvalene and gold nanoparticles modified screen-printed carbon electrodes for malic acid determination in wine," *Sens. Actuators B, Chem.*, vol. 202, pp. 971–975, Jun. 2014.

[91] P. Ekabutr, O. Chailapakul, and P. Supaphol, "Modification of disposable screen-printed carbon electrode surfaces with conductive electrospun nanofibers for biosensor applications," *J. Appl. Polym. Sci.*, vol. 130, no. 6, pp. 3885–3893, 2013.

[92] Q. Gao, Y. Guo, W. Zhang, H. Qi, and C. Zhang, "An amperometric glucose biosensor based on layer-by-layer GOx-SWCNT conjugate/redox polymer multilayer on a screen-printed carbon electrode," *Sens. Actuators B, Chem.*, vol. 153, no. 1, pp. 219–225, 2011.

[93] I. M. Apetrei and C. Apetrei, "The biocomposite screen-printed biosensor based on immobilization of tyrosinase onto the carboxyl functionalised carbon nanotube for assaying tyramine in fish products," *J. Food Eng.*, vol. 149, pp. 1–8, Oct. 2015.

[94] A. J. Bandodkar, R. Nuñez-Flores, W. Jia, and J. Wang, "All-printed stretchable electrochemical devices," *Adv. Mater.*, vol. 27, no. 19, pp. 3060–3065, 2015.

[95] R. K. Pal, A. A. Farghaly, C. Wang, M. M. Collinson, S. C. Kundu, and V. K. Yadavalli, "Conducting polymer-silk biocomposites for flexible and biodegradable electrochemical sensors," *Biosens. Bioelectron.*, vol. 81, pp. 294–302, Jan. 2016.

[96] A. R. Meier, M. Matteucci, R. F. Vreeland, R. Taboryski, and M. L. Heien, "Rapid voltammetric measurements at conducting polymer microelectrodes using ultralow-capacitance poly(3,4-ethylenedioxythiophene):Tosylate," *Langmuir*, vol. 32, no. 32, pp. 8009–8017, 2016.

[97] F. Gao, R. Yuan, Y. Chai, M. Tang, S. Cao, and S. Chen, "Amperometric third-generation hydrogen peroxide biosensor based on immobilization of Hb on gold nanoparticles/cysteine/poly(p-aminobenzene sulfonic acid)-modified platinum disk electrode," *Colloid Surf. A*, vol. 295, nos. 1–3, pp. 223–227, 2007.

[98] D. Polcari *et al.*, "Disk-shaped amperometric enzymatic biosensor for *in vivo* detection of d-serine," *Anal. Chem.*, vol. 86, no. 7, pp. 3501–3507, 2014.

[99] J. W. Severinghaus and P. B. Astrup, "History of blood gas analysis. IV. Leland Clark's oxygen electrode," *J. Clin. Monitor*, vol. 2, no. 2, pp. 125–139, 1986.

[100] B. Feng and Y. N. Liu, "A disposable cholesterol enzyme biosensor based on ferrocene-capped gold nanoparticle modified screen-printed carbon electrode," *Int. J. Electrochem. Sc.*, vol. 10, no. 6, pp. 4770–4778, 2015.

[101] B. Weng *et al.*, "Wholly printed polypyrrole nanoparticle-based biosensors on flexible substrate," *J. Mater. Chem. B*, vol. 2, no. 7, pp. 793–799, 2014.

[102] P. Kanyong, R. M. Pemberton, S. K. Jackson, and J. P. Hart, "Development of a sandwich format, amperometric screen-printed uric acid biosensor for urine analysis," *Anal. Biochem.*, vol. 428, no. 1, pp. 39–43, 2012.

[103] A. Erlenkötter, M. Kottbus, and G. C. Chemnitius, "Flexible amperometric transducers for biosensors based on a screen printed three electrode system," *J. Electroanal. Chem.*, vol. 481, no. 1, pp. 82–94, 2000.

[104] M. F. M. Noh and I. E. Tothill, "Development and characterisation of disposable gold electrodes, and their use for lead(II) analysis," *Anal. Bioanal. Chem.*, vol. 386, nos. 7–8, pp. 2095–2106, 2006.

[105] P. Kotzian *et al.*, "Oxides of platinum metal group as potential catalysts in carbonaceous amperometric biosensors based on oxidases," *Sens. Actuators B, Chem.*, vol. 124, no. 2, pp. 297–302, 2007.
[106] J. Barton *et al.*, "Screen-printed electrodes for environmental monitoring of heavy metal ions: A review," *Microchimica Acta*, vol. 183, no. 2, pp. 503–517, 2016.
[107] S. Centi, A. I. Stoica, S. Laschi, and M. Mascini, "Development of an electrochemical immunoassay based on the use of an eight-electrodes screen-printed array coupled with magnetic beads for the detection of antimicrobial sulfonamides in honey," *Electroanalysis*, vol. 22, no. 16, pp. 1881–1888, 2010.
[108] S. Piermarini, L. Micheli, N. H. S. Ammida, G. Palleschi, and D. Moscone, "Electrochemical immunosensor array using a 96-well screen-printed microplate for aflatoxin B1 detection," *Biosens. Bioelectron.*, vol. 22, no. 7, pp. 1434–1440, 2007.
[109] J. Kim *et al.*, "Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics," *Biosens. Bioelectron.*, vol. 74, pp. 1061–1068, Jun. 2015.
[110] K. Malzahn, J. R. Windmiller, G. Valdés-Ramírez, M. J. Schöning, and J. Wang, "Wearable electrochemical sensors for *in situ* analysis in marine environments," *Analyst*, vol. 136, no. 14, pp. 2912–2917, 2011.
[111] W. Jia *et al.*, "Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration," *Anal. Chem.*, vol. 85, no. 14, pp. 6553–6560, 2013.
[112] J. S. Shim, M. J. Rust, and C. H. Ahn, "A large area nano-gap interdigitated electrode array on a polymer substrate as a disposable nano-biosensor," *J. Micromech. Microeng.*, vol. 23, no. 3, p. 035002, Jan. 2013.
[113] C. Lete, B. Lakard, J. Y. Hihn, F. J. del Campo, and S. Lupu, "Use of sinusoidal voltages with fixed frequency in the preparation of tyrosinase based electrochemical biosensors for dopamine electroanalysis," *Sens. Actuators B, Chem.*, vol. 240, pp. 801–809, Mar. 2017.
[114] R. Popovtzer, T. Neufeld, D. Biran, E. Z. Ron, J. Rishpon, and Y. Shacham-Diamand, "Novel integrated electrochemical nano-biochip for toxicity detection in water," *Nano Lett.*, vol. 5, no. 6, pp. 1023–1027, 2005.
[115] M. Benlarbi, L. J. Blum, and C. A. Marquette, "SU-8-carbon composite as conductive photoresist for biochip applications," *Biosens. Bioelectron.*, vol. 38, no. 1, pp. 220–225, 2012.
[116] G. Vásquez, A. Rey, C. Rivera, C. Iregui, and J. Orozco, "Amperometric biosensor based on a single antibody of dual function for rapid detection of Streptococcus agalactiae," *Biosens. Bioelectron.*, vol. 87, pp. 453–458, Feb. 2017.
[117] J. C. Chou, R. T. Chen, Y. H. Liao, J. S. Chen, M. S. Huang, and H. T. Chou, "Dynamic and Wireless Sensing Measurements of Potentiometric Glucose Biosensor Based on Graphene and Magnetic Beads," *IEEE Sens. J.*, vol. 15, no. 10, pp. 5718–5725, Mar. 2015.
[118] A. Tarasov *et al.*, "Riordan, M. H. Mooney, and E. M. Vogel, "A potentiometric biosensor for rapid on-site disease diagnostics," *Biosens. Bioelectron.*, vol. 79, pp. 669–678, Jan. 2016.
[119] E. Ogabiela and S. B. Adeloju, "A potentiometric phosphate biosensor based on entrapment of pyruvate oxidase in a polypyrrole film," *Anal. Methods*, vol. 6, no. 14, pp. 5290–5297, 2014.
[120] N. Nesakumar, S. Sethuraman, U. M. Krishnan, and J. B. B. Rayappan, "Cyclic voltammetric acetylcholinesterase biosensor for the detection of captan in apple samples with the aid of chemometrics," *Anal. Bioanal. Chem.*, vol. 407, no. 16, pp. 4863–4868, 2015.
[121] M. Zaib, A. Saeed, I. Hussain, M. M. Athar, and M. Iqbal, "Voltammetric detection of As(III) with Porphyridium cruentum based modified carbon paste electrode biosensor," *Biosens. Bioelectron.*, vol. 62, pp. 242–248, Mar. 2014.
[122] X. Sun, C. Zhai, and X. Wang, "Amperometric acetylcholinesterase biosensor based on O-carboxymethyl chitosan-gold nanoparticle nanocomposite by in-situ synthesis method," *IEEE Sensors J.*, vol. 13, no. 1, pp. 172–179, Feb. 2013.
[123] N. Nasirizadeh and H. R. Zare, "Differential pulse voltammetric simultaneous determination of noradrenalin and acetaminophen using a hematoxylin biosensor," *Talanta*, vol. 80, no. 2, pp. 656–663, 2009.
[124] G. Ertürk, D. Berillo, M. Hedström, and B. Mattiasson, "Microcontact-BSA imprinted capacitive biosensor for real-time, sensitive and selective detection of BSA," *Biotechnol. Rep.*, vol. 3, pp. 65–72, Nov. 2014.
[125] M. Labib, M. Hedström, M. Amin, and B. Mattiasson, "A multipurpose capacitive biosensor for assay and quality control of human immunoglobulin G," *Biotechnol. Bioeng.*, vol. 104, no. 2, pp. 312–320, 2009.
[126] M. Labib, M. Hedström, M. Amin, and B. Mattiasson, "A novel competitive capacitive glucose biosensor based on concanavalin A-labeled nanogold colloids assembled on a polytyramine-modified gold electrode," *Anal. Chim. Acta*, vol. 659, nos. 1–2, pp. 194–200, 2010.
[127] M. Hoyos-Nogués *et al.*, "Impedimetric antimicrobial peptide-based sensor for the early detection of periodontopathogenic bacteria," *Biosens. Bioelectron.*, vol. 86, pp. 377–385, Feb. 2016.
[128] A. Hushegyi, D. Pihákova, T. Bertok, V. Adam, R. Kizek, and J. Tkac, "Ultrasensitive detection of influenza viruses with a glycan-based impedimetric biosensor," *Biosens. Bioelectron.*, vol. 79, pp. 644–649, May 2016.
[129] S. R. Teixeira *et al.*, "Polyaniline-graphene based α-amylase biosensor with a linear dynamic range in excess of 6 orders of magnitude," *Biosens. Bioelectron.*, vol. 85, pp. 395–402, Nov. 2016.
[130] M. S. Cheng, S. H. Lau, K. P. Chan, C. S. Toh, and V. T. Chow, "Impedimetric cell-based biosensor for real-time monitoring of cytopathic effects induced by dengue viruses," *Biosens. Bioelectron.*, vol. 70, pp. 74–80, Dec. 2015.

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