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Electrochemical and optical biosensors for biological sensing applications

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ABSTRACT: Biosensors are analytical tools that play an important role in advanced applications, especially in the monitoring of biological molecules related to health conditions. A variety of biosensors have been described in this review to emphasize the important applications in the biological field. These biosensors are categorized into four groups depending on the bioreceptor molecules, including enzymatic biosensors, immuno-based biosensors, DNA-based biosensors, and other interesting biosensors. Many platforms and materials have been developed with various benefits. The detection principles of the biosensors in this review are focused on optical and electrochemical techniques due to their characteristic advantages. Information of using novel materials for fabrication of biosensors is also provided.

KEYWORDS: Biosensors, enzymatic biosensors, immuno-based biosensors, DNA-based biosensors, electrochemical detection, optical detection

INTRODUCTION

Biosensors are analytical tools for converting biological responses to detectable signals. The recognition system of a biosensor utilizes a biochemical mechanism that is highly specific and independent of physical limitations such as pH and temperature [1, 2]. Research and development of biosensors have been universally studied, contributing to advancements in drug discovery, monitoring disease-causing bacteria and markers, and detecting pollutants. The characteristics of biosensors are (i) selectivity to the analytes of interest, (ii) reproducibility to produce identical responses, (iii) stability under ambient disturbances, (iv) sensitivity to analytes down to nano to femto-levels to ensure their existence in the sample, and (v) linearity in the range of analyte concentrations, which is related to the accuracy of the measured response [3].

The typical components of a biosensor are shown in Fig. 1, including the analyte of interest, bioreceptor molecules, transducer, electronic system, and display [2]. In a biosensor, the most important component is the bioreceptor, which is designed to specifically interact with the analyte of interest. The key requirement of bioreceptors is high selectivity to the analyte in the matrix of the sample and contaminant. Biosensors are categorized into four groups based on their bioreceptors, including enzymes, antibodies/antigens, nucleic acids, and others [1]. Various biosensor platforms for the application of biological compound detection have been employed on a variety of transducers, such as optical, electrical, electrochemical, and thermal transducers. Optical spectroscopy and electrochemistry are the most preferred transducers used in biosensors due to their characteristic strength [4]. Optical biosensors, including fluorescence and colorimetry, work on the principle of the generation of an optical signal from the specific on-site interaction of biosensor elements such as enzyme substrates and metal nanoparticles [5]. In electrochemical biosensors, electrochemical signals are produced by the redox reaction of electroactive species in the biosensor system [6]. In this review, we emphasize an overview of optical and electrochemical biosensors for the application of biological compound detection categorized by their bioreceptor mechanisms.

Enzymatic biosensors

An "enzyme biosensor" is an analytical device integrating an enzyme as a bioreceptor with a trans-

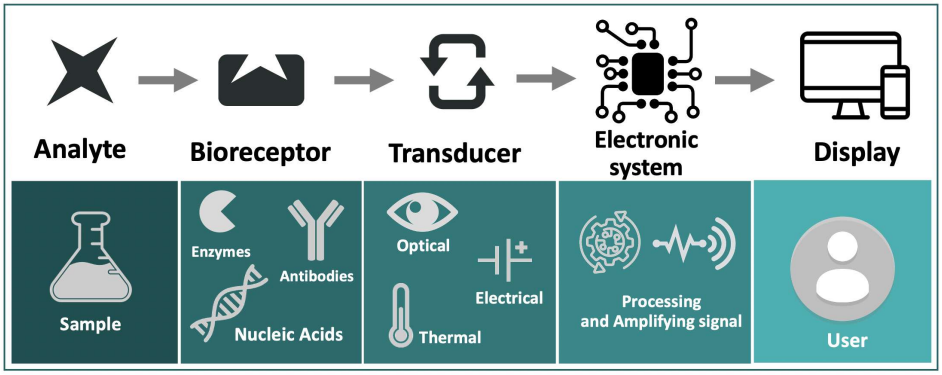

Fig. 1 Typical components of a biosensor.

Table 1 Summarized data of the enzymatic biosensors based on electrochemical and optical detection.

Detection	Technique	Analyte(s)	Enzyme	LOD	Linear range	Year
Electro-chemical	Amperometry	Lactate	Lactate oxidase	19 μM	50 μM–10 mM	2019 [7]
	Amperometry	Lactate	Lactate oxidase	5.0 μM	5 μM–5.0 mM	2019 [8]
	Amperometry	Cholesterol	Cholesterol oxidase	0.52 mM	0.6–6.0 mM	2016 [9]
	Amperometry	Cholesterol	Cholesterol oxidase	0.99 mg/dl	3.9–773.4 mg/dl	2015 [10]
	Amperometry	Cholesterol	Cholesterol oxidase	0.25 mg/dl	0.4–270.7 mg/dl	2015 [11]
	Amperometry	Glucose	Glucose oxidase	0.29 mM	1–10 mM	2015 [12]
	Amperometry	Cholesterol	Cholesterol oxidase	1 μM	50 μM–10 mM	2014 [13]
	Amperometry	Glucose	Glucose oxidase	–	0–10 mM	2011 [14]
	Amperometry	Cholesterol	Cholesterol oxidase	1 nM	1 μM–7 mM	2011 [15]
	Amperometry	Glucose	Glucose oxidase	0.21 mM	0–100 mM	2009 [16]
		Lactate	Lactate oxidase	0.36 mM	0–50 mM	
		Uric acid	Uricase	1.38 mM	0–35 mM	
Optical	Fluorescence spectroscopy	Organophosphorus and carbamate insecticides (pirimicarb, dichlorvos and carbaryl)	Acetylcholinesterase and Choline oxidase	0.05 mg/l (pirimicarb) 0.01 mg/l (dichlorvos) 0.01 mg/l (carbaryl)	–	2017 [17]
	Colorimetry	Glucose	Glucose oxidase	0.5 mM	–	2010 [18]
		Lactate	Lactate oxidase	1.0 mM		
		Uric acid	Uricase	0.1 mM		

ducer (e.g., electrode) to generate a detectable signal correlated with a substrate/inhibitor/co-factor concentration. The glucose sensor is one of the most universal examples of the enzyme-based biosensors that could save millions of lives from diabetes. In essence, there are two main classes of enzymatic assays: enzyme catalysis and enzyme inhibition. For enzyme catalysis, the presence of the analyte can increase the enzyme catalytic activity. Accordingly, the response signal (such as glucose, lactate, uric acid, and cholesterol) is increased. In contrast, for enzyme inhibition, the presence of analyte can inhibit the catalytic activity of the enzyme-substrate since the analyte takes part as an inhibitor in the substrate-enzyme system. Organophosphorus pesticides, for instance, are a group of common inhibitors that can inhibit the catalytic activity of acetylcholinesterase and its substrate. Thus, a decrease in the response signal is observed. Selected examples of enzymatic biosensors published by our group are presented in Table 1.

Immuno-based biosensors

Immunosensors are biosensors that were established based on the high affinity of antibodies to form a stable complex with their antigens [1]. There is a specific antibody for each antigen; thus, the detection of antigen/antibody of interest can be performed in the presence of others. The application of antigen/antibody formation is important in medical diagnostics for many diseases [19]. Several optical and electrochemical transducers have been employed.

One of the most attractive optical biosensors is the combination of antigen-antibody specific binding and the catalytic reaction of labeling enzymes that enable highly selective and sensitive biochemical assays. Alkaline phosphatase (ALP) is

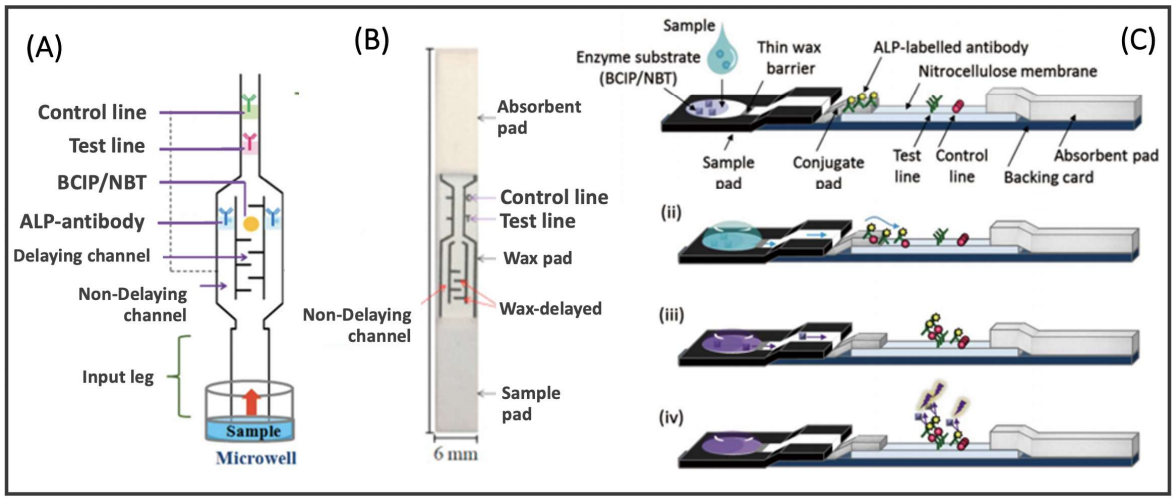

Fig. 2 Illustration of (A) automated paper-based biosensor for hCG detection [20], (B) automated wax-printed paper-based lateral flow device for AFP detection [21] and (C) wax-assisted one-step lateral flow test devices for mouse IgG detection [22].

a frequently used enzyme for immunosystem labeling [20–22]. The introduction of 5-bromo-4-chloro-3′-indolyl phosphate *p*-toluidine salt/nitro-blue tetrazolium chloride (BCIP/NBT) and ALP substrate into a system containing ALP-labeled antibodies produced an insoluble blue to purple product that can be observed visually [20–22]. In the common scheme of ALP/BCIP/NBT-based immunosensors, the operation requires the addition of an enzyme-substrate after antigen-antibody complex formation, necessitates a complicated multi-step process including mixing, washing, and incubation [20]. Therefore, many automated immunosensors have been developed by creating a delay platform and pre-depositing enzyme substrates to allow the sequential reagent delivery system by a single introduction of the sample solution, which has been applied for the detection of various biological molecules such as human chorionic gonadotropin (hCG) [20], alpha-fetoprotein (AFP) [21], and mouse IgG [22]. The designs of automated immunosensors are shown in Fig. 2.

Although the use of the catalytic reaction of a labeling enzyme provides high sensitivity, some of the disadvantages include instability of the enzyme and substrate and the increased complexity of the assay. Moreover, the addition of a substrate solution is required to produce a measurable signal. Metal nanoparticles are popular labeling materials for antigens/antibodies in immunoassays. Compared to an enzyme-labeled immunosensor, the color of particular metal nanoparticles occurs naturally, and there is no need for the substrate solution. Gold nanoparticles (AuNPs) have gained much attention as metal nanoparticles in colorimetric immunosensors because of their long-term stability, easily controllable size distribution, and good compatibility with biological molecules, such as antibodies, antigens, proteins, DNA, and RNA [19, 23]. AuNP-conjugated antibodies/antigens have been applied in various immunoassay platforms for the detection of *Salmonella typhi* [23], ractopamine [24] and cortisol [25]. However, the sensitivity of AuNP-based immunosensors was not sufficient in a particular application. Many researchers have proposed procedures to enhance the sensitivity, such as modifying the surface of AuNPs with a europium (III)-chelate fluorophore-doped silica shell for the detection of human thyroid-stimulating hormone (hTSH) [26] and using a silver enhancement solution after antigen-antibody complex formation for the detection of cortisol [27]. This approach demonstrated the versatility of metal nanoparticles in the application of immunosensors.

Electrochemical detection is another popular platform employed in biosensors for ultrasensitive detection. The important key of electrochemical immunosensors is the presence of electroactive species in the system. The electroactive species could exist either as a labeling agent of an antibody/antigen or working solution or the generated electroactive species products. Various electroactive species have been used as labeled agents, such as AuNPs for the detection of hCG [28], *Salmonella typhi* [29] and

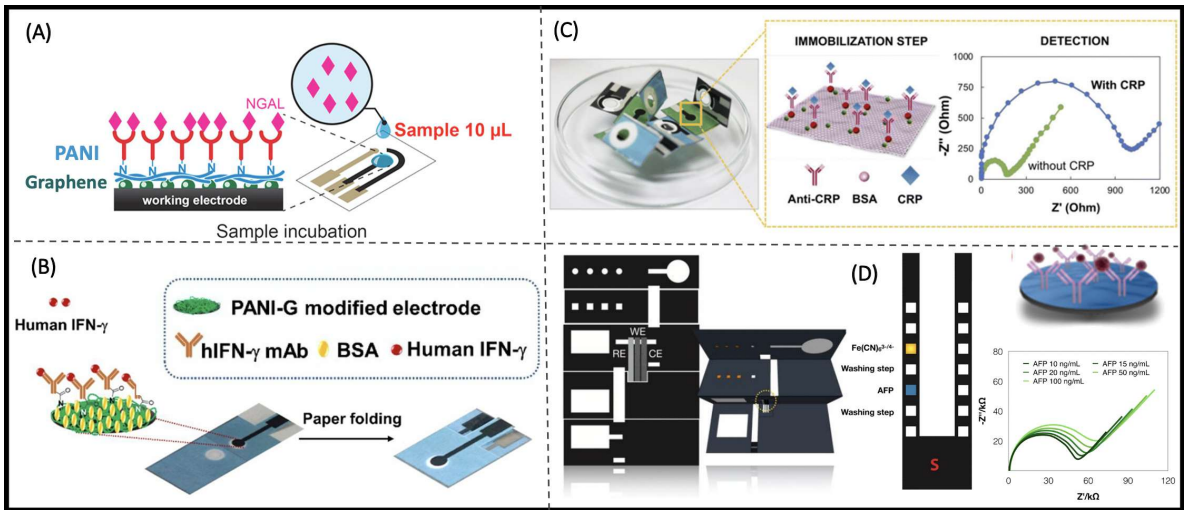

Fig. 3 Illustration of (A) G/PANI-modified screen-printed carbon electrode for NGAL detection [35], (B) PANI-modified screen-printed graphene electrode for IFN-γ detection [36], (C) origami paper-based immunosensor for CRP detection [37] and (D) stopped-flow 3D sequential microfluidic platform for label-free electrochemical immunosensor of AFP [38].

leptospirosis [30], platinum nanoparticles (PtNPs) for the detection of hCG [31], anthraquinone for the detection of c-reactive protein (CRP) [32] and Cd-Se/ZnS quantum dots for the detection of phosphorylated bovine serum albumin [33]. Using these labeled materials, the electrochemical signals directly generated their unique electrochemical properties. Moreover, the catalytic enzyme reaction was applied in an electrochemical immunosensor via the reaction of enzyme-labeled antibodies and a particular substrate to generate electroactive species products such as the detection of mouse IgG using ALP as the labeled enzyme and 2-phospho-L-ascorbic acid as the substrate [34].

Label-free immunosensors are platforms that eliminate complicated and time-consuming labeling preparation. The direct conjugation of the target molecule is achieved by capture of antibodies at the electrode surface. In one of these designs, the electrochemical signal was monitored by measuring the changes in the charge transfer efficiency between the electrode and the redox couple $[Fe(CN)_6]^{3-/4-}$ using an electrochemical technique. Modifications of the electrode surface with various nanomaterials were performed to anchor antibodies on the electrochemical transducer surface and enhance the sensitivity of the detection, as shown in Fig. 3. The fabrication of the biorecognition surface was performed via EDC/NHS chemistry. Polyaniline (PANI) and graphene (G) were presented as modifiers on electrodes and applied in a different design for the detection of neutrophil gelatinase-associated lipocalin (NGAL) (Fig. 3A) [35] and human interferon-gamma (IFN-γ) (Fig. 3B) [36]. Excellent sensitivities were observed due to the electrocatalytic properties and high surface area of the PANI/G nanocomposite. AuNPs were also presented as a modifier for the immobilization of antibodies. The electrodeposition of AuNPs was performed followed by the immobilization of antibodies via L-cysteine and EDC/NHS. This approach was applied in an origami paper-based immunosensor for the detection of CRP, as shown in Fig. 3C [37]. In addition, a stopped-flow 3D sequential microfluidic platform has been developed and applied in label-free electrochemical immunosensors (Fig. 3D) [38]. Using this device, the detection of AFP could be achieved using one-step manipulation, which eliminates the complex procedure of multiple-step reagent manipulation.

DNA-based biosensors

Biosensors play a vital role in several areas of life science, especially in medical diagnosis. Currently, biosensors are extensively used as tools to accurately identify a disease. However, to access a related biomarker of a disease at an early stage or a superficial level, a sensitive biosensor is required. A biosensor consists of three main parts: a biorecognition element, transducer, and a procedure for displaying data. Among these compo-

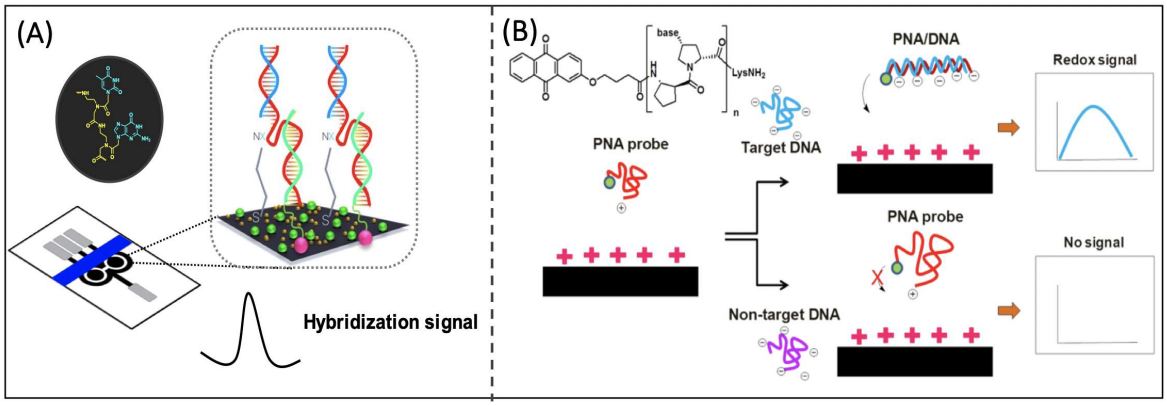

Fig. 4 Illustration of the hybridization-induced conformation (A and B) for the detection of target DNAs [40, 43].

nents, the recognition layers (e.g., enzyme, antibody, DNA, or DNA-analogs and aptamers) are the most significant parts and have been utilized for sensitive and selective binding with the target molecules. According to previous studies, peptide nucleic acid (PNA) probes have been shown to offer higher sensitivity, specificity, and fast hybridization kinetics. Additionally, the hybridization process of PNA requires a shorter probe length than DNA. Recently, a new conformationally restricted pyrrolidinyl PNA was developed. The structure of this newly introduced PNA is made up of a α/β-peptide backbone derived from D-proline/2-aminocyclopentanecarboxylic acid (acpcPNA). This structural PNA system exhibits advantages, including a more durable binding affinity and a higher specificity towards a complementary target DNA than the initially discovered PNA or other DNA analogs. Because of these excellent properties, acpcPNA has been widely applied as a sensor probe to detect target biomarkers in combination with various detection methods, such as electrochemical detection and colorimetric detection. Hence, the utilization of acpcPNA probes combined with these strategies for disease identification will be the focus. Electrochemical detection is a promising method for the sensitive and selective determination of target DNAs. The detection principles of this approach are based on the hybridization-induced conformational or resistant changes in redox tags or mediators used (e.g., anthraquinone (AQ) or $[Fe(CN)_6]^{3-/4-}$, resulting in differences in signal responses. For medical diagnosis, acpcPNA has been successfully used as a probe to selectively detect the high-risk group of human papillomavirus DNAs (HPV DNA) and *Mycobacterium tuberculosis* DNA in practical samples for the evaluation of cervical cancer and tuberculosis, respectively (Fig. 4A) [39–42]. In addition, acpcPNA has been utilized as a specific probe for the detection of isothermally amplified shrimp white spot syndrome virus DNA in agricultural applications (Fig. 4B) [43]. These fabricated platforms might be an alternative tool for broader applications in medical diagnosis.

To the best of our knowledge, colorimetric detection is particularly attractive and can achieve the rapid detection of a disease, as naked-eye observation can only interpret a screening result. To date, colorimetric assays based on the aggregation of nanoparticles, such as silver (AgNPs) and gold nanoparticles (AuNPs) enhancing the signal, have received considerable interest in medical diagnosis. In 2017, Teengam reported a colorimetric assay for the simultaneous detection of virus MERS-CoV, MTB, and HPV DNAs based on acpcPNA-induced nanoparticle aggregation [44]. This proposed platform was successfully applied to determine real DNA samples and provided satisfactory results. By using this method, three DNA sequences related to the target diseases can simultaneously be evaluated, reducing turn-around-time (TAT).

Other applications

There are biosensors that have not been used in the aforementioned bioreceptors. Numerous chemical substances related to health conditions have been demonstrated. The direct colorimetric assay presented the simplest detection of biological molecules. As the simplest demonstration, the determination of the albumin (AL) to creatinine (CR) ratio has been employed for the screening of diabetes. Bromocresol green was used to evaluate

Table 2 The merits of other applications of biosensors.

Receptor	Analyte(s)	Transducer	Description	Ref.
–	Albumin (AL) and Creatinine (CR)	Colorimetry	Bromocresol green for total AL and CR Jaffé picric acid for selective CR	[45]
APBA and ESM	Total hemoglobin and glycated hemoglobin (HbA1c)	EIS	Affinity sensor Label-free Paper-based SPCE	[46]
APBA	HbA1c	EIS	Affinity sensor Label-free Gold microelectrode array (IDA) chips	[47]
CDP-choline	CRP	EIS	Affinity sensor Label-free Phosphocholine-modified SPCE	[48]
PMPC-SH	CRP	DPV	Affinity sensor Label-free AuNPs modified SPCE	[49]
–	Glucose	Chronoamperometry	Nonenzymatic sensor CoPc/G/IL paper-based SPCE	[51]
–	Glucose	Chronoamperometry	Nonenzymatic sensor Pt/Au/BDD electrode	[52]
–	Creatinine	Amperometry	Nonenzymatic sensor CuO/IL/ERGO modified paper-based SPCE	[53]
–	Dopamine	SWV	Nonenzymatic sensor G/PANI/PS nanofiber-modified SPCE	[55]
–	Dopamine	SWV	Nonenzymatic sensor SDS modified ePAD	[54]
–	Norepinephrine and Serotonin	SWASV	Nonenzymatic sensor BDDPE	[56]
–	Norepinephrine, serotonin and *p*-aminophenol	DPV	Nonenzymatic sensor Janus-ePAD BDDPE	[57]

EIS: electrochemical impedance spectroscopy, DPV: differential pulse voltammetry, SWV: square wave voltammetry, SWASV: square wave anodic stripping voltammetry, SPCE: screen-printed carbon electrode, APBA: 3-aminophenyl boronic acid, ESM: boronate-modified eggshell membrane, CDP-choline: cytidine 5′-diphosphocholine sodium salt dihydrate, PMPC-SH: thiol-terminated poly(2-methacryloyloxyethyl phosphorylcholine), CoPc/G/IL: cobalt phthalocyanine-ionic liquid-graphene composite, Pt/Au/BDD electrode: Bimetallic Pt-Au nanocatalysts electrochemically deposited on boron-doped diamond electrode, CuO/IL/ERGO: copper oxide/ionic liquid/electrochemically reduced graphene oxide composite, G/PANI/PS: graphene/polyaniline/polystyrene, SDS modified ePAD: sodium dodecyl sulfate-modified electrochemical paper-based analytical device, BDDPE: boron doped-diamond paste electrode.

the total Al and CR, while Jaffé picric acid was used for selective detection of CR. The distinguished color change can be observed by the naked eye [45].

Affinity sensors are point-of-care devices based on either a biological recognition element or chemical recognition that are highly suitable for monitoring biomarkers [46]. For example, boronic acid and phosphocholine have been presented as chemical recognition sites for the detection of glycated hemoglobin [46, 47] and c-reactive protein [48, 49], respectively. Through the modification of these recognition molecules on the electrode surface, electrochemical detection was performed to examine the concentration of the target analyte.

Nonenzymatic electrochemical assays are another platform that is applied as a biosensor. Nonenzymatic biosensors are based on the catalytic reaction of the analyte of interest by a variety of materials on the electrochemical transducer [50]. The concentration of the analytes was determined by direct electrochemical detection. Nonenzymatic biosensors have been applied for the detection of many substances, such as glucose [51, 52], creatinine [53], dopamine [54, 55], and neurotransmitters [56, 57]. The merits of other applications are shown in Table 2.

CONCLUSION

Various biosensor platforms have been developed and categorized based on their bioreceptors. Each type of biosensor presents fascinating properties. The development of biosensors mainly includes miniaturizing biosensors, automating operations, using novel biorecognition molecules, employing new material devices, and improving the analytical performance of biosensors towards target molecules. With a wide range of target molecules, many bioreceptors are used including enzymes, antigens/antibodies, nucleic acids, and synthetic receptors, and a variety of detection methods have been employed including electrochemical, colorimetric and spectroscopic detection. Thus, the development of biosensors plays an important role in many applications, such as those regarding the environment and food contaminants, especially in the diagnosis of many diseases. Future work should include the integration of technology that enables biosensors to be empowered in a wide range of applications with simple, user-friendly, inexpensive, less time analysis, low reagent and sample volumes.

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