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Review Article

Electrochemical biosensors for rapid pathogen detection

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Abstract

Rapid pathogen detection is an emerging issue in clinical, environmental, and food industry sectors. Biosensors can represent a solution to culture-based and molecular methods as they respond to sensitivity, specificity, and rapidity needs. Screen-printed electrodes have been used in association with nanoparticles to increase the signal and improve sensitivity reaching low numbers of the targets. Antibodies, DNA probes, and aptamers are mainly used to functionalize the working electrodes to ensure high specific pathogen detection by the use of voltammetry, impedance spectroscopy, amperometry, and conductivity. Electrochemical biosensors can be miniaturized to construct portable devices useful for *in situ* assays.

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Genosensors, Aptasensors, Immunosensors, Pathogens.

Introduction

Bacteria responsible for multidrug resistance like *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species (ESKAPE), and some foodborne pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* spp. are responsible for 700,000 deaths per year globally and have a big economic impact [1,2]. To control pathogenic bacteria spreading, new detection methods are urgently demanded. Moreover, pandemics due to respiratory viruses have demonstrated the need of sensitive, specific, easy to use, and rapid methods for virus detection. Classical microbiological methods require trained operators and a long time for analyses. In addition, stressed bacteria can enter in the viable but not culturable status that cannot be detected using culture methods. A plethora of molecular biology methods has been developed with this aim, from antigen–antibody–based methods (enzyme-linked immunosorbent assay) to polymerase chain reaction–based methods, which can suffer from high cost, time-consuming labors, and inhibition of DNA polymerase for the presence of different compounds in the sample matrix.

Biosensors have emerged as advantageous analytical devices able to analyze biological samples by combining a recognition biological component (called bioreceptor) with a transducer, which converts biological activity into a quantifiable signal, usually optical or electrical [1,3–7]. Biosensors for pathogen detection can be classified based on the bioreceptor to immunosensors (antibody–antigen), genosensors (DNA probe), aptasensors (DNA or RNA aptamer), and so on, or on the transducer used in optical, piezoelectric, and electrochemical sensors [8]. Electrochemical biosensors (EBs) can ensure fast response, robustness, cost-effectiveness, high selectivity, high sensitivity, and on-site detection [9,10]. In addition, they require low volumes of sample and can be easily miniaturized, which makes them useful for multiplex and point-of-care sensing. Most EBs use voltammetry, impedance spectroscopy, amperometry, and conductivity. EBs may operate in a label-free mode, when the transducer directly converts the recognition event into an electrical signal and in a labeled-based mode when electrochemical labels (such as nanoparticles or enzymes) are coupled with the biorecognition event to generate a readable signal. In both cases, in their operating range, EBs provide signal readout proportional to the analyte concentrations enabling quantitative detection.

In this short review, we present the advances in the field of EBs for the rapid detection of bacteria and viruses in the recent few years.

Electrode functionalization

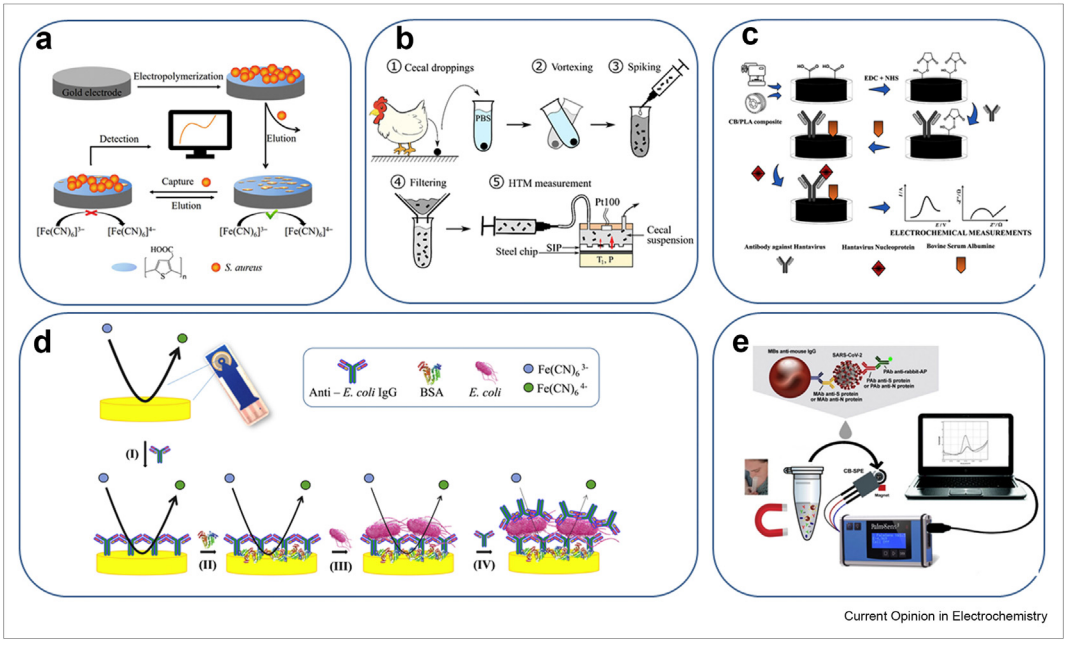
The choice of the working electrode and its surface modification are the key factors ensuring high

selectivity, low limit of detection (LOD), and long-term stability of EBs. Working electrodes are usually made of noble metals (gold, silver, platinum), semiconducting metal oxides (ZnO, indium tin oxide, tin dioxide), and carbon-based materials (graphene, glassy carbon, black carbon). Gold screen-printed electrodes (SPEs) are largely used for pathogen detection as they are affordable, easy to functionalize with biological molecules, and allow a good sensitivity. In recent years, printed technologies using biodegradable electrodes (such as cellulose paper and nitrocellulose membrane), combined with nanomaterials or conducting polymers have allowed the development of low-cost point-of-care devices with enhanced sensing characteristics [10–12]. Using flexible printing, the working, reference and counter electrodes can be created within few mm^2 and the pathogen can be detected in only few µL of sample [13,14].

Electrodes can be further modified with conducting polymers, metal or carbon-based nanoparticles to improve specificity, electrocatalytic properties, stability, and conductivity, or to enable efficient functionalization with biological materials. Over the past few years, molecularly imprinted polymers possessing specific recognition sites complementary to the template have demonstrated their great potential in pathogen detection. A gold electrode surface modified with the bacteria-imprinted conductive poly (3-thiopheneacetic acid) (BICP) film template enabled detection of *S. aureus* in contaminated milk samples with the LOD of only 2 CFU/mL within 10 min (Figure 1a) [15]. An LOD of 1.1×10^3 CFU/mL for *Campylobacter coli* and 2.7×10^4 CFU/mL for *Campylobacter jejuni*, directly in chicken cecal droppings within a 60-min analysis (Figure 1b) was obtained by Givanoudi et al. [16] with surface-imprinted polymer receptors.

The attachment of biological molecules on gold electrodes is usually done by the formation of self-assembled layers through the gold-thiol chemistry. Self-assembled mono- or multilayers provide support for the immobilization of recognition elements. For instance, bioorthogonal conjugation methods, such as copper-catalyzed click reaction, enable electrode modification for covalent immobilization of antibodies for *E. coli* sensing [17]. Nanocomposites, including hybrid ones, are used for electrode modification to increase its surface area for immobilization of many recognition elements, which improve sensor sensitivity without obstructing electron exchange, like recently demonstrated for *Helicobacter pylori* detection [18]. Various SPEs incorporation new materials are constantly developed for biological assays, like these based on carbon nano-onions [19].

Figure 1



Examples of electrochemical biosensors using various functionalizations and protocols for pathogen detection. **(a)** Gold electrode modified with BICP film for *S. aureus* detection (adapted with permission from Ref. [15]). **(b)** Schematic workflow of establishing the dose–response curve of the EB system for direct detection of *Campylobacter* cells (adapted with permission from Ref. [16]). **(c)** Buildup of polylactic acid/carbon black working electrodes (adapted with permission from Ref. [22]). **(d)** Stepwise functionalization and detection by an immunosensor *E. coli* detection in water (adapted with permission from Ref. [25]). **(e)** Immunosensor coupled to magnetic beads for SARS-CoV-2 detection (adapted with permission from Ref. [27]).

Electrochemical immunosensors

Electrochemical immunosensors may directly generate a signal upon the antigen binding to the electrode carrying immobilized antibodies. Such an immunosensor was developed for the detection of viral protein PB1-F2 by immobilizing specific anti−PB1-F2 antibodies on the surface of the gold microelectrode modified with conducting polypyrrole-bearing ferrocene as a redox marker [20,21]. Recently, the detection of hantavirus *Araucaria* nucleoprotein by an immunosensor constructed using a 3D conductive filament of carbon black and polylactic acid was reported (Figure 1c) [22]. The antibody was anchored by the amino-link directly at the filament surface after its activation. Cyclic voltammetry and impedance spectroscopy were used for the evaluation of each step of the sensor construction and quantitative detection of the nucleoprotein with an LOD of 22 µg/mL in human serum samples. The utilization of antibodies directed toward bacterial surface epitopes decreases the overall time of detection, as no extraction/purification of bacterial biomarkers is needed. A laser-induced graphene electrode functionalized with antibodies directed toward *Salmonella* Typhimurium was shown to detect live *Salmonella* cells in chicken broth with an LOD of 13 CFU/mL within 22 min without the need for sample preconcentration or redox labeling [23].

In the indirect assay, the antigen is immobilized on the electrode and its binding to a primary antibody is revealed through the labeled secondary antibody. Recently, such a type of electrochemical immunosensor was introduced for the detection of tuberculosis [24]. A screen-printed carbon electrode was modified by quantum dot (CdSe/ZnS QD) and functionalized silica nanoparticles (SiNPs) and linked to the enzyme catalase through antigen−antibody binding for the detection of *Mycobacterium tuberculosis* secretory protein CFP10−ESAT6 (antigen) using a differential pulse voltammetry.

To increase the sensitivity and specificity, immunosensors may work as a capture 'sandwich' assay. For this, a capturing antibody is preimmobilized on the electrode and a sandwich with the analyte is formed between the first antibody (named capturing antibody) and the second antibody (named detecting antibody). The detection antibody is usually labeled with a redox marker or an enzyme to enable the detection. Cimafonte et al. [25] described an impedimetric immunosensor using a gold SPE for the detection of *E. coli* in drinking water. Anti-*E. coli* antibodies directed against a bacterial surface epitope were covalently attached on the electrode surface by the photochemical immobilization technique (Figure 1d). By measuring the resistance modifications because of the antibody capturing the bacterium *E. coli* was detected with the sensitivity of 3×10^1 CFU/mL after the addition of the detecting antibody to create a sandwich in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe.

Finally, the electrochemical immunoassay may combine magnetic bead-based pathogen preconcentration. This strategy provides higher test sensitivity when a few analyte entities need to be detected in a large volume of sample [26]. Magnetic beads decorated with the capturing antibody are added to the sample to bind the analyte. The external magnetic field moves then magnetic beads in a small volume and a simple washing step eliminates any matrix. Subsequently, the beads carrying analyte are deposited on the working electrode surface using a miniaturized magnet and electrochemical measurements are performed. The high efficiency of such detection was recently shown for SARS-CoV-2 detection in saliva [27]. Using a magnetic bead-based EB with a carbon black-based screen-printed working electrode, two SARS-CoV-2 proteins (spike, S and nucleocapsid, N) were detected. The sandwich format was obtained with anti-S and anti-N antibody immobilized on magnetic beads (Figure 1e). The binding was evaluated using secondary antibodies labeled with alkaline phosphatase enzyme. The miniaturized EB detected S and N protein with an LOD equal to 19 ng/mL and 8 ng/mL in untreated saliva, respectively, within 30 min. Murasova et al. [28] combined an electrochemical immunosensor with specific and selective magnetoimmunocapturing for the detection of *Salmonella* Typhimurium in milk. The capturing antibodies were labeled with Quantum dots (CdTe/COOH) for their excellent electrical properties and ability to provide high current responses. Square-wave anodic stripping voltammetry detected the metal ion signals that were proportional to the amounts of captured bacteria cells with an LOD of 4 CFU/mL. The whole analysis took 2.5 h.

Electrochemical genosensors

The detection of DNA hybridization, using electrochemical genosensors, is the basis for the pathogen DNA/RNA detection. The most common approach is the immobilization of thiolated single-strand DNA probes (ssDNA) over a gold electrode surface (or surface containing AuNPs) by the spontaneous formation of self-assembled monolayers and the subsequent hybridization of complementary DNA sequences [29].

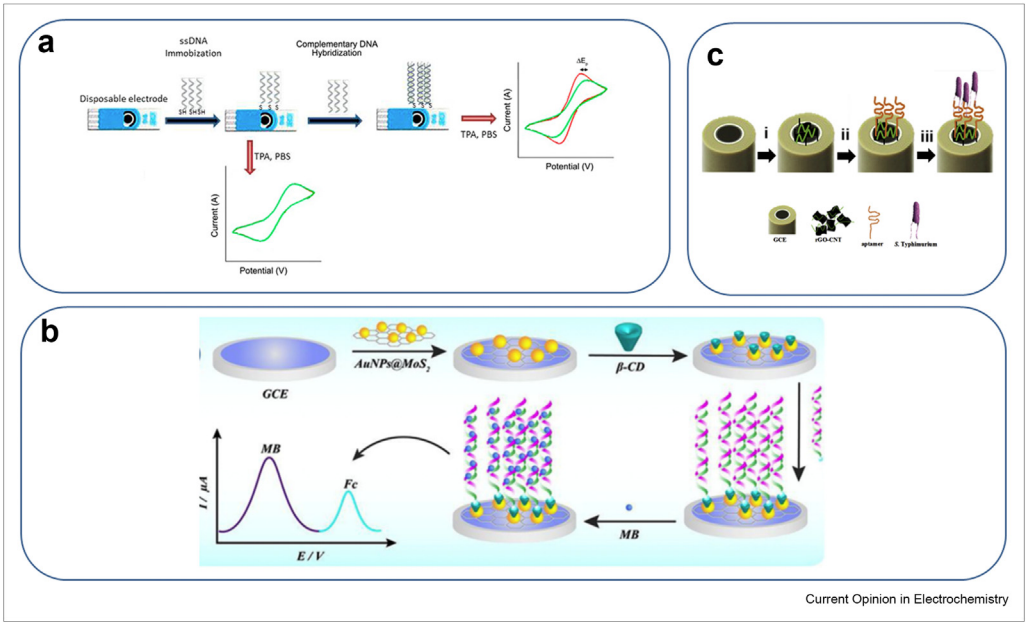
Saini et al. [30] detected *Listeria monocytogenes* in milk using a screen-printed CNF/AuNP-based electrode functionalized with ssDNA probes, targeting the *plcA* gene of *L. monocytogenes*. The ssDNA probes were modified with an amino group at 5' end to form a peptide bond with the carboxyl group of the CNF/AuNP surface. An LOD of 82 fg/6 µL was obtained in 30 min

using methylene blue (MB) as a redox probe. MB covalently binds guanine bases in ssDNA. Its electrochemical reduction decreases upon hybridization because of unavailable guanine bases in double-stranded DNA (dsDNA). Alternatively, an oxidation indicator tripropylamine was used for label-free detection of DNA of hepatitis A virus in water by cyclic voltammetry [31] (Figure 2a). The LOD of 0.65 pM and 6.94 fg/μL was obtained for the complementary ssDNA and viral cDNA, respectively. An AuNP-modified, SPE was used to detect *E. coli* DNA using electrochemical impedance spectroscopy by Ariffin et al. [32]. The carbon SPE was modified by adding a layer of AuNPs followed by a layer of aminated hollow silica microspheres, which was after functionalized with ssDNA probes using glutaraldehyde as a crosslinker. The LOD was 1.95×10^{-21} μM DNA *E. coli* in water samples. A genosensor was used for the detection of *Haemophilus influenzae* in human plasma samples. A solution of Zn-based metal–organic frameworks/carboxymethyl cellulose compound was used to modify the Au electrode surface to improve sensitivity. The electrode surface was functionalized with ssDNA probes and then blocked with mercaptohexanol to avoid nonspecific attachment. An LOD of 1.48 fM was obtained with cyclic voltammetry [33].

Some recent EBs for pathogen detection are coupled with advanced molecular biology techniques. Chaibun et al. [34] proposed an EB coupled with the rolling circle amplification (RCA) for the detection of SARS-CoV-2 in clinical samples. RCA was used to amplify targeting genes encoding for S and N protein, after RNA extraction using a commercial kit and reverse transcription to produce cDNA. Amplicons were detected through their hybridization with the redox-active labeled DNA probes using differential pulse voltammetry. The method detected one copy/μL of viral N or S genes within 2 h. Fu et al. [35] reported a loop-mediated isothermal amplification–based EB for quantification of *Streptococcus agalactiae* (Figure 2b). DNA was extracted using a genomic DNA extraction commercial kit. The LOD of 0.23 fg/μL of genomic DNA was obtained using an electrode modified with AuNPs@MoS_2. DNA was extracted using the DNeasy Blood and Tissue commercial kit before been subjected to recombinase-assisted amplification–based CRISPR/Cas12a.

An ultrasensitive CRISPR/Cas12a based EB for *L. monocytogenes* detection showed the LOD of 0.68 aM genomic DNA and 940 CFU/g in spike food samples within 2 h [36].

Figure 2



Examples of electrochemical biosensors based on immobilized DNA sequences. **(a)** A disposable DNA-based biosensor realized through thiol-gold coupling of the thiolated-ssDNA probe on a screen-printed gold electrode. The hybridization of the complementary hepatitis A virus DNA sequences induced changes in CV peak potential (ΔEp) of the redox indicator TPA (with permission from Ref. [31]). **(b)** Schematic diagram of the glassy carbon electrode functionalization with reduced graphene oxide–carbon nanotube (rGO-CNT) using drop-casting **(i)**, with amino-aptamer to form ssDNA/rGO-CNT/GCE **(ii)**, and electrode incubation with S. Typhimurium **(iii)** (with permission from Ref. [39]). **(c)** Schematic illustration of the principle of ratiometric LAMP E-sensor for *Group B* Streptococci detection (with permission from Ref. [35]).

Electrochemical aptasensors

Aptasensors have the advantage to skip the DNA extraction step, which increases the test simplicity and reduces the time for response. Aptamers, small single-stranded DNA (ssDNA) or RNA, specifically bind to various targets (from molecules to whole cells) because of their specific 3D structure [37,38], which ensures the specificity of aptasensors. Appaturi et al. [39] immobilized aptamers on a reduced graphene oxide–carbon nanotubes/glassy carbon electrode to detect *Salmonella* Typhimurium cells (Figure 2c) with differential pulse voltammetry reaching an LOD of 10^1 CFU/mL in 10 min. Aptamer concentration optimization (5 µmol/L) was necessary because higher concentrations led to the current density saturation. Das et al. [40] detected *P. aeruginosa* in water using an aptamer-mediated colorimetric and electrochemical detection and reached an LOD of 60 CFU/mL. Despite aptamers are largely used at the academic level for the construction of EBs, there are still several challenges that need to be addressed to enable aptasensor practical applications. For instance, aptamers can be degraded, or their 3D aptamer structure can be modified in complex media [38].

Conclusions

We presented a review of EBs for pathogen detection, their recognition elements, and transduction strategies. EBs present a great potential for online multiplex monitoring besides point-of-care single-detection applications. Bacteria are the most assessed pathogens using EBs, but the detection of viruses, especially COVID-19, has been increasingly examined over the past 2 years. EBs can be miniaturized to portable devices to constitute an alternative to culturing or polymerase chain reaction–based method as reported recently [41–43]. Our review focuses on working electrode modifications with nanocomposites, especially carbon- and metal-based, which are excellent building materials used to either enhance EB specificity or its electrochemical signals. Moreover, in this review, successful collaborations between microbiologists and biosensor constructors to obtain ultrasensitive devices are showcased, such as devices coupling RCA, Loop-Mediated Isothermal Amplification (LAMP), or CRISPR techniques with electrochemical detection. Recent advances in electrochemical sensors for pathogen detection demonstrate that EBs offer great potential for preventing the spread of highly contagious diseases and improving global healthcare.

Author's contribution

Jasmina Vidic: Writing — Reviewing and Editing. Marisa Manzano: Writing — Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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