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Review

Pathogen detection with electrochemical biosensors: Advantages, challenges and future perspectives

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ABSTRACT

Detection of pathogens, e.g., bacteria and viruses, is still a big challenge in analytical medicine due to their vast number and variety. Developing strategies for rapid, inexpensive, specific, and sensitive detection of the pathogens using nanomaterials, integrating with microfluidics devices, amplification methods, or even combining these strategies have received significant attention. Especially, after the health-threatening COVID-19 outbreak, rapid and sensitive detection of pathogens became very critical. Detection of pathogens could be realized with electrochemical, optical, mass sensitive, or thermal methods. Among them, electrochemical methods are very promising by bringing different advantages, i.e., they exhibit more versatile detection schemes and real-time quantification as well as label-free measurements, which provides a broader application perspective. In this review, we discuss the recent advances for the detection of bacteria and viruses using electrochemical biosensors. Moreover, electrochemical biosensors for pathogen detection were broadly reviewed in terms of analyte, bio-recognition and transduction elements. Different fabrication techniques, detection principles, and applications of various pathogens with the electrochemical biosensors were also discussed.

Contents

1. Introduction

The recent COVID-19 pandemic has again proved the fact that despite great advances in medical sciences, infection diseases are still one of the main problems in healthcare system around the world. In fact, it is estimated about 15% of total mortality in the world is caused by infectious disease [1]. Unexpectedly, they became even a bigger problem due to the changes in today's modern lifestyle and socioeconomical activities, which accelerates the spread of infection much faster around the world [2]. Due to this, many advances in detection and treatment of infectious disease have been studied and reported in the past decades, including developing different types of vaccines, innovative technologies, e.g., single-cell based studies, CRISPR technologies, RNA interference that help us to explore infectious disease more [3–6]. Moreover, CAR- and TCR-T cell-based therapies have been also investigated as new candidates [7]. Particularly, new revolutionary techniques are being developed through, e.g., the discovery

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of novel biomarkers, application of nanotechnology, and recent advancements in device developments to realize portable, rapid, accurate, and inexpensive point-of-care platforms. Molecular diagnostics using DNA and RNA biomarkers is the most advanced types of detection mechanism for infectious diseases. Currently, conventional methods based on antibody, e.g., enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Even though these methods provide large sensitivities, they are time-consuming, labor-intensive, and require relatively expensive equipment. Moreover, sample pre-treatment limits their application to laboratory environment.

Biosensors are the devices which can measure and/or quantify biomarkers specialized for infectious diseases. They are realized via the combination of constituting elements. First, selective recognition is achieved by ligand. Various recognition ligands, e.g., nucleic acids, antibodies, enzymes are massively employed. Second, a sensitive transducer which converts the biochemical signals that occur between the targeted analytes and the bioreceptor into measurable electrical signals for analyte identification and quantification.

Biosensors allow selective and sensitive detection of targeted analyte in a cost-effective and rapid manner. One important feature of biosensors is to enable real-time analyses of analyte without the need for complicated and expensive sample preparation. Another important feature to be considered here is their potential for enabling portable in-situ analyses, which is very critical for point-of-care diagnostics. In addition to their use in medical and point-of-care applications, they are utilized in monitoring prognosis, disease treatment, quality control for food and environmental samples, drug discovery, forensics, and biomedical research [8]. Based on their transducers, biosensors can be categorized into different types, e.g., the most common types are electrochemical and optical [9].

Electrochemical biosensors combine an analyte-receiving mechanism and an electrochemical transducer together, where the interaction between the targeted analyte and the transducer generates an electrochemical signal in current, potential, resistance or impedance format. There are wide range of electrochemical biosensor schemes with different signal mechanisms, e.g., voltammetric cyclic voltammetry (CV), differential pulse voltammetry (DPV), stripping voltammetry, alternating current voltammetry (ACV), polarography, square wave voltammetry (SWV), and linear sweep voltammetry (LSV).

Electrochemical biosensors have received significant attention thanks to providing rapid, accurate and sensitive responses in a cost-effective manner [10–12]. Electrochemical biosensors could use different types and forms of nanomaterials and nanocomposites to enhance the sensitivity of the detection mechanisms and to provide better detection limits through different strategies [13,14]. Electrochemical biosensors can be also combined with microfluidic systems to develop miniaturized components in a single platform. Integration of two platforms provides advantages compared to traditional electrochemical sensing systems, e.g., disposability, need for low number of sample, cost-effectiveness and rapid analysis. More importantly, this integration also brings the multiplexing modality, e.g., simultaneous detection of multiple target species from a single sample.

So far, electrochemical platforms are the most popular biosensors, and they have been introduced for the detection of large numbers of biomarkers and diagnosis of diseases, e.g., infectious diseases and cancers [11,13,15–18]. In this paper, we reviewed the recent advances in electrochemical biosensing for pathogen detection through different innovative strategies and approaches.

2. Pathogen detection with electrochemical biosensors

Pathogens, e.g., viruses, bacteria, fungi, or protozoa are the main cause for the pandemic diseases. Among those, viruses and bacteria are the largest in number. Methods for sensitive, rapid and on-site detection of pathogens is very critical for diagnosis and treatment of infectious diseases before spreading and globally effecting the human health. In that sense, electrochemical biosensors are massively utilized to detect these two deadly pathogens.

2.1. Bacteria detection

In this section, we extensively reviewed various electrochemical biosensors for the detection of different species of bacteria. In electrochemical biosensors, the working electrode is a fundamental component used as a solid support for biomolecule immobilization. Among varieties of detection methods and strategies, nanomaterials have an important role in the detection of pathogens, e.g., their biomarkers, toxins, by providing large surface area, high surface to volume ratio, larger loading capacity, and mass transport of reactants [9].

Especially, 3D-structured nanomaterials, e.g., graphene, have been used in electrochemical nano-biosensors, which could further enhance the sensitivity compared 2D-structures [19]. In another study, pyocyanin toxin from cystic fibrosis patients was detected with an amperometric electrochemical nano-biosensor. A screen printed electrode was used as working electrode, which was made of nano-grass structures covered with gold a nano-layer. The sensor could detect the pyocyanin toxin pnly in 60 s without pretreatment with a limit of detection, e.g., 172 nM, demonstrating its shorter response time and higher sensitivity compared to conventional methods [20].

Regarding electrochemical nanobiosensor of bacteria, recently an electrochemical immunosensor was developed for the detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7) using CV with the combination of nanomaterials [21]. In this work, pencil graphite electrodes (PGEs) were modified with chitosan (Chi), multi-walled carbon nanotubes (MWCNTs), and gold nanoparticles (AuNPs) - polypyrrole (Ppy) to enhance adsorption capacity of the antibodies for higher sensitivity. Here, the modified electrodes were activated with 2% glutaraldehyde. The amine group of the activated electrodes were interacted with the amine group functionalized monoclonal anti-*E. coli* O157:H7. Then, the modified electrodes were blocked with bovine serum albumin (BSA) and incubated with *E. coli* O157:H7 specimen. The experimental steps were shown in Fig. 1A. Currents vs. potential map obtained from different electrodes were shown in Fig. 1B. Here, PPy/AuNP/MWCNT@Chi electrodes provide highest redox peaks (curve d and e) compared others due to their better ability of enhancing electron transfer. Limit of detection (LOD) was determined as 30 colony-forming units (CFU)/mL. Selectivity of the developed immunosensor was tested, and no cross reaction was detected between the developed *E. coli* O157:57 immunosensor and other bacteria species, e.g., *Escherichia coli* O124, *Pseudomonas aeruginosa, Salmonella enteritidis, Shigella, and Burkholderia cepacia* (Fig. 1C). By using this nanocomposite film composed of PPy/AuNP/MWCNT@Chi, they could generate higher current responses and better biocompatibility compared to their classical counterparts adapted for *E. coli* O157:57 detection.

Different chemical groups such as silanes, thiols, amines, and conducting polymers could be used for electrode modification. Proper immobilization is very important for biomolecule activity and biocompatibility. Moreover, biomolecules should maintain its biological activity upon proper immobilization, which need effective surface chemistry methods. One example of surface modification method, e.g., an electrochemical-based impedance immunosensor for the detection of stressed *Staphylococcus aureus* was introduced [22]. Stressed and resuscitation are different from each other in terms of cell size, cell and small granule number, and integrity of the outer membrane. For these particular reasons, discrimination of stressed and resuscitation bacteria from each other is quite important in food and environmental samples. In this work, gold electrodes were treated with 16- mercaptohexanoic acid (thiol)/ethanol solution, and the SAM thiol layer on the surface was activated with mixture of 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.1 M N-hydroxysuccinimide (NHS). Modified electrodes were incubated with anti-*S. aureus* antibody, and the electrode modification was determined with CV and electrochemical impedance spectroscopy (EIS).

Finally, the modified electrodes were incubated with stressed and resuscitated *S. aureus*. Stressed bacteria were resuscitated after the incubation in tryptic soy broth and the sensor response for *S. aureus* was determined with EIS. In EIS measurements, charge transfer resistance (R_{ct}) increased with bacteria concentrations compared to bare electrodes, where LOD was

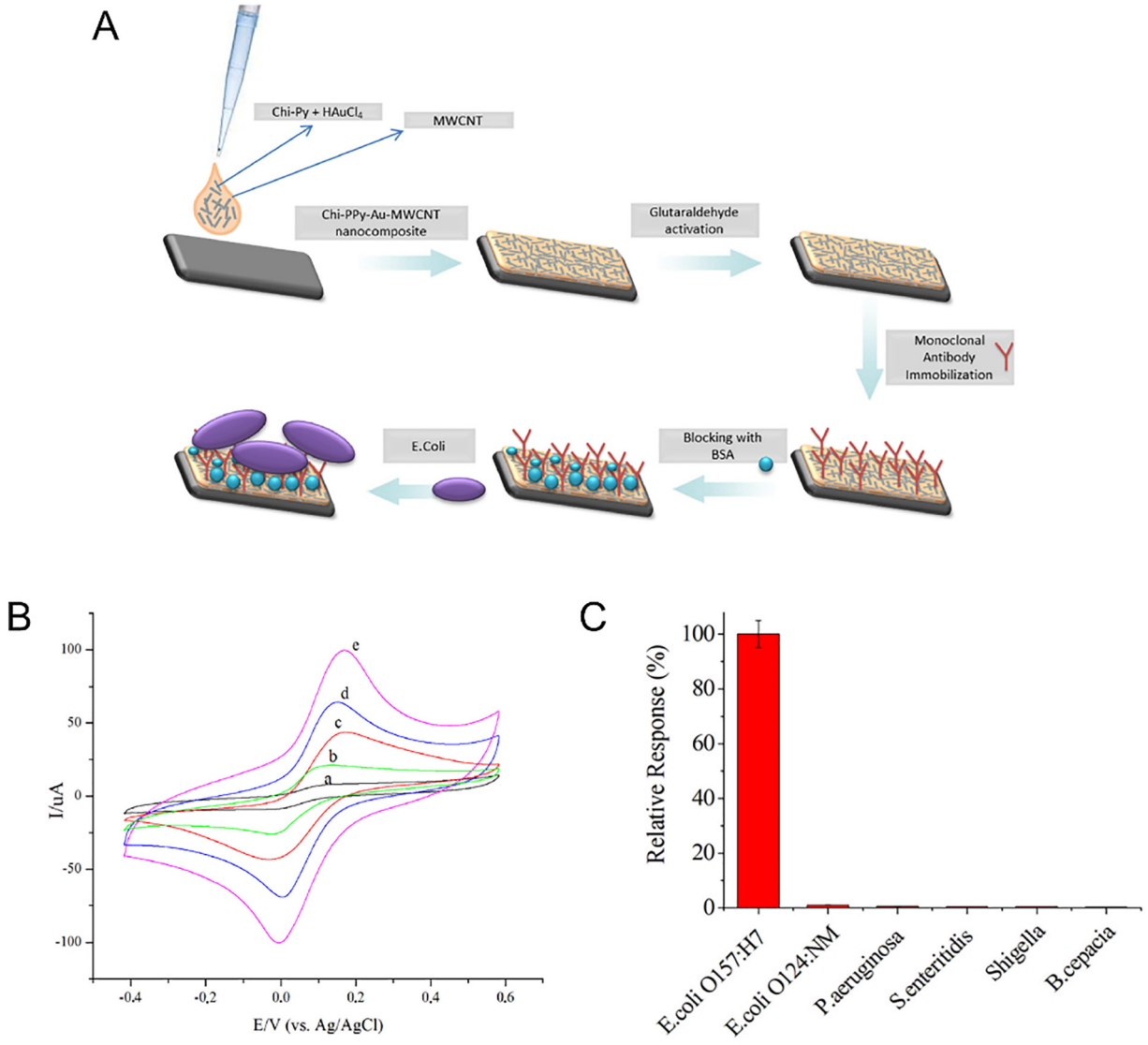

Fig. 1. (A) Schematic representation of the development of chitosan (Chi), multi-walled carbon nanotubes (MWCNTs), and gold nanoparticles (AuNPs) with polypyrrole (Ppy) modified electrodes for the detection of *E. coli.* (B) Cyclic voltammograms of different modified electrodes: (a) Chi-PPy, (b) bara electrode, (c) Chi-PPy-MWCNT, (d) Chi-PPy-MWCNT-AuNPs (1:1), (e) Chi-PPyMWCNT-AuNPs (1:2) in 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution at a scan rate of 50 mV/s. (C) Selectivity of the developed sensor toward other bacteria species. Reprinted from [21] with permission: Copyright © 2017 Elsevier B.V.

determined as 1.0×10^1 CFU/mL. The developed immunosensor could be successfully used to recognize stressed and resuscitation bacteria using impedance spectroscopy measurements.

Moreover, Dong et al. developed an electrochemical impedance immunosensor for the detection of *Salmonella typhimurium* in milk samples [23]. Modified glassy carbon electrodes (GCEs) were used as working electrodes, while MWCNTs were used as modifying agents. One of the main drawbacks of carbon nanotubes is their insolubility in water. Aggregation and low uniformity could be also other factors encountered in carbon-based systems. In order to address these problems, carbon nanotubes are generally combined with other nanomaterials, polymers or underwent with chemical modification via sulfonic acid groups or hydroxyl groups agents. In this work, MWCNTs and poly(amidoamine) (PAMAM) dendrimer solution were mixed in chitosan solution with an ultrasonic stirring. The mixture solution was dripped into a modified GCE, and the solvent was vaporized, i.e., AuNPs spread onto the modified GCEs. The modified electrodes were then incubated with EDC and NHS. The modified electrodes were incubated with anti-*S. typhimurium* antibody, and S. typhimurium cultures were pipetted into the sensor for immobilization. When bacteria is immobilized on an electrode surface, they reduce the output current and thus, increase the impedance of the interface. In EIS measurements, R_{ct} increased with bacterial concentration. The designed sensor system enhanced the stability of the immobilized bio-molecules. Compared to bare electrodes, addition of MWCNT, PAMAM and AuNPs significantly enhanced the sensitivity of the immunosensor. The LOD of the biosensor was 5.0×10^2 CFU/mL and the recovery was between 94.5% and 106.6%.

Cimafonte et al., for the first time, designed an impedimetric immunosensor for *E. coli* detection in drinking water [24]. In this work, gold screen-printed electrodes (Au-SPEs) were utilized as working, reference and counter electrodes, where the electrodes were mounted in a fluidic cell. Impedance measurements were performed by flowing various solutions into the golden part of the electrodes. Before the surface modification, anti- *E. coli* antibodies were activated by Photochemical Immobilization Technique (PIT) to bind antibodies upright onto the gold surfaces in a covalent way. PIT is an alternative method to SAM for binding antibodies onto gold surfaces. By means of the specific reduction of disulfide bonds in immunoglobulins produced by UV light activating nearby amino acids in the PIT method, antibodies can attach perpendicularly to the environmentally contacting surfaces of metals. After the UV activation, a solution of

UV-activated anti- *E. coli* antibodies was incubated on the gold surface of Au-SPEs. After the washing step, a small volume of drinking water was incubated with various concentrations of *E. coli*. For enhancing the resulting signal, anti- *E. coli* was transported with stabile influx rate to the gold surface of the electrodes. Thus, a sandwich complex was formed, i.e., impedance was augmented. *E. coli* detection was monitored with EIS and CV, where LOD was found as 3.0×10^1 CFU/mL. Analytical performance of the developed immunosensor has been compared with other impedimetric immunosensor for the detection of *E. coli*, e.g., the developed sensor had the shorter functionalization time. Here, PIT is used for the first time in biosensing and it is a quite rapid functionalization method since only 30 s is required for antibody activation, and the whole functionalization procedure lasts 1 h.

Nanomaterials on the electrode surface could increase the number of immobilized biomolecules, amplify sensing signals, and provide stronger electrical conductivity. Accordingly, a label-free electrochemical immunosensor (Fig. 2) was developed for the detection of *E. coli* O157:H7 with 3-dimentional silver nano-flowers (3D Ag) to immobilize bacterial antibodies through a bio-mineralization method [25]. The reason of using nano-flowers is to have wider porosity, larger surface area, and better intrinsic conductivity, and unique platform for functionalization. 3D Ag was also synthesized via the use of green chemistry, which was eco-friendly method. They have a very stable form, and provide a highly specific surface area and excellent biocompatibility. In this work, $[Fe(CN)_6]^{3-/4-}$ was used as a redox probe, and the alterations in resistances were measured with EIS. This simple, fast and highly selective sensor had a linear range between 3.0×10^2 and 3.0×10^8 CFU/mL, and the detection limit is determined as 1.0×10^2 CFU/mL. The good conductivity and bio-interface of Ag nano-flowers could be also used for the detection of other biomarkers.

Shukla et al., for the first time, developed an electrochemical immunosensor for *Cronobacter sakazakii* detection with CV and DPV [26]. For GCE working electrode modification, Au/GO suspension was dripped on the electrode surfaces. The Au/GO coated electrodes were further modified with thiol group involving anti-*C. sakazakii* antibody via incubation in moisture conditions. Finally, the modified electrodes were incubated with *C. sakazakii*. The presence of *C. sakazakii* was determined via the use of changes in the peak currents along with the bacteria concentration. The developed structure provided a larger electrode surface area. One of the limitation is that the multiplexing ability for the developed assay needs to be confirmed for simultaneous analyses of bulk samples. The platform has an LOD, e.g., 2.0×10^1 CFU/mL.

In another study, Soares et al. developed a novel detection method for *Salmonella enterica* serovar Typhimurium in chicken meat with EIS [12]. The authors used a label-free Laser-Induced Graphene (LIG) electrodes, which can be easily manufactured from commercial polymers, functionalized with high-specificity antibodies. They used a one-step, direct-write graphene fabrication method which converts sp^3 carbon found in polyimide (PI) into highly conductive sp^2-hybridized carbon found in graphene through CO_2 laser induction to benefit from the unique properties of graphene, i.e., high mechanical strength, good electrical conductivity, large specific surface area, good impermeability, and biocompatibility. Fig. 3 shows the development of the nanosensor via depositing the graphene on the PI film to realize the working electrodes and the attachment of linkers before the capture-antibody immobilization. Porous graphene was produced from PI using laser induction. The linear range of the nanosensor was between 25 and 10^5 CFU/mL, and LOD was determined as 1.3×10^1 CFU/mL. In addition to this broad linear range and low detection limit achieved with the use of nanomaterial (graphene layer) based electrodes, the response time of the detection method was very short, e.g., 22 min. LIG immusensor fabrication is simpler and more affordable compared to the other nanomaterial-based techniques, and LIG provides rapid and selective detection. Moreover, LIG electrodes, produced by laser induction on the polyimide film, prevented the need for high-temperature, vacuum environment, and metal seed catalysts compared to other complex fabrication procedures or post-printing processes.

In recent years, the paper-based electrochemical sensors are taking attention due to enabling the miniaturization of the detection system and thus minimal need of sample. These sensors can also be easily integrated with portable devices making them well suited for on-site detection. In addition, paper can be cut or designed according to the desired size, so the working area or confined zones can be arranged [27–29]. Furthermore, the paper-based electrochemical sensors are eco-friendly due to its biodegradable structure.

Rengaraj et al. developed a hydrophobic paper-based electrochemical impedance biosensor for the detection of bacterial pollution in water samples with EIS [30]. Here, SPEs were modified with paper-based probes that were made by screen-printing of three layers of a carbon-based conductive ink onto paper. Thanks to the hydrophobicity of the paper substrate, electrode functionalization was realized much easier and the adsorption of non-specific residues were eliminated. In addition, the hydrophobicity of the paper substrate facilitated the electrode functionalization. For the electrode modification, electrodes were oxidized with linear sweep voltammetry with Ag/AgCl in electrolyte solution involving $K_2Cr_2O_7$ and HNO_3. The electrodes were then incubated with EDC/NHS to activate carboxylic groups followed by a lectin modification. Then, ethanolamine hydrochloride solution was applied to the surface of the electrode to prevent non-specific adsorption. In EIS measurements, R_{ct} increased with bacteria concentrations and LOD was found as 1.9×10^3 CFU/mL which is lower

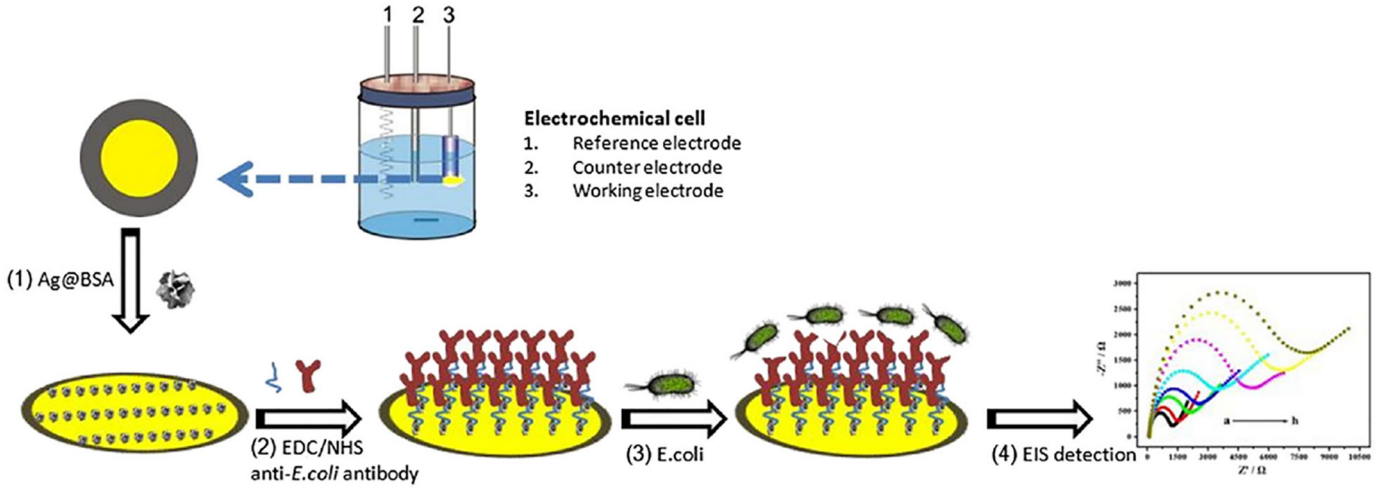

Fig. 2. Assembling steps of the label-free immunosensor for the detection of *E. coli* based on the application of antibody AG nano-flowers on Au electrodes. Reprinted from [25] with permission: CC BY 4.0 open access publication.

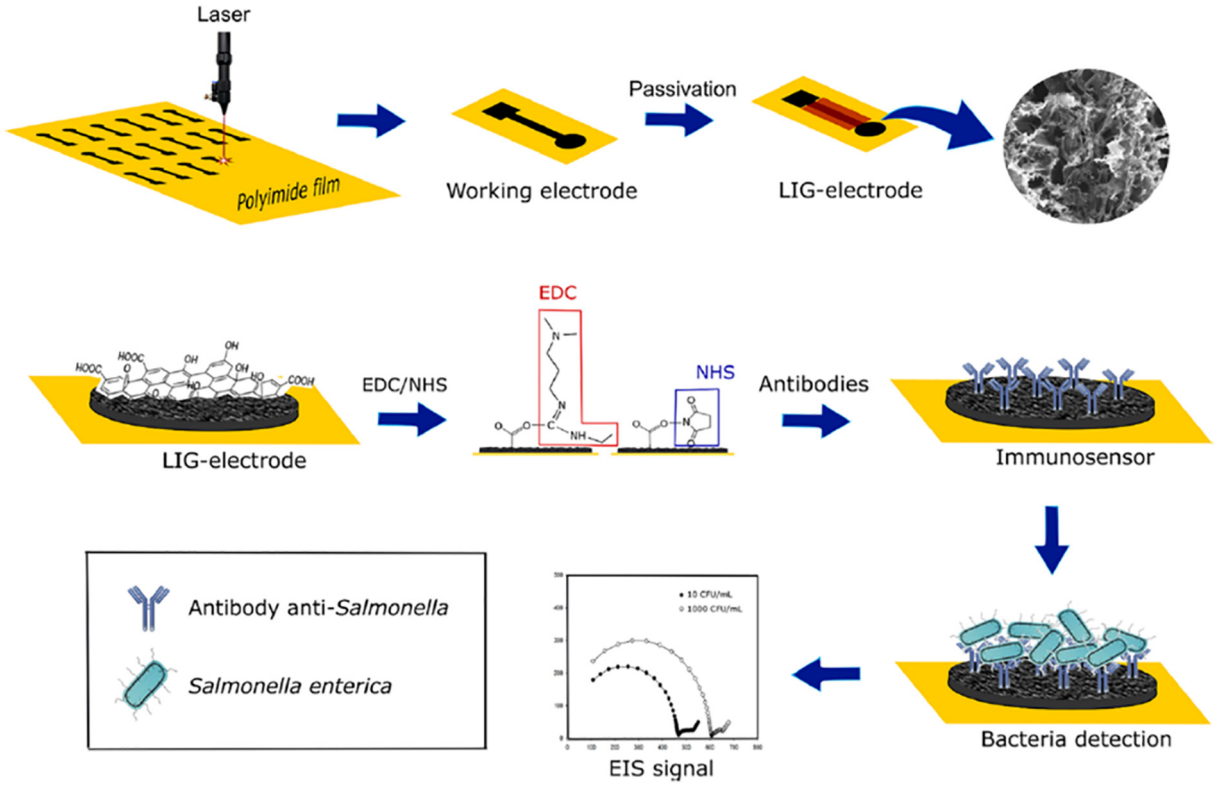

Fig. 3. Schematic illustration of the electrode fabrication and the detection scheme of the electrochemical immunosensor based on laser-induced graphene working electrode modified with linkers and capturing antibodies to detect *Salmonella* bacteria in chicken food products. Reprinted from [12] with permission: Copyright © 2020 American Chemical Society.

than the impedimetric sensors previously reported for the detection of bacterial pollution. The paper based sensing method could be used for portable testing kits due to its simplicity, low-cost and speed. In addition, the paper electrode is eco-friendly due to its biodegradable structure.

In another detection scheme based on paper-based electrochemical biosensors, Alatraktchi et al. introduced a method for the detection of pyocyanin toxin for bacteria *Pseudomonas aeruginosa* with CV and SWV methods. Electrodes were produced by screen-printing carbon ink on paper. Pyocyanin signal obtained with the paper-based sensor was compared with that of obtained with commercial carbon- and gold-based screen printed sensors. The developed biosensor showed three fold improvement in limit of detection, e.g., 95 nM, compared to conventional ceramic-based electrodes without the need for sample pretreatment. With its high sensitivity and easy-to-manufacture electrode, this method is very promising for the detection of bacteria toxin detection [28].

Outbreak of food-borne pathogens became a serious health problem, i.e., detection of pathogens in a rapid and sensitive manner in food samples is massively needed. For these reasons, Wang et al. introduced an electrochemical immunosensor based on antibody-hierarchical mesoporous silica for *S. aureus* detection with DPV [31]. GCEs were used as working electrode that was modified with antibody-hierarchical mesoporous silica. The antibody-hierarchical mesoporous silica structure provided better sensitivity and selectivity. In addition, the designed structure prevented the generation of waste materials during the manufacturing process Thanks to the preparation of the hierarchical mesoporous silica by a biological template method, the overall process was environmentally friendly. Moreover, the platform supported an LOD, 1.1×10^1 CFU/mL with short detection time, e.g., 20 min. The sensor was also stable for 15 days.

Tufa et al. introduced a sensitive sandwich-type electrochemical immunosensor for the detection of *Mycobacterium tuberculosis* with DPV (Fig. 4) [32]. They used graphene quantum dot (GQD)-coated Fe_3O_4@Ag core-shell nanostructures for electrode modification, while AuNPs conjugated to an anti-CFP-10 antibody were employed as a label for signal augmentation. In the study, Fe_3O_4 increased the surface-volume ratio, Ag enhanced electrical conductivity and prevented Fe_3O_4 nanoparticles from aggregation, and GQD improved the loading capacity of antibodies onto the electrode surface. This nanotriplex complex system showed large sensitivities, e.g., 0.33 ng/mL detection limit, and a broad linear range of bacteria surface antigen, e.g., 0.005–500 μg/mL.

Modification of electrode surfaces with nano-structures has also been investigated for pathogen detection. Especially, two dimensional nanomaterials such as molybdenum disulfide (MoS_2) provides multifold enhancement in the sensitivity due to the existence of bandgap in its structure. In addition, energy state can easily be tuned from semiconducting to fully conducting, which enables the use of MoS_2 in biosensing systems for easier electron transfer. Singh et al. developed a lab-on-chip electrochemical immunosensor combined with a microfluidics system for the detection of *Salmonella typhimurium* in nourishment samples [33]. Functionalized MoS_2 nano-sheets (NS) using cetyltrimethylammonium bromide (CTAB) assisted chemical exfoliation method to obtain highly dispersible suspension were deposited onto patterned hydrolyzed indium tin oxide (ITO) microelectrodes integrated with polydimethylsiloxane (PDMS) microfluidic device. Capturing antibodies were immobilized inside the microfluidic channels, and EIS was utilized as the detection method (Fig. 5A). Fig. 5B shows the Nyquist plots of fMoS_2-NS/ITO and anti-S.typhimurium/f-MoS_2-NS/ITO microfluidic immunochip. R_{ct} value of anti S.typhimurium coated electrodes were larger than the f-MoS_2-NS/ITO microfluidic chip due to the blocking of charge carrier. Thanks to the use of a nanomaterial and a microfluidics device, this lab-on-chip device has a detection limit of 1.56 CFU/mL, and a broad detection range of 10^1–10^7 CFU/mL.

In another study, a paper-based electrochemical biosensor for the detection of foodborne pathogen, *S. aureus* was studied with CV and DPV [34]. Paper based carbon paste electrodes (CPE) were employed as the working electrodes. Covalent binding was achieved between the single walled carbon nanotubes (SWCNTs) and anti-*S. aureus* with EDC and NHS. Activated SWCNTs were incubated with anti-*S. aureus* antibodies. Antibody and

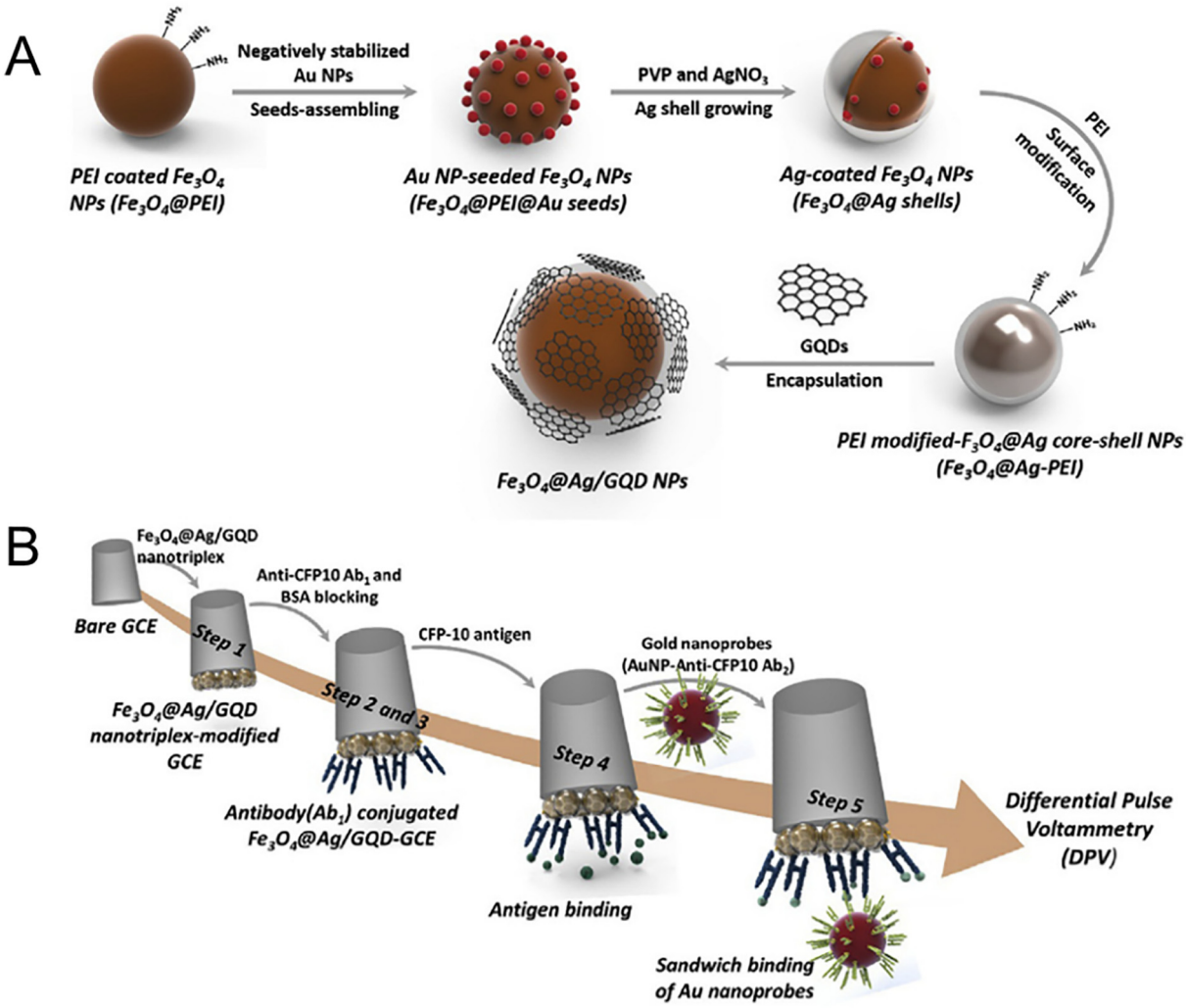

Fig. 4. Schematic illustration of (A) the preparation of Fe_3O_4@Ag/GQD nanoparticles and, (B) modification of GCE electrodes for the detection of *Mycobacterium tuberculosis* using a sandwich-type electrochemical immunosensor. Reprinted from [32] with permission: Copyright © 2018 Elsevier B.V.

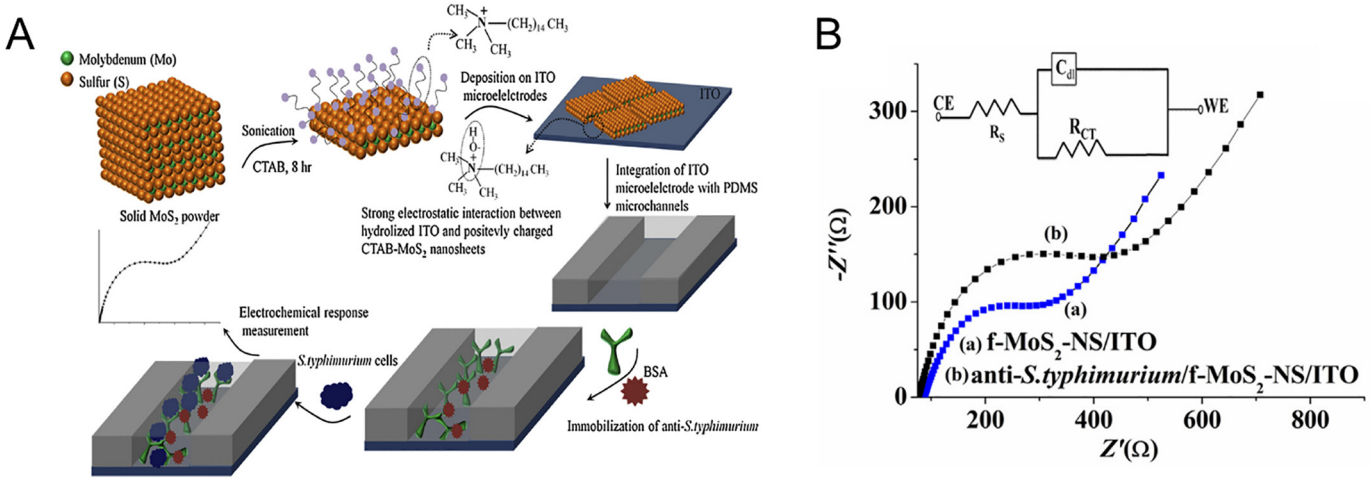

Fig. 5. (A) Synthesis of the nano-sheet and the fabrication of the lab-on-chip device for the detection of *Salmonella typhimurium* in nourishment samples using an electrochemical immunosensing method. (B) EIS results of fMoS_2-NS/ITO and anti-*S. typhimurium*/f-MoS_2-NS/ITO microfluidic immunochip in $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution. Reprinted from [33] with permission: Copyright © 2017 Elsevier B.V.

SWCNTs covalent attachment improved the stability of the immunosensor. Anti-*S. aureus* antibody-SWCNT conjugates were dripped onto the electrodes. Finally, the modified electrodes were incubated with various *S. aureus* concentrations. Using milk solutions with different *S. aureus* concentrations, the peak currents increased with *S. aureus* concentration in DPV measurements. For this platform, LOD was determined as 1.3×10^1 CFU /mL in milk samples. The use of anti-*S. aureus* antibody-SWCNT conjugates could increase the robustness of the paper-based immunosensor by shortening the functionalization steps as well as could increase the stability of the biosensor by enhancing the survival rate of biomolecules.

Due to the need for a minimum sample preparation step, label-free detection systems with high sensitivity took significant attention for the incorporation of such systems in portable devices. Biosensors combined with laboratory-on-a-chip (LoC) devices, or Point of Care Diagnostics (POCs) offer tremendous advantages such as rapid, portable, high throughput, and automatized detection methods. For considering these issues, an electrochemical detection of *Streptococcus mutans* was realized with printed-circuit-board (PCB) electrodes [35]. In this novel PCB based electrochemical biosensor, the thiolated primary antibody was immobilized to the electrode surface and the bacteria detection was realized with EIS. The platform has an LOD of 1.0×10^3 CFU/mL. Thanks to its simplicity, sensitivity and selectivity, the PCB platform could be a good candidate for point-of-care applications in field.

Metal oxides such as conductive zinc oxide (ZnO) nanostructures have been used for biosensing applications due to superior properties such UV light sensitivity, and wide band gap (~3.37 eV) [36,37]. In addition, ZnO configuration can be tuned into numerous shapes which provide the use of this structure in different applications. For instance, Chauhan et al., for the first time, designed an electrochemical biosensor for the detection of cytotoxin associated gene A protein (CagA) in human serum samples with CV, EIS and SWV [38]. CagA is a virulence factor and important biomarker of *H. pylori* infection, where screen-printed Au electrodes (SP-AuE) were used as working electrodes. For SP-AuE modification, zinc oxide tetrapods (ZnO-T) was synthesized, where a 365-nm UV light was applied to ZnO-T to enhance conductivity. Ion ray was applied to the modified electrodes, and ion irradiated ZnO-T were realized. The tetrapodal shape is a 3-dimensional configuration that possesses connective four arms from the core center at an average angle of ~110°. These structures can be used for the development of multifunctional 3D-networks, which prevents agglomeration and provides high conductivity. After the modification of electrodes, they were incubated with CagA antigen, and CagA antibodies were introduced to the electrodes. Experimental steps of this detection scheme are shown in Fig. 6A. As shown in Fig. 6B, in CV measurements, the peak current decreased with anti-CagA concentration. In real sample analyses, recovery was 98.6% and LOD was determined as 0.2 ng/mL. Here, the irradiated electrodes have better electroconductivity and specificity compared to the un-irradiated electrodes.

Combining nanomaterials with polymers have been widely used for biosensing of bacteria in order to benefit from synergetic effect of the mentioned materials. Kang et al. developed an electrochemical immunosensor for *Bacillus cereus* detection with chronoamperometry in milk samples [39]. In the study, AuNPs mixed with horseradish peroxidase (HRP) yielding AuNPs-HRP composite that was mixed with Thionine (Thi)-chitosan (Chit). GCEs were modified with chitosan used as a bridge cross-linker by dropping. Chitosan modified electrodes were then interacted with gold solution via electrostatic adsorption. The electrodes were modified with anti-*B. cereus*, and Thi-Chit/AuNPs -HRP mixture was dipped into the modified electrodes. The electrodes were interacted with anti-*B. cereus* by dripping. The platform has an LOD of 1.0×10^1 CFU/mL, which is smaller compared to the ones supported by the immunosensors reported earlier. Using chitosan and AuNPs incorporated with an antibody increased the effective surface area of the electrodes. In addition, AuNPs promoted electron transfer rate due to its channel effect. The developed immunosensor exhibited high reproducibility and long-term stability, making it an ideal platform for food quality and safety control applications.

Click chemistry reaction are highly attractive reactions for immobilizing bioreceptor molecules onto the electrodes surfaces. Svalova et al. designed an electrochemical immunosensor for *E. coli* detection in drinking water and milk using CV and EIS based on copper-catalyzed “click” reaction (Fig. 7) [40]. For electrode modification, vinylbenzylazide (VBA) was electro-deposited with CV in lithium perchlorate. Cu nanoparticles (CuNP) were then sent to the polymer-coated electrodes. A click reaction occurred between the azide groups in PVBA and the acetylene fragment of the ester. Electrochemically oxidized copper particles were added to the polymer film, which catalyzed this reaction. Finally, anti-*E. coli* antibodies were incubated on the modified electrodes. The system has an LOD of 6.3 CFU/mL. The developed method reduced the immobilization time from a few hours to 30 min, and prevented denaturation of the immunoreceptor.

Bakhori et al., for the first time, developed an electrochemical immunosensor based on CdSE/ZnS quantum dots (QD) and silica nanoparticles (SiNPS) for *Mycobacterium tuberculosis* detection [41]. Screen-printed carbon electrodes were modified with CdSE/ZnS (QD) and SiNPs to amplify

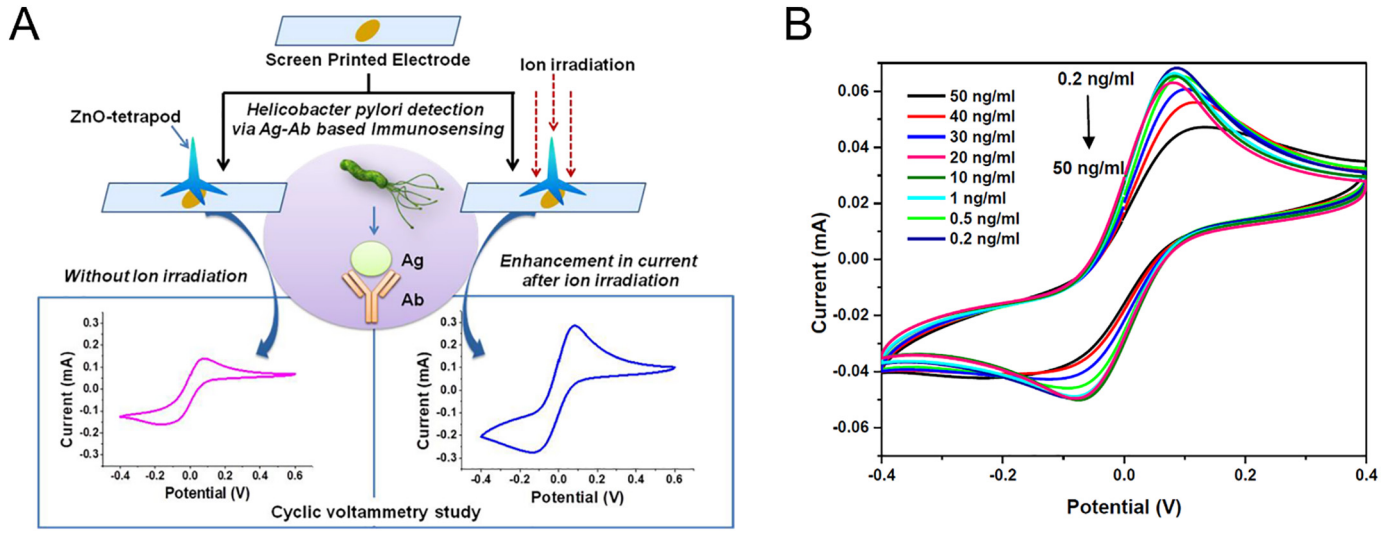

Fig. 6. (A) Schematic illustration of the preparation of the CagA antigen@ZnO-T/SP-AuE immunosensor. (B) CV of CagA antigen coated modified electrodes with different CagA-antibody concentrations between in 5 mM $[Fe(CN)_6]^{3-/4-}$ electrolyte. Reprinted from [38] with permission: Copyright © 2018 American Chemical Society.

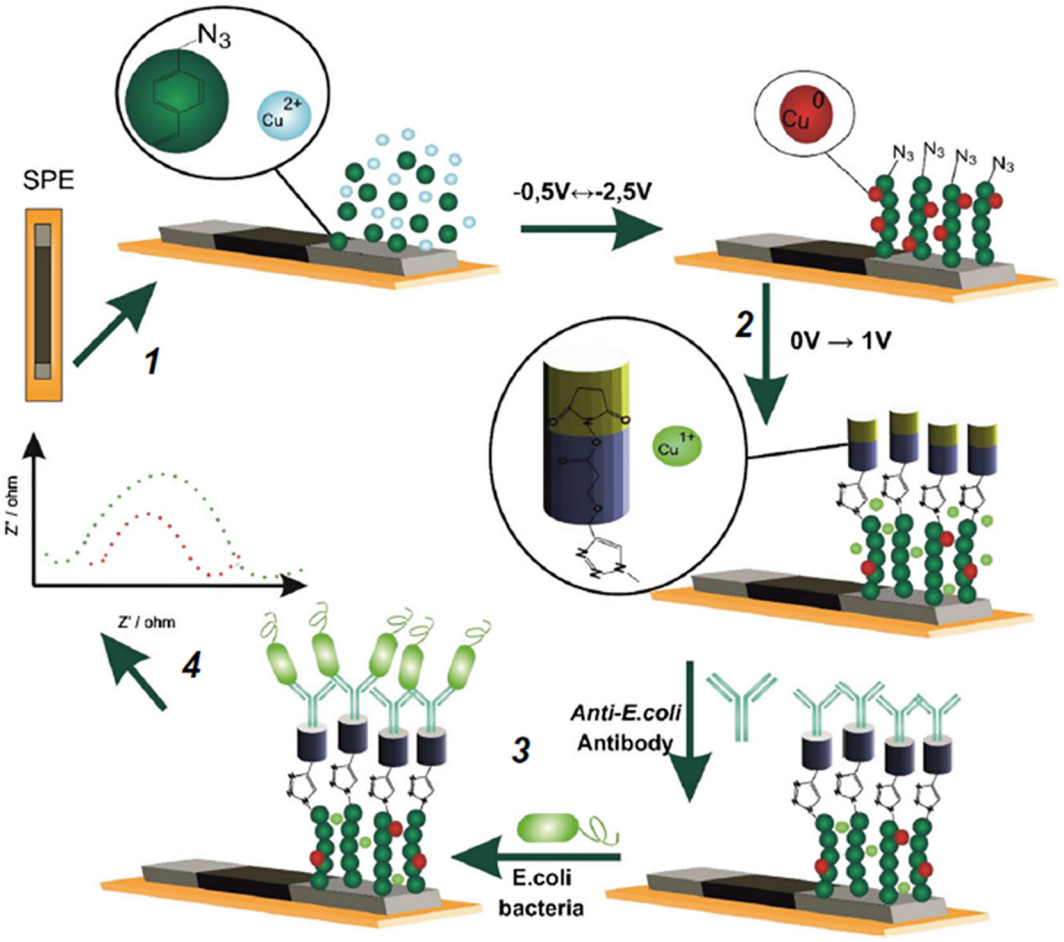

Fig. 7. Schematic illustration of the development of covalently immobilized anti-*E. coli* based impedimetric immunosensor for *E. coli* detection. Reprinted from [40] with permission: © 2020 Springer Nature Switzerland AG.

the detection signal as well as to increase the selectivity of the sensor. These nano-modification provided a strong electrocatalytic effect, high sensitivity and selectivity. Bacteria detection was determined with DPV, where LOD was found as 1.5×10^{-10} g/mL.

Detection of pathogens with graphene based biosensors have been taking attention. For instance, Jijie et al. developed an graphene based electrochemical immunosensor for *Escherichia coli* UTI89 detection in aqueous and serum samples with DPV [42]. In this work, gold electrodes were used as working electrodes. In order to achieve GO/polyethylenimine (PEI) dispersion, GO and PEI combination was agitated. Au electrodes were modified with reduced graphene oxide (rGO)/PEI by electrophoretic deposition (EPD). To perform EPD, GO/PEI aqueous dispersion was used, where Au electrodes are the cathode and the Pt foil is the anode. The modified electrodes were then plunged in a certain amount of pyrene-poly(ethylene glycol) (PEG) solution. Anti-fimbrial *E. coli* antibody immobilization on the electrode was realized with EDC/NHS chemistry. EDC/NHS was the cross-bonding agent between -NH_2 groups of PEI and -COOH groups of anti-fimbrial *E. coli* antibodies. Finally, the electrodes were treated with antibody that was incubated with various *E. coli* concentrations, and in DPV measurements, the peak current decreased with *E. coli* UTI89 concentration. LOD was determined as 1.0×10^{1} CFU/mL.

In another study with rGO modified electrodes, Muniandy et al. developed a label-free electrochemical aptasensor for *S. Typhimurium* detection in raw chicken meat with DPV [43]. For GCE modification, azophloxine (AP) was dissolved in GO solution. For pH adjustment, hydrazine was added to the solution to obtain reduced GO (rGO). GCEs were then modified with rGO-AP solution with drop-casting in order to enrich its free charge-carrier density, thus potentially enhancing its solubility in hydrophilic medium and electrical conductivity. The electrodes were further modified with different concentrations of aptamer (ssDNA) solutions. Aptamer modified electrodes were treated with various *S. Typhimurium* concentrations. In DPV measurements, the peak current increased with *S. Typhimurium* concentration, and LOD was found as 1.0×10^{1} CFU/mL.

Xu et al. developed an electrochemical immunosensor for *Escherichia coli* O157:H7 and *Salmonella Typhimurium* detection with EIS [44], where screen-printed interdigitated microelectrodes and magnetic beads (MBs) were used in the detection scheme. Streptavidin coated MBs were modified with biotinylated antibodies (Ab) and the conjugation of glucose oxidase (GOx) with Ab was realized with a GOx conjugation kit. GOx was selected as the labeling enzyme due to its long term stability, high turnover number, and specificity. Bacteria mixed with MBs-Ab conjugates, and magnetic separation was applied to MBs-Ab/bacteria cell conjugate. The sandwich complex was then exposed to magnetic separation to remove extra ions in the specimen. Here, LOD was found as 2.05×10^{3} CFU/g for *E. coli* O157:H7, and 1.04×10^{3} CFU/mL for *S. Typhimurium*. The important feature of the designed system is that the electrode can be regenerated multiple times for long-term use. In addition, using MBs–Ab conjugates together had achieved high capture efficiencies.

Electrochemical genosensors, aptasensors, and other biosensor detection systems have been also developed for bacteria detection. For instance, Nazari-Vanani et al. developed a novel electrochemical DNA biosensor for *Enterococcus faecalis* detection with DPV in urine samples [45]. Here, gold-disk electrodes covered with ice crystals-like gold nanostructure (Au/nano) were used as working electrodes. In order to ensure DNA

hybridization, toluidine blue (TB) was used as redox indicator. An electrodeposition method was developed to fabricate a novel ice crystals-like gold nanostructures. For electrode modification from Au to Au/nano, Au electrodes were treated with H_2SO_4, $HAuCl_4$, and sorbitol. For electrode modification with probe-DNA (p-DNA), a dithiothreitol (DTT) solution was first added into the p-DNA solution, and agitated. The p-DNA/DTT solution was applied to the Au/nano electrodes by dropping, where the modified electrodes were interacted with 6-mercapto-1-hexanol (MCH). The p-DNA modified electrodes were incubated in the complementary-DNA (c-DNA), where they were treated with TB solution. In DPV measurements, peak current decreased with c-DNA concentration, and LOD was determined as 4.7×10^{-20} M for c-DNA, and 30.1 ng/μl for genome. The developed biosensor showed excellent sensitivity and selectivity with a low detection limit and could be potentially used as an alternative method for *Enterococcus faecalis* detection without using PCR amplification in clinical samples.

Henihan et al. developed a novel electrochemical biosensor for bacterial ribosomal RNA (rRNA) detection in *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* without labelling and amplification with kinetic EIS [46]. Electrodes were first treated with thiol-modified peptide nucleic acid (PNA) oligonucleotides, and hybridization was performed with 16S rRNA solution. For kinetic EIS measurements, screen-printed dual gold working electrodes were used. In EIS measurements, hybridization with *E. coli* rRNA increased R_{ct} more compared to the hybridization with other rRNAs and the system provides an LOD as 50 pM within 10 min at ambient temperature.

Recently, nicking endonucleases have been used to amplify the obtained signals in biosensors. Luo et al. developed a new signal-out electrochemical sensing for *Enterobacteriaceae* detection in milk samples with DPV, EIS, and SWV integrating Exonuclease III-assisted target recycling amplification [47]. In the sample preparation first, the gold electrodes were treated with thiolated capture probe. The electrodes were then dipped into MCH to obtain aligned DNA monolayer. After washing, salmon sperm DNA was sent to the electrodes, and the electrodes were modified with commix of *Escherichia coli* Exonuclease III (Exo III) and target DNA. The modified electrodes were treated with biotinylated detection probe, and streptavidin-alkaline phosphatase (ST-AP). After target DNA hybridizes with capture DNA, Exo III can selectively digest the capture DNA. A successful target recycling was achieved with Exo III which released the target to undergo a new hybridization and cleavage cycle on sensor surface, Experimental steps were schematically shown in Fig. 8A. Electrochemical DNA biosensors characterization were realized with EIS (Fig. 8B), and SWV (Fig. 8C). Here, bare electrodes exhibited an almost linear behavior

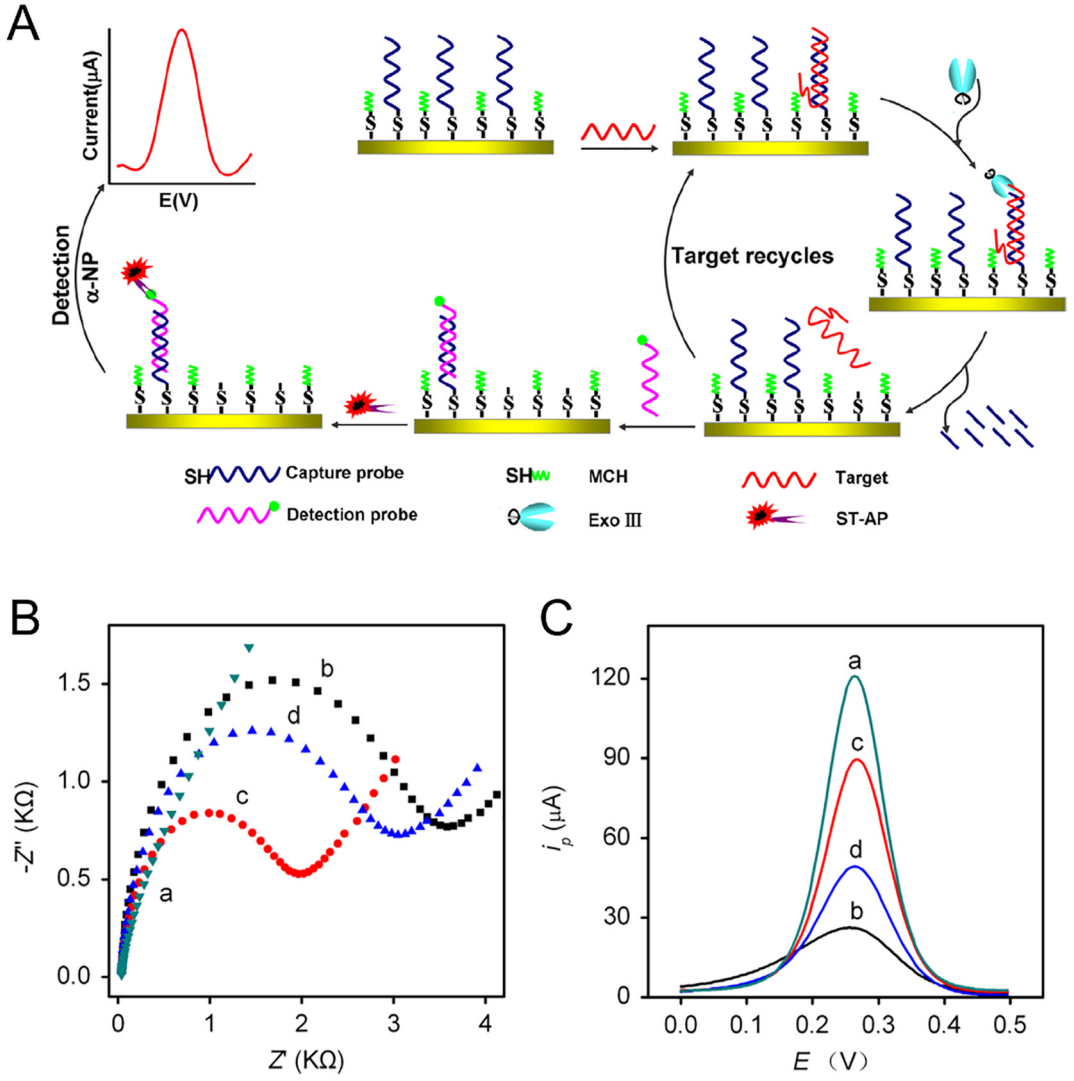

Fig. 8. (A) Schematic illustration of the electrochemical DNA biosensor for the detection of Enterobacteriaceae bacteria. (B) EIS, and (C) SWVs for (a) bare electrode, (b) capture DNA modified electrode, (c) capture DNA modified electrode after the reaction with target DNA and Exo III, and (d) further reaction with detection probe. Reprinted from [47] with permission: Copyright © 2013 Elsevier B.V.

(curve a). As the thiolated capture DNA was self-assembled onto the bare electrodes, the R_{ct} increased (curve b), while it decreased significantly (curve c) when the biosensor was hybridized with target DNA and reacted with Exo III. Upon the hybridization of the biotinylated detection probe with the remaining capture DNA, R_{ct} further increased (curve d). In DPV measurements, α-naphthyl phosphate (α-NP) was used. Here, the peak current decreased with target DNA concentration. The system LOD was determined as 8.7 f. for target DNA, and 40 CFU/mL for milk specimens.

Ranjbar and Shahrokhian developed an electrochemical aptasensor for *Staphylococcus aureus* detection with CV and EIS in human serum specimen [48]. For GCEs modification, CTAB coated AuNPs were used. Solid carbon nanoparticles (CNPs) were treated with dimethylformamide (DMF) via ultrasonic stirring, and AuNPs were added to the CNP suspension with agitating. Solid AuNPs/CNPs were resolved with cellulose nanofibers (CNFs) solution in DMF, and GCEs were then modified with AuNPs/CNPs/CNFs. For obtaining a stable response, modified electrodes were cycled, and thiolated aptamer was added to the modified electrodes. After modification with aptamer, electrodes were incubated with *S. aureus*. The fabricated nanocomposite was a biocompatible scaffold with high surface area for immobilization of the thiolated aptamer. In EIS measurements, R_{ct} increased with *S. aureus* concentration, and LOD was found as 1 CFU/mL. The most notable feature of the designed aptasensor was that the ability of the aptamer to discriminate between live and dead bacteria cells.

Muniandy et al. designed an electrochemical aptasensor for *Salmonella enterica serovar Typhimurium* detection in chicken meat with DPV [49]. In the system, rGO and titanium dioxide (TiO_2) nanostructures were used for GCEs modification. To achieve aptamer immobilization, aptamer solution were added to the modified electrodes, and the interaction between ssDNA aptamers of the phosphate groups and TiO groups was realized by binding. For bacteria detection, rGO/TiO_2, and the aptamer coated electrodes were modified with *S. Typhimurium* (Fig. 9). The peak current decreased with *S. Typhimurium* concentrations in DPV measurements, and LOD was found as 1.0×10^1 CFU/mL. The rGO-TiO2 nanocomposite sensor platform provided an improved signal response compared to rGO or TiO platforms alone, which can be attributed to the increase in the electron transfer rate with rGO-TiO2 nanocomposite platform. In addition, the interaction between phosphate groups on DNA aptamers and TiO groups embedded on the surface of rGO prevented the aptamer labelling with thio- or amine groups; thus, the cost of the process could be reduced.

An electrochemical aptasensor was developed for the first time for the detection of *Pseudomonas aeruginosa* from blood serum specimens with EIS [50]. For GCE modification, nano-sized chitosan particles (NCs) were prepared, and the modified GCEs were treated with glutaraldehyde and plunged into aptamer solution. Aptamer and NC modified electrodes were interacted with *P. aeruginosa* solution, and in EIS measurements, R_{ct} value increased with *P. aeruginosa* concentration. In real sample analyses, recovery was found between 93.2% and 124%, and LOD was found as 3 CFU/mL. The developed aptasensor showed excellent stability with satisfactory reproducibility, and selectivity.

Zarei et al., for the first time, developed an electrochemical aptasensor for *Shigella dysenteriae* detection in water, unpasteurized and pasteurized skim milk [51]. In this work, GCEs were modified with AuNPs via electrodeposition, and the aptamer solution was added to the AuNP-modified electrodes via dropping. For *S. dysenteriae* detection, the aptamer and AuNPs modified electrodes were immersed in *S. dysenteriae* solution. The concentration of aptamer, immobilization time to the electrode surface and, target binding time were optimized to get the best analytical signal. In EIS measurements, R_{ct} value increased with *S. dysenteriae* concentration, and LOD was determined as 1.0×10^1 CFU/mL. The aptasensor based biosensor can discriminate live bacteria from their dead cells.

Hatami et al. developed an electrochemical DNA biosensor for *Mycobacterium tuberculosis* (TB) detection with CV and DPV. Here, metal oxide formation is used, for the first time, in the electrochemical sensing of TB [52]. In the sample preparation, GCE surface modification was realized with electrodeposition with ZnO. ZnO coated electrodes were further modified with AuNPs, and ZnO/AuNP modified electrodes were dipped into the probe DNA solution. For *M. tuberculosis* detection, ssDNA/AuNPs/ZnO modified electrodes were incubated with DNA of the targeted bacteria.

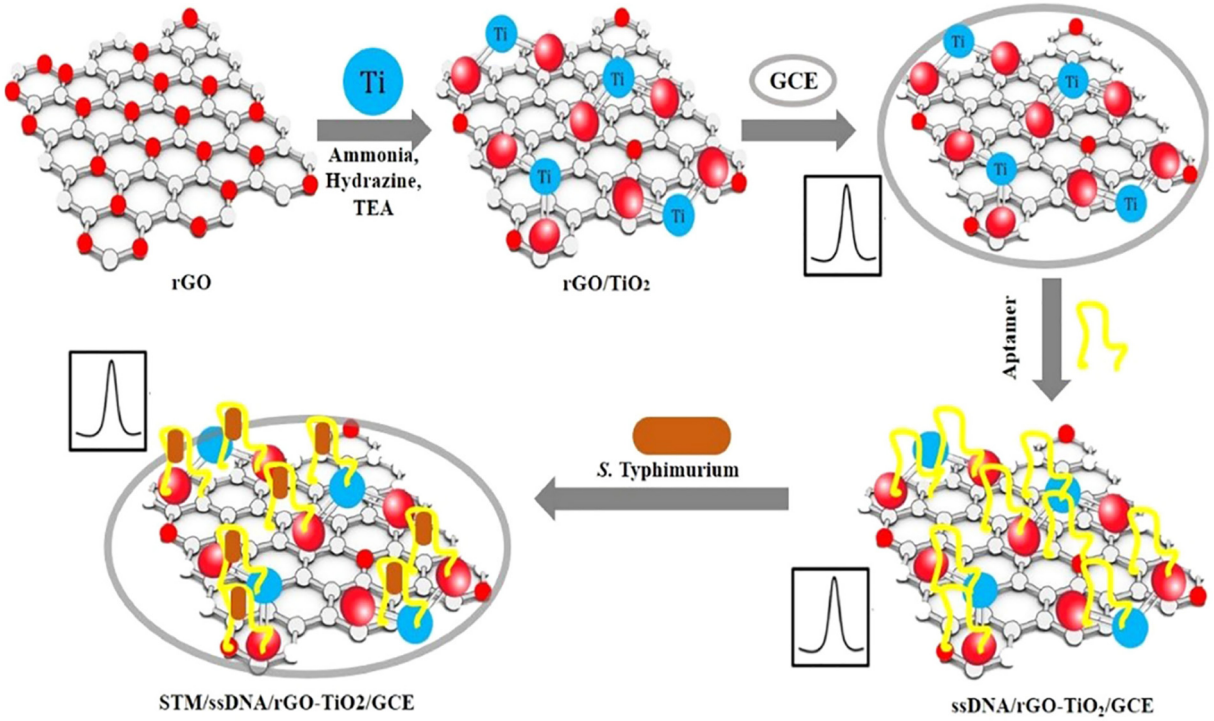

Fig. 9. Schematic illustration of the development of rGO and TiO_2 modified electrochemical aptasensor for the determination of *S. Typhimurium*. Reprinted from [49] with permission: Copyright © 2020 Elsevier B.V.

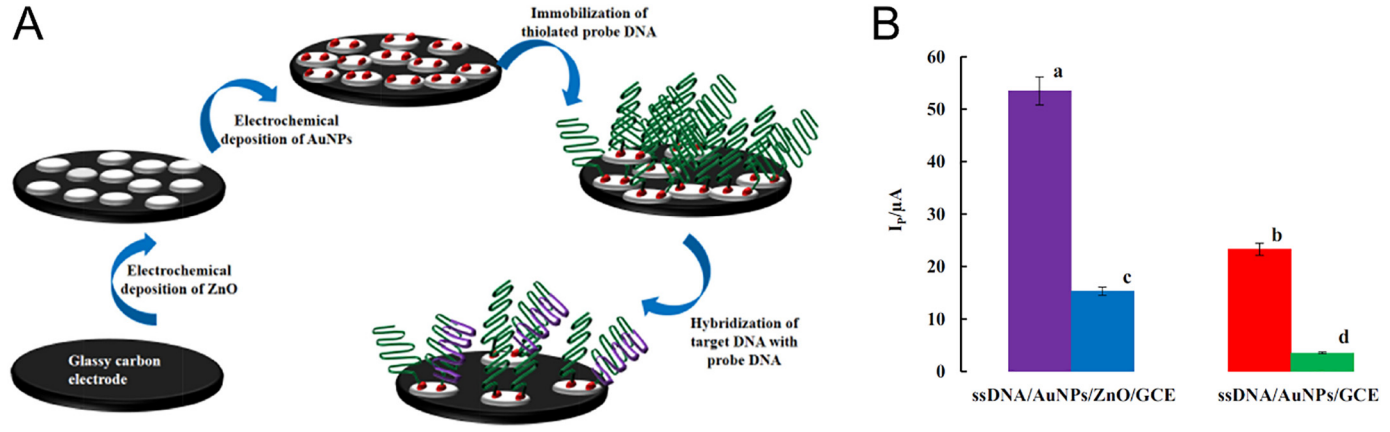

Fig. 10. (A) Schematic illustration of the development of ZnO/AuNP modified electrochemical DNA biosensor for the detection of *Mycobacterium tuberculosis*. (B) Comparison of the peak current in DPV between ZnO modified and ZnO-free electrodes in the absence (a,b) and presence (c,d) of complementary DNA. Reprinted from [52] with permission: Copyright © 2020 Elsevier B.V.

Experimental steps are schematically shown in Fig. 10A. In DPV measurements, peak current decreased with target bacteria concentration, and LOD was determined as 1.18 pM. More importantly, the enhancement in the peak current due to the deposition of ZnO could be clearly seen when comparing with ZnO-free electrodes (Fig. 10B). Here, the further decrease in the peak current due to the presence of the complementary DNA also demonstrated metal oxide's ability to create larger signal difference for the targeted analytes.

Hajihosseini et al., for the first time, developed an electrochemical DNA biosensor for *Helicobacter pylori* detection with DPV [53]. For the electrode modification, GO was dripped onto GCE surface, and GO-modified GCEs were further modified with AuNPs by deposition. *H. pylori* probe was interacted with the modified electrodes, and the ssDNA/GO/AuNPs modified electrodes were dipped into the hybridization solution involving the oligonucleotide of the targeted bacteria. Oracet blue (OB) was used as a redox indicator to enhance the electrochemical signals of DNA. In DPV measurements, OB peak current increased with DNA concentration of the targeted bacteria, and LOD was found as 27 pM. The developed biosensor confirmed that OB-based DNA biosensors are capable of detecting the single-base mismatch in the target DNA. The authors also stated that the best method for probe self-assembly was the drop self-assembly and the best hybridization method was solution based hybridization method.

In another study, one novel latent electrochemical redox probe, ferrocene carbamate phenyl acrylate (FCPA), was introduced, which was used as an effective transducer for the selective detection of non-redox active biomarkers. By using a specific probe that consists of a recognition unit attached via a linker to a latent redox active reporter, real-time tracking of cellular Cysteine (Cys) production in *E. coli* W3110 was realized. Such novel electrochemical molecular switch successfully discriminated Cys over other biothiols in complex biological systems [54].

Molecularly imprinted polymers (MIPs), artificial recognition elements, are polymers that are fabricated via chemical, photochemical or electrochemical methods in the presence of a target template material of interest. Recently, they have been used for pathogen detection due to its excellent properties such as simple fabrication process together with their stability [55–60].

For instance, Golabi et al. developed an electrochemical biosensor for *Staphylococcus epidermidis* detection with the use of MIPs [61]. Here, cell imprinted polymers (CIPs) were electrochemically prepared with 3-aminophenylboronic acid (3-APBA) monomer. Boronic acid interaction with 1,2- or 1,3-diols enabled the detection of bacteria containing diol, where gold disc electrodes (GDE) were used as working electrodes. After the CIP production, synthesized CIPs were handled with fructose to extract the cells from surface. In order to separate bacterial cells from the polymer structure, CIPs were rinsed with deionized water. The modified CIP electrode was incubated with bacteria cells, and the bacteria detection was determined by EIS. In EIS measurements, R_{ct} increased with bacteria concentration with a linear behavior between 10^3 and 10^7 CFU/mL.

Saini et al., for the first time, introduced an electrochemical DNA biosensor based on plcA gene (a virulent gene) for *Listeria monocytogenes* detection with CV and EIS [62]. Here, SPEs were employed, where ssDNA probe with -NH_2 immobilized on the electrodes, and hybridization was carried out with target DNA. The platform possesses an LOD as low as 13.7 fg/μL. The developed biosensor had lower LOD compared to the reported biosensors for the detection of L. *monocytogenes*.

Wu et al. introduced an electrochemical biosensor for the detection of *E. coli* and *S. aureus* directly from blood samples [63]. They developed porous nickel electrodes modified with Zn-doped CuO nanoparticles, where the Zn-CuO nanoparticles can bind to the bacteria with high affinity. After binding, Zn-CuO nanoparticles penerate to the bacteria via nanopiercing, which leads to bacterial lysis and death. Possessing three features, e.g., capture, killing and detection all in one structure, the platform could be a very critical candidate for both diagnosis and treatment of pathological diseases. In EIS measurement, R_{ct} decreased with bacteria concentrations, and the biosensor could detect the bacteria concentrations between 10^1 and 10^5 CFU/mL.

When constructing a biosensor system based on hybridization, the most important issue to be considered is that the immobilization amount of probe sequence onto the electrode surface in order to recognize its target. In addition, the immobilization amount of probe, the molecular orientation of probe DNA should be also properly designed for efficient hybridization. For these purposes, nanomaterial based systems for the control of DNA immobilization and hybridization have been used. For instance, Mobed et al. designed a novel electrochemical DNA biosensor for *Legionella pneumophila* mip gen detection [64]. The platform utilizes gold nanostructures with cysteamine. Modification of gold working electrodes with nanostructures provided strong stability for different number of cycles. In the biosensor, hybridization between probe and target sequences was determined with CV and SWV, and a very low limit of quantification was determined as 1 zM.

Ariffin et al. introduced an electrochemical DNA biosensor for *E. coli* detection [65]. The genosensor employs silica microspheres that was treated with APTES that was applied to screen-printed electrodes with AuNPs. AuNPs provided lower R_{ct} values compared to the bare screen-printed electrodes shown with EIS measurements, where ssDNA probe was attached to the sensor interacting with hollow silica microspheres. Hybridization with target bacteria sequence was determined with EIS, where LOD was determined as 1.95×10^{-21} M.

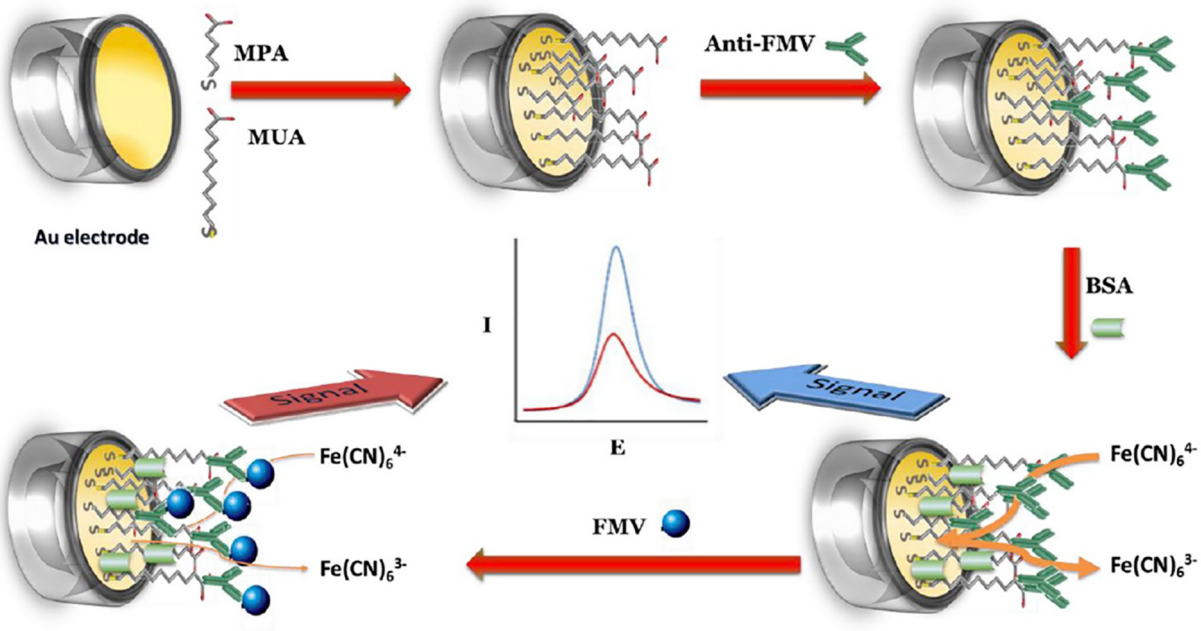

Fig. 11. Schematic illustration for the development of electrochemical immunosensor for the detection of FMV. Reprinted from [66] with permission: Copyright © 2018 Elsevier B.V.

2.2. Virus detection

In this section, we extensively reviewed recent electrochemical biosensors for the detection of viruses. Haji-Hashemi et al., for the first time, developed an electrochemical immunosensor for Fig mosaic virus (FMV) detection with CV, DPV and EIS [66]. In order to build the immunosensor, gold electrodes were dipped into a mixture of 11-mercapto undecanoic acid (MUA) and 3-mercapto propionic acid (MPA) solutions. The electrodes were interacted with EDC and NHS solutions to obtain NHS ester groups on the electrode surface. For FMV detection, antibody modified electrodes were treated with FMZ solutions of various concentrations. The experimental steps are shown in Fig. 11. In DPV measurements, the peak current decreased with virus concentration, and LOD was determined as 0.03 nM. The proposed immunosensor exhibited high selectivity, good reproducibility, high sensitivity and low detection limit. Moreover, the results were in good agreement with RT-PCR method, which confirmed the validity of the proposed immunosensor for detection of fig mosaic disease in real samples.

Fani et al. developed an electrochemical DNA biosensor for Human T-Lymphotropic Virus-1 (HTLV-1) detection with DPV [67]. Screen-printed

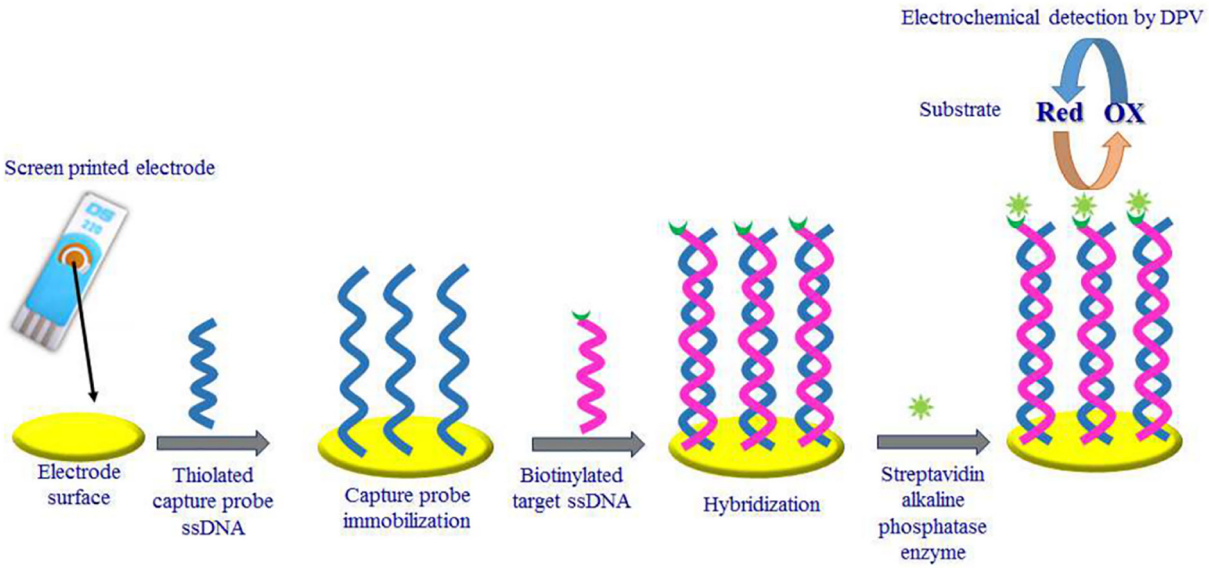

Fig. 12. Scheme for the development of electrochemical DNA biosensor for the determination of Ebola virus. Reprinted from [68] with permission: Copyright © 2018 Elsevier B.V.

carbon electrodes (SPCEs) were modified with reduced graphene oxide (rGO), polypyrrole, L-cystein, and AuNPs by electrodeposition with CV. For probe modification, the electrodes were immersed into DNA solution, and for hybridization with target DNA, probe-covered electrodes were dipped into a solution involving target DNA, anthraquinone-2-sulfonic acid sodium salt monohydrate (AQMS) and NaCl, where AQMC was used as redox-active label. For real sample analysis, peripheral blood mononuclear cell specimens were extracted and the acquired DNA was treated with NaOH, and incubated to denature DNA. The acquired ssDNA target was then applied to the probe modified electrodes. In DPV measurements, the peak current increased with target DNA concentrations, and LOD was found as 40×10^{-18} mol/L.

Ilkhani et al., for the first time, developed an electrochemical DNA biosensor for Ebola virus detection with DPV [68]. First, thiolated single strand probe were immobilized onto gold screen-printed electrodes. The probe modified electrodes were treated with MCH solution to enhance stability of the probe on the electrode surface. For hybridization, the probe-modified electrodes were treated with biotinylated target DNA. After the hybridization, the modified electrodes were interacted with streptavidin-alkaline phosphatase. In DPV measurements, electrodes were treated with 4-aminophenyl phosphate solution by incubation, and reduction occurred. 4-aminophenol was obtained by the enzymatic reaction, its signals were analyzed in DPV measurements. The experimental steps are shown in Fig. 12. In these measurements, the peak currents of 4-aminophenol increased with increasing target DNA concentrations. The system possesses an LOD as 4.7 nM.

Recently, using novel materials combined with the biosensor systems are under of interest. For instance, graphene quantum dots (GQDs), which are zero-dimension graphene nanocrystals, have been used for the detection of variety of biomolecules due to their excellent properties such as biocompatibility and low-cost preparation methods [69,70]. In addition, they have potentials in replacing those well-known metal chalcogenides–based quantum dots. They generally have contain functional groups such as carboxyl, hydroxyl, carbonyl, epoxide at their edges as reaction sites, and this feature make them good candidate for bacteria and virus detection. For example, Ghanbari et al., for the first time, introduced an electrochemical aptasensor for hepatitis C virus (HCV) core antigen detection with EIS and DPV in human serum specimens [71]. Here, GCEs were modified with GQDs and aptamer. GQDs and aptamer were bonded one other via π-π interactions, chemisorption between GQDs and aptamers amino

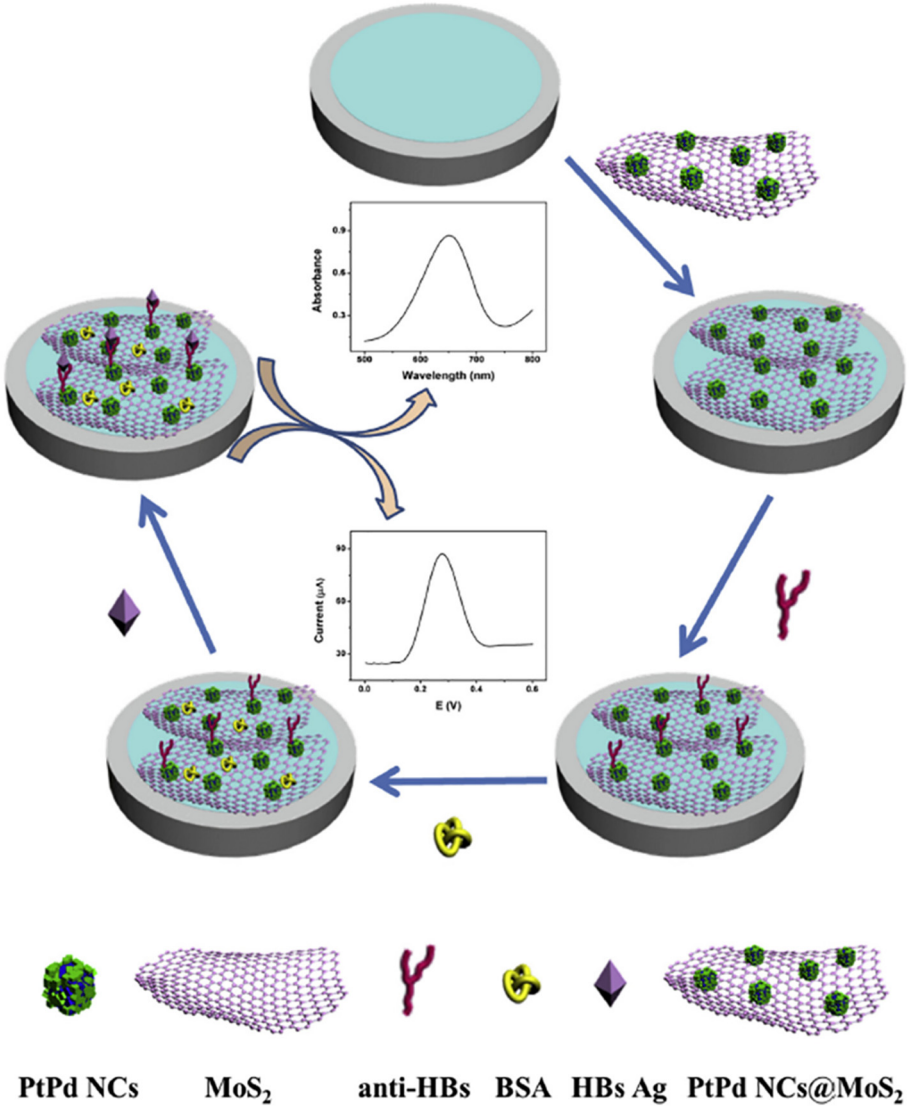

Fig. 13. Schematic illustration of the label-free electrochemical immunosensor employing modified GCE electrodes with PtPd nanocubes on molybdenum disulfide nanosheet (MoS_2) and hepatitis B antigen. Reprinted from [73] with permission: Copyright © 2019 Elsevier B.V.

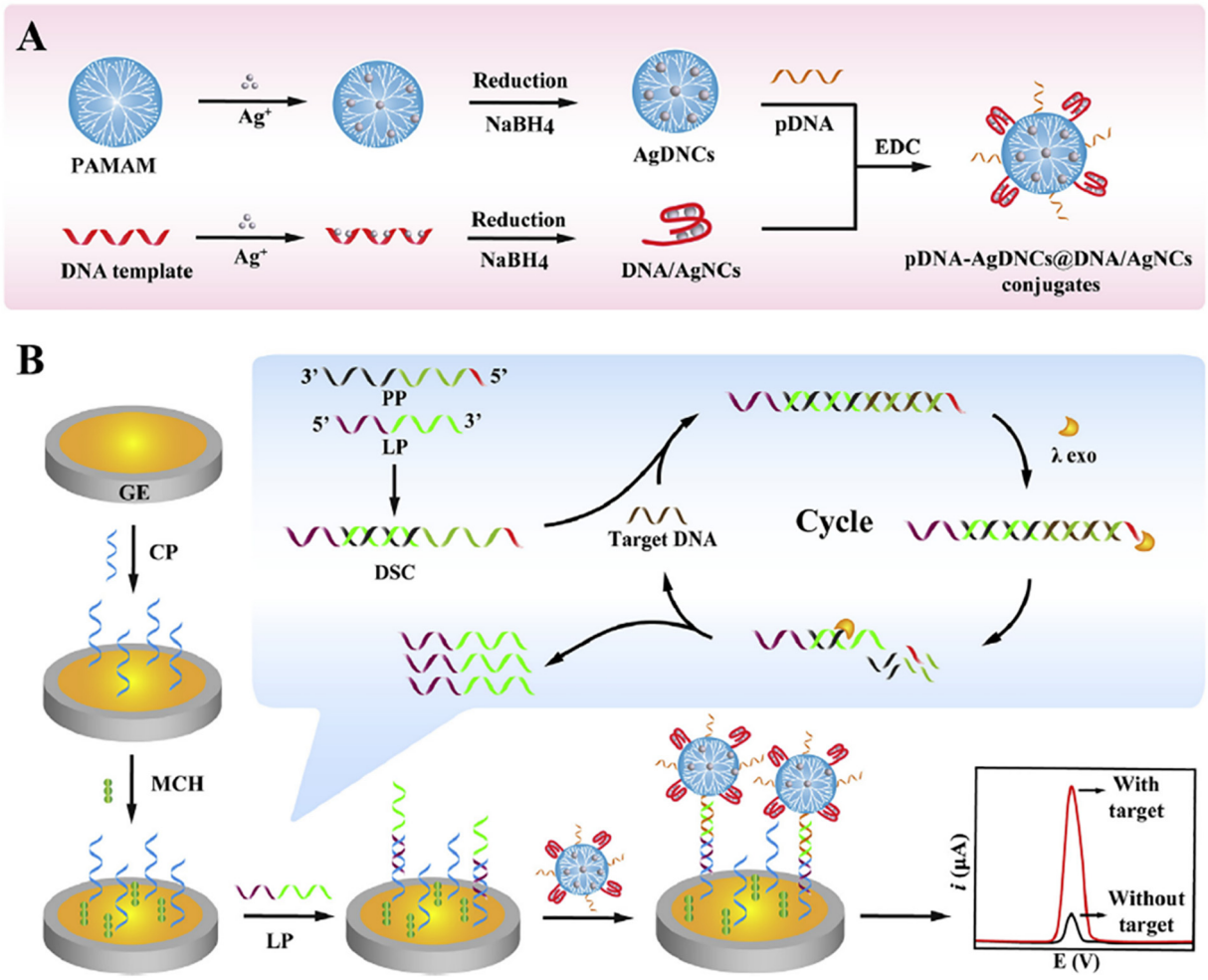

Fig. 14. (A) Conjugation of PAMAM dendrimers-encapsulated silver nanocomposites (AgDNCs) and DNA-encapsulated Ag nanoclusters (DNA/AgNCs). (B) Modification steps of the gold working electrode and amplification of target DNA using an enzyme-based method. Reprinted from [74] with permission: Copyright © 2019 Elsevier B.V.

groups, i.e., aptamer and GQDs modified electrodes were interacted with antigen. In EIS measurements, R_{ct} value increased with antigen concentrations, and LOD was found as 3.3 pg/mL, while recovery was found between 99%–109%.

Karash et al. developed an electrochemical aptasensor for avian influenza virus H5N1 detection with EIS in chicken tracheal specimens [72]. In the biosensor, gold interdigitated array (IDA) microelectrodes were used, where they were modified with streptavidin by physical adsorption to build aptasensor. The streptavidin modified electrodes were treated with biotinylated H5N1 aptamers, while AuNPs were suspended with H5N1 aptamers to enhance the signal, and thiocyanuric acid was added in the aptamer modified AuNPs. For virus detection, H5N1 viruses were immobilized on the electrode. The system was found to have an LOD as 0.25 Hematology Apheresis Unit (HAU) for pure virus and 1 HAU for swab specimens.

Another label-free immunosensor was introduced by Tan et al. for the detection of hepatitis B [73]. In this work, GCE was modified with PtPd nanocubes (PtPd NCs) on molybdenum disulfide nanosheets (MoS_2) to enhance its peroxidase activity along with higher conductivity and surface area to increase the sensitivity of the biosensor system. The system has an LOD, e.g., 10.2 fg/mL while exhibited a linear response between 32 fg/mL and 100 ng/mL of hepatitis B surface antigen. Along with its high sensitivity, the system provides specificity, reproducibility and stability in a rapid manner (Fig. 13).

Que. et al. introduced a novel electrochemical nanobiosensor for the detection of Epstein-Barr virus DNA [74]. They used gold electrodes modified with thiolated DNA capture probes to detect target DNA. For the electrochemical readout, they developed a new combination of the nanomaterials and an exonuclease-assisted amplification method to enhance the sensitivity of the mechanism. In order to fabricate the electrochemical tag using nanomaterials, they attached Ag ions on the branches of polyamidoamine (PAMAM) dendrimers and reduced them to create the conjugates of PAMAM dendrimers-encapsulated silver nanocomposites (AgDNCs). They then modified the surface of this complex with a DNA strand, which was also conjugated to form a DNA-encapsulated Ag nanoclusters (DNA/AgNCs). DNA probes were immobilized on the surface of the AgDNCs. This complex acts as an electrochemical label as shown in Fig. 14A. Through a series of hybridization of DNA probes and the use of exonuclease enzyme, the isothermal amplification was performed to enhance the DPV measurement. Fig. 14B shows the fabrication and amplification steps. Overall, a low detection limit, e.g., 0.38 fM, and a wide linear range between 1 f. and 1 nM with high selectivity were achieved due to the use of two types of nanoparticles and the DNA amplification method.

The use of electrochemical biosensors in the detection of bacteria and virus. In Table 1, we summarize the systems based on electrochemical transducers for bacteria and virus detection.

3. Conclusion

In this review, electrochemical biosensors for the detection of bacteria and viruses were discussed in detail. In addition, applications of different types of nanomaterials and polymers with different surface modifications

Table 1
Electrochemical detection of bacteria and virus with different methods and their sensitivities.

Target pathogen	Recognition element	Method	Limit of detection	Reference
Escherichia coli	Anti-*E. coli* antibody	DPV	3 CFU/mL	[75]
Escherichia coli	Anti-*E. coli* monoclonal antibody	CV	2.84×10^3 CFU/mL	[76]
Escherichia coli	Monoclonal antibody, HRP labeled secondary anti- *E. coli* antibody	Amperometry	50 CFU/mL	[77]
Escherichia coli	ssDNA probe	EIS	1×10^{-17} M	[78]
Escherichia coli	*E. coli* aptamer	DPV	2 CFU/mL	[79]
Escherichia coli	DNA capture probe	CV	4 CFU/mL	[80]
Escherichia coli B	*T4 bacteriophage*	DPV	14 ± 5 CFU/mL	[81]
Salmonella typhimurium	*S. typhimurium aptamer*	EIS	3 CFU/mL	[82]
Salmonella typhimurium Salmonella enteridis	Amino-functionalized Salmonella DNA aptamer	CV, EIS	6.7×10^1 CFU/mL *S. typhimurium* 5.5×10^1 CFU/mL *S. enteridis*	[83]
Salmonella typhimurium	Anti-*S. Typhimurium* antibody, HRP labeled secondary antibody	Amperometry	1.0×10^1 CFU/mL	[84]
Salmonella typhimurium	Anti-specific outer membrane antigen antibody	EIS	1.0×10^1 CFU/mL	[85]
Salmonella typhimurium	Biotinylated aptamers	EIS	8.0×10^1 CFU/mL	[86]
Salmonella typhimurium	Monoclonal antibody	EIS	1.0×10^3 CFU/mL	[87]
Staphylococcal enterotoxin B	SEB aptamer	DPV	0.21 fM	[88]
Piscirickettsia salmonis	ssDNA primer	Chronoamperometry	5×10^{-8} μg/mL	[89]
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* specific aptamers	Amperometry	6.0×10^1 CFU/mL	[90]
Avian Influenza Virus (H5N1)	Monoclonal antibody	EIS	2^{-1} HAU/50 μL	[91]
Citrus tristeza Virus	Polyclonal antibody	DPV	0.27 nM	[92]
Citrus tristeza Virus	Thiolated ssDNA probe	EIS	100 nM	[93]
Chikungunya Virus	ssDNA probe	EIS, CV	3.4 nM	[94]
Dengue Virus	Anti-nonstructural antibody monoclonal antibodies	EIS	0.3 ng/mL	[95]
Foot and Mouth Disease Virus	Viral imprinted polymer	LSV	1.98 ng/mL	[96]
Hepatitis A Virus	ssDNA capture probe	CV	0.65 pM (complementary ssDNA) 6.94 fg/mL (viral cDNA)	[97]
Hepatitis B Virus	Thiolated ssDNA probe	CV	2.0×10^{-12} mol/L	[98]
Hepatitis B Virus	Peptide nucleic acid	Electrochemical lateral flow assay	7.23 pM	[99]
Hepatitis B Virus Surface Antigen (HBsAg)	Primary antibody of HBs (Ab1) and hemin bio-barcoded AuNPs probe labeled anti-HBsAg antibody (Ab2)	SWV	0.19 pg/mL	[100]
Hepatitis C Virus	ssDNA probe	CV	7 fM	[101]
Hepatitis C Virus (HCV)	DNA aptamer and molecular imprinting	DPV	1.67 fg/mL	[102]
H1N1 Influenza virus	Monoclonal antibody	Chronoamperometry	0.5 PFU/mL	[103]
H1N1 Influenza Virus	Specific monoclonal antibody	DPV	113 PFU/mL	[104]
H5N1 Avian Influenza Virus	Biotinylated DNA aptamers against H5N1	EIS	0.0128 HAU	[105]
Human Enterovirus 71 (EV71)	Monoclonal antibodies of EV71	Chronoamperometry	0.01 ng/mL	[106]
Human T-lymphotropic virus	Hairpin capture DNA probe	EIS	1.71×10^{-13} M	[107]
Inactivated H1N1 Virus	DNA aptamers against inactivated H1N1 virus	EIS	0.9 pg/mL	[108]
Middle East Respiratory Syndrome Corona Virus (MERS-CoV)	MERS-CoV antigen	SWV	1.0 pg/mL	[109]
Newcastle Disease Virus (NDV)	Anti-NDV monoclonal antibody	DPV	10(0.68) EID50/0.1 mL	[110]
Norovirus	Norovirus specific aptamers	SWV	N/A	[111]
Respiratory Syncytial Virus (RSV)	Antibody specific to the F protein	CV, EIS	1.1×10^3 PFU/mL	[112]
Type 5 Adenovirus	Anti-adenovirus 5 antibody	EIS	30 adenovirus particles/mL	[113]
Zika Virus	SH-probe ssDNA	EIS, CV, DPV	25 nM	[114]
Zika Virus	Surface imprinted polymer (SIP)	EIS, CV	2×10^{-4} PFU/mL	[115]

for enhancing the sensitivity of the electrochemical biosensors have been reviewed.

Electrochemical biosensors have been used extensively in recent years as an alternative to conventional methods for the detection of pathogens because of their high sensitivity, fast response time, and low cost. They also do not require laborious interpretation and equipment resources, exhibit more versatile detection schemes which provides broader applications, can be easily manipulated by the personnel without previous training, and are capable of real time quantification.

Electrochemical methods such as EIS, DPV, CV, SWV and amperometry have been used for pathogen detection. In order to enhance the signals obtained, nanomaterials or polymers have been generally applied with these methods. Among them, the most preferred electrochemical based technique is EIS. The main strategy for an impedimetric biosensor is the immobilization of bioreceptors (e.g., antibodies) onto the surface of the electrode which bypasses the labeling procedure that is normally required by other electrochemical biosensors. In EIS, the binding event between the bioreceptor molecules and target could be read-out in the electrical signals. The accumulation of negative charges during the binding process of the target toward bioreceptor molecules immobilized on electrode surfaces causes a repulsion of the redox species, thus inhibiting the redox reaction and enhancing the charge transfer resistance value. However, despite the advantages such as allowing label-free measurements, the major drawbacks of impedimetric biosensors are that the consistency of the fabricated biosensors is greatly affected by the surface condition of the electrodes and the unspecific absorption of compounds in biological samples, and it is very difficult to reproduce and regenerate the electrodes.

Despite advantages of electrochemical biosensors, there are some issues to be considered for the analyte detection, For instance, analyte loss is still a problem due to the need for transporting the sample to the electrode surface. One of the issues to be considered for pathogen detection is that the developed biosensor system should allow multiple detection. Simultaneous and multiple detection could be accomplished by using multiple transducers that exhibit different biorecognition elements which make the system

more complex. For this reason, multiple biorecognition elements on a single electrode should be designed. Most of the studies for pathogen detection in food or environmental samples are tested in liquids such as milk, fruit juices or broths, and used as a proof-of-concept. However, the developed biosensor systems which can detect analyte of the interest in complex samples without serious interferences should be developed commonly. Moreover, most of studies have been calculating LOD and linear range, however, the validation parameters such as precision, accuracy, repeatability, selectivity/specificity, linearity and the limit of quantification should be also investigated.

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.

References

[1] W.H. Organization, Global Health Estimates 2015: Disease Burden by Cause, Age, Sex, by Country and by Region, 2000–2015, World Health Organization, Geneva, 2016.

[2] M. Eckhardt, J.F. Hultquist, R.M. Kaake, R. Hüttenhain, N.J. Krogan, A systems approach to infectious disease, Nat. Rev. Genet. 21 (6) (2020) 339–354.

[3] D. Malavia, N.A. Gow, J. Usher, Advances in molecular tools and in vivo models for the study of human fungal pathogenesis, Microorganisms 8 (6) (2020) 803.

[4] J. Dietvorst, L. Vilaplana, N. Uria, M.-P. Marco, X. Muñoz-Berbel, Current and near-future technologies for antibiotic susceptibility testing and resistant bacteria detection, TrAC Trends Anal. Chem. 127 (2020) 115891.

[5] C. Lemoine, A. Thakur, D. Krajišnik, R. Guyon, S. Longet, A. Razim, S. Górska, I. Pantelić, T. Ilić, I. Nikolić, Technological approaches for improving vaccination compliance and coverage, Vaccines 8 (2) (2020) 304.

[6] W.N. Lin, M.Z. Tay, R. Lu, Y. Liu, C.-H. Chen, L.F. Cheow, The role of single-cell technology in the study and control of infectious diseases, Cells 9 (6) (2020) 1440.

[7] A. Bertoletti, A.T. Tan, Challenges of CAR- and TCR-T cell–based therapy for chronic infections, J. Exp. Med. 217 (5) (2020).

[8] N. Bhalla, P. Jolly, N. Formisano, P. Estrela, Introduction to biosensors, Essays Biochem. 60 (1) (2016) 1–8.

[9] L. Xu, N. Shoaei, F. Jahanpeyma, J. Zhao, M. Azimzadeh, K.T. Al, Optical, electrochemical and electrical (nano) biosensors for detection of exosomes: a comprehensive overview, Biosens. Bioelectron. 112222 (2020).

[10] S. Jafari, M. Dehghani, N. Nasirizadeh, M.H. Baghersad, M. Azimzadeh, Label-free electrochemical detection of Cloxacillin antibiotic in milk samples based on molecularly imprinted polymer and graphene oxide-gold nanocomposite, Measurement 145 (2019) 22–29.

[11] S. Topkaya, M. Azimzadeh, Biosensors of in vitro detection of cancer and bacterial cells, Nanobiosensors for Personalized and Onsite Biomedical Diagnosis, Institution of Engineering and Technology 2016, pp. 73–94.

[12] R.R. Soares, R.G. Hjort, C.C. Pola, K. Parate, E.L. Reis, N.F. Soares, E.S. McLamore, J.C. Claussen, C.L. Gomes, Laser-induced Graphene electrochemical Immunosensors for rapid and label-free monitoring of *Salmonella enterica* in chicken broth, ACS Sens. 5 (7) (2020) 1900–1911.

[13] S.N. Topkaya, M. Azimzadeh, M. Ozsoz, Electrochemical biosensors for cancer biomarkers detection: recent advances and challenges, Electroanalysis 28 (7) (2016) 1402–1419.

[14] S. Yazdanparast, A. Benvidi, M. Azimzadeh, M.D. Tezerjani, M.R. Ghaani, Experimental and theoretical study for miR-155 detection through resveratrol interaction with nucleic acids using magnetic core-shell nanoparticles, Microchim. Acta 187 (8) (2020) 1–10.

[15] M. Amiri, A. Bezaatpour, H. Jafari, R. Boukherroub, S. Szunerits, Electrochemical methodologies for the detection of pathogens, ACS Sens. 3 (6) (2018) 1069–1086.

[16] F.A. Alatraktchi, W.E. Svendsen, S. Molin, Electrochemical detection of Pyocyanin as a biomarker for *Pseudomonas aeruginosa*: a focused review, Sensors (Basel) 20 (18) (2020).

[17] S. Kuss, H.M.A. Amin, R.G. Compton, Electrochemical detection of pathogenic bacteria-recent strategies, Adv. Challeng. 13 (19) (2018) 2758–2769.

[18] F. McEachern, E. Harvey, G. Merle, Emerging technologies for the electrochemical detection of bacteria, Biotechnol. J. 15 (9) (2020) e2000140.

[19] S. Hemanth, A. Halder, C. Caviglia, Q. Chi, S.S. Keller, 3D carbon microelectrodes with bio-functionalized graphene for electrochemical biosensing, Biosensors 8 (3) (2018).

[20] F.A. Alatraktchi, M. Dimaki, N. Støvring, H.K. Johansen, S. Molin, W.E. Svendsen, Nanograss sensor for selective detection of *Pseudomonas aeruginosa* by pyocyanin identification in airway samples, Anal. Biochem. 593 (2020) 113586.

[21] A. Güner, E. Çevik, M. Şenel, L. Alpsoy, An electrochemical immunosensor for sensitive detection of *Escherichia coli* O157:H7 by using chitosan, MWCNT, polypyrrole with gold nanoparticles hybrid sensing platform, Food Chem. 229 (2017) 358–365.

[22] K. Bekir, H. Barhoumi, M. Braiek, A. Chrouda, N. Zine, N. Abid, A. Maaref, A. Bakhrouf, H.B. Ouada, N. Jaffrezic-Renault, H.B. Mansour, Electrochemical impedance immunosensor for rapid detection of stressed pathogenic *Staphylococcus aureus* bacteria, Environ. Sci. Pollut. Res. Int. 22 (20) (2015) 15796–15803.

[23] J. Dong, H. Zhao, M. Xu, Q. Ma, S. Ai, A label-free electrochemical impedance immunosensor based on AuNPs/PAMAM-MWCNT-Chi nanocomposite modified glassy carbon electrode for detection of Salmonella typhimurium in milk, Food Chem. 141 (3) (2013) 1980–1986.

[24] M. Cimafonte, A. Fulgione, R. Gaglione, M. Papaianni, R. Capparelli, A. Arciello, S. Bolletti Censi, G. Borriello, R. Velotta, B. Della Ventura, Screen printed based impedimetric immunosensor for rapid detection of *Escherichia coli* in drinking water, Sensors (Basel) 20 (1) (2020) 274.

[25] H. Huang, M. Liu, X. Wang, W. Zhang, D.-P. Yang, L. Cui, X. Wang, Label-free 3D Ag nanoflower-based electrochemical immunosensor for the detection of *Escherichia coli* O157: H7 pathogens, Nanoscale Res. Lett. 11 (1) (2016) 507.

[26] S. Shukla, Y. Haldorai, V.K. Bajpai, A. Rengaraj, S.K. Hwang, X. Song, M. Kim, Y.S. Huh, Y.-K. Han, Electrochemical coupled immunosensing platform based on graphene oxide/gold nanocomposite for sensitive detection of Cronobacter sakazakii in powdered infant formula, Biosens. Bioelectron. 109 (2018) 139–149.

[27] V.B.C. Lee, N.F. Mohd-Naim, E. Tamiya, M.U. Ahmed, Trends in paper-based electrochemical biosensors: from design to application, Anal. Sci. 34 (1) (2018) 7–18.

[28] F.A. Alatraktchi, J.S. Noori, G.P. Tanev, J. Mortensen, M. Dimaki, H.K. Johansen, J. Madsen, S. Molin, W.E. Svendsen, Paper-based sensors for rapid detection of virulence factor produced by *Pseudomonas aeruginosa*, PLoS One 13 (3) (2018), e0194157.

[29] E. Cesewski, B.N. Johnson, Electrochemical biosensors for pathogen detection, Biosens. Bioelectron. 159 (2020) 112214.

[30] S. Rengaraj, Á. Cruz-Izquierdo, J.L. Scott, M. Di Lorenzo, Impedimetric paper-based biosensor for the detection of bacterial contamination in water, Sensors Actuators B Chem. 265 (2018) 50–58.

[31] H. Wang, Y. Xiu, Y. Chen, L. Sun, L. Yang, H. Chen, X. Niu, Electrochemical immunosensor based on an antibody-hierarchical mesoporous SiO2 for the detection of *Staphylococcus aureus*, RSC Adv. 9 (28) (2019) 16278–16287.

[32] L.T. Tufa, S. Oh, J. Kim, K.-J. Jeong, T.J. Park, H.-J. Kim, J. Lee, Electrochemical immunosensor using nanotriplex of graphene quantum dots, Fe3O4, and Ag nanoparticles for tuberculosis, Electrochim. Acta 290 (2018) 369–377.

[33] C. Singh, M.A. Ali, V. Kumar, R. Ahmad, G. Sumana, Functionalized MoS2 nanosheets assembled microfluidic immunosensor for highly sensitive detection of food pathogen, Sensors Actuators B Chem. 259 (2018) 1090–1098.

[34] J. Bhardwaj, S. Devarakonda, S. Kumar, J. Jang, Development of a paper-based electrochemical immunosensor using an antibody-single walled carbon nanotubes bioconjugate modified electrode for label-free detection of foodborne pathogens, Sensors Actuators B Chem. 253 (2017) 115–123.

[35] G. Dutta, A.A. Jallow, D. Paul, D. Moschou, Label-free electrochemical detection of S. mutans exploiting commercially fabricated printed circuit board sensing electrodes, Micromachines (Basel) 10 (9) (2019) 575.

[36] C.T. Lee, Y.S. Chiu, S.C. Ho, Y.J. Lee, Investigation of a photoelectrochemical passivated ZnO-based glucose biosensor, Sensors (Basel) 11 (5) (2011) 4648–4655.

[37] B. Haghighi, S. Bozorgzadeh, Fabrication of a highly sensitive electrochemiluminescence lactate biosensor using ZnO nanoparticles decorated multiwalled carbon nanotubes, Talanta 85 (4) (2011) 2189–2193.

[38] N. Chauhan, S. Gupta, D.K. Avasthi, R. Adelung, Y.K. Mishra, U. Jain, Zinc oxide tetrapods based biohybrid interface for voltammetric sensing of helicobacter pylori, ACS Appl. Mater. Interfaces 10 (36) (2018) 30631–30639.

[39] X. Kang, G. Pang, Q. Chen, X. Liang, Fabrication of *Bacillus cereus* electrochemical immunosensor based on double-layer gold nanoparticles and chitosan, Sensors Actuators B Chem. 177 (2013) 1010–1016.

[40] T.S. Svalova, M.V. Medvedeva, A.A. Saigushkina, I.V. Kozitsin, N.N. Malysheva, V.O. Zhdanovskikh, A.V. Okhokhonin, A.N. Kozitsina, A label-free impedimetric immunosensor based on covalent immobilization of anti-*E. coli* antibody via a copper-catalyzed azide-alkyne cycloaddition reaction, Anal. Bioanal. Chem. 412 (21) (2020) 5077–5087.

[41] N. Mohd Bakhori, N.A. Yusof, J. Abdullah, H. Wasoh, S.K. Ab Rahman, Surface enhanced CdSe/ZnS QD/SiNP electrochemical immunosensor for the detection of *Mycobacterium tuberculosis* by combination of CFP10-ESAT6 for better diagnostic specificity, Materials (Basel) 13 (1) (2019) 149.

[42] R. Jijie, K. Kahlouche, A. Barras, N. Yamakawa, J. Bouckaert, T. Gharbi, S. Szunerits, R. Boukherroub, Reduced graphene oxide/polyethylenimine based immunosensor for the selective and sensitive electrochemical detection of uropathogenic *Escherichia coli*, Sensors Actuators B Chem. 260 (2018) 255–263.

[43] S. Muniandy, I.J. Dinshaw, S.J. Teh, C.W. Lai, F. Ibrahim, K.L. Thong, B.F. Leo, Graphene-based label-free electrochemical aptasensor for rapid and sensitive detection of foodborne pathogen, Anal. Bioanal. Chem. 409 (29) (2017) 6893–6905.

[44] M. Xu, R. Wang, Y. Li, Rapid detection of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in foods using an electrochemical immunosensor based on screen-printed interdigitated microelectrode and immunomagnetic separation, Talanta 148 (2016) 200–208.

[45] R. Nazari-Vanani, N. Sattarahmady, H. Yadegari, H. Heli, A novel and ultrasensitive electrochemical DNA biosensor based on an ice crystals-like gold nanostructure for the detection of *Enterococcus faecalis* gene sequence, Colloids Surf. B: Biointerfaces 166 (2018) 245–253.

[46] G. Henihan, H. Schulze, D.K. Corrigan, G. Giraud, J.G. Terry, A. Hardie, C.J. Campbell, A.J. Walton, J. Crain, R. Pethig, K.E. Templeton, A.R. Mount, T.T. Bachmann, Label- and amplification-free electrochemical detection of bacterial ribosomal RNA, Biosens. Bioelectron. 81 (2016) 487–494.

[47] C. Luo, H. Tang, W. Cheng, L. Yan, D. Zhang, H. Ju, S. Ding, A sensitive electrochemical DNA biosensor for specific detection of Enterobacteriaceae bacteria by exonuclease III-assisted signal amplification, Biosens. Bioelectron. 48 (2013) 132–137.
[48] S. Ranjbar, S. Shahrokhian, Design and fabrication of an electrochemical aptasensor using Au nanoparticles/carbon nanoparticles/cellulose nanofibers nanocomposite for rapid and sensitive detection of *Staphylococcus aureus*, Bioelectrochemistry 123 (2018) 70–76.
[49] S. Muniandy, S.J. Teh, J.N. Appaturi, K.L. Thong, C.W. Lai, F. Ibrahim, B.F. Leo, A reduced graphene oxide-titanium dioxide nanocomposite based electrochemical aptasensor for rapid and sensitive detection of *Salmonella enterica*, Bioelectrochemistry 127 (2019) 136–144.
[50] M. Sarabaegi, M. Roushani, A nano-sized chitosan particle based electrochemical aptasensor for sensitive detection of *P. aeruginosa*, Anal. Methods 11 (43) (2019) 5591–5597.
[51] S.S. Zarei, S. Soleimanian-Zad, A.A. Ensafi, An impedimetric aptasensor for Shigella dysenteriae using a gold nanoparticle-modified glassy carbon electrode, Microchim. Acta 185 (12) (2018) 538.
[52] Z. Hatami, E. Ragheb, F. Jalali, M.A. Tabrizi, M. Shamsipur, Zinc oxide-gold nanocomposite as a proper platform for label-free DNA biosensor, Bioelectrochemistry 133 (2020) 107458.
[53] S. Hajihosseini, N. Nasirizadeh, M.S. Hejazi, P. Yaghmaei, A sensitive DNA biosensor fabricated from gold nanoparticles and graphene oxide on a glassy carbon electrode, Mater. Sci. Eng. C 61 (2016) 506–515.
[54] T.S.T. Balamurugan, C.-H. Huang, P.-C. Chang, S.-T. Huang, Electrochemical molecular switch for the selective profiling of cysteine in live cells and whole blood and for the quantification of aminoacylase-1, Anal. Chem. 90 (21) (2018) 12631–12638.
[55] X. Yang, J. Sun, F. Cui, J. Ji, L. Wang, Y. Zhang, X. Sun, An eco-friendly sensor based on CQD@MIPs for detection of N-acylated homoserine lactones and its 3D printing applications, Talanta 219 (2020) 121343.
[56] Y. Guo, M. Girmatsion, H.W. Li, Y. Xie, W. Yao, H. Qian, B. Abraha, A. Mahmud, Rapid and ultrasensitive detection of food contaminants using surface-enhanced Raman spectroscopy-based methods, Crit. Rev. Food Sci. Nutr. (2020) 1–14.
[57] A. Kushwaha, J. Srivastava, A.K. Singh, R. Anand, R. Raghuwanshi, T. Rai, M. Singh, Epitope imprinting of Mycobacterium leprae bacteria via molecularly imprinted nanoparticles using multiple monomers approach, Biosens. Bioelectron. 145 (2019) 111698.
[58] A.H.A. Hassan, L. Sappia, S.L. Moura, F.H.M. Ali, W.A. Moselhy, M. Sotomayor, M.I. Pividori, Biomimetic magnetic sensor for electrochemical determination of scombrotoxin in fish, Talanta 194 (2019) 997–1004.
[59] A.G. Ayankojo, J. Reut, V. Ciocan, A. Opik, V. Syritski, Molecularly imprinted polymer-based sensor for electrochemical detection of erythromycin, Talanta 209 (2020) 120502.
[60] C. Tancharoen, W. Sukjee, P.-t. Yenchitsomanus, A. Panya, P.A. Lieberzeit, C. Sangma, Selectivity enhancement of MIP-composite sensor for explosive detection using DNT-dengue virus template: a co-imprinting approach, Mater. Lett. 285 (2021) 129201.
[61] M. Golabi, F. Kuralay, E.W.H. Jager, V. Beni, A.P.F. Turner, Electrochemical bacterial detection using poly(3-aminophenylboronic acid)-based imprinted polymer, Biosens. Bioelectron. 93 (2017) 87–93.
[62] K. Saini, A. Kaushal, S. Gupta, D. Kumar, PlcA-based nanofabricated electrochemical DNA biosensor for the detection of *Listeria monocytogenes* in raw milk samples, 3 Biotech 10 (7) (2020) 327.
[63] A. Bhalkikar, T.-S. Wu, C.M. Marin, T.J. Fisher, M. Wang, I.H. Wells, A. Sarella, Y.-L. Soo, C.L. Cheung, Ozone-mediated synthesis of ceria nanoparticles, Nanoscale 10 (21) (2018) 9822–9829.
[64] A. Mobed, M. Hasanzadeh, S. Hassanpour, A. Saadati, M. Agazadeh, A. Mokhtarzadeh, An innovative nucleic acid based biosensor toward detection of legionella pneumophila using DNA immobilization and hybridization: a novel genosensor, Microchem. J. 148 (2019) 708–716.
[65] E. Yuhana Ariffin, L.Y. Heng, L.L. Tan, N.H. Abd Karim, S.A. Hasbullah, A highly sensitive impedimetric DNA biosensor based on hollow silica microspheres for label-free determination of *E. coli*, Sensors (Basel) 20 (5) (2020).
[66] H. Haji-Hashemi, M.R. Safarnejad, P. Norouzi, M. Ebrahimi, M. Shahmirzaie, M.R. Ganjali, Simple and effective label free electrochemical immunosensor for fig mosaic virus detection, Anal. Biochem. 566 (2019) 102–106.
[67] M. Fani, M. Rezayi, Z. Meshkat, S.A. Rezaee, M. Makvandi, K.A. Angali, A novel electrochemical DNA biosensor based on a gold nanoparticles-reduced graphene oxide-polypyrrole nanocomposite to detect human T-lymphotropic virus-1, IEEE Sensors J. 20 (18) (2020) 10625–10632.
[68] H. Ilkhani, S. Farhad, A novel electrochemical DNA biosensor for Ebola virus detection, Anal. Biochem. 557 (2018) 151–155.
[69] P. Zhang, X. Zhao, Y. Ji, Z. Ouyang, X. Wen, J. Li, Z. Su, G. Wei, Electrospinning graphene quantum dots into a nanofibrous membrane for dual-purpose fluorescent and electrochemical biosensors, J. Mater. Chem. B 3 (12) (2015) 2487–2496.
[70] A. Muthurasu, V. Ganesh, Horseradish peroxidase enzyme immobilized graphene quantum dots as electrochemical biosensors, Appl. Biochem. Biotechnol. 174 (3) (2014) 945–959.
[71] K. Ghanbari, M. Roushani, A. Azadbakht, Ultra-sensitive aptasensor based on a GQD nanocomposite for detection of hepatitis C virus core antigen, Anal. Biochem. 534 (2017) 64–69.
[72] S. Karash, R. Wang, L. Kelso, H. Lu, T.J. Huang, Y. Li, Rapid detection of avian influenza virus H5N1 in chicken tracheal samples using an impedance aptasensor with gold nanoparticles for signal amplification, J. Virol. Methods 236 (2016) 147–156.
[73] Z. Tan, H. Dong, Q. Liu, H. Liu, P. Zhao, P. Wang, Y. Li, D. Zhang, Z. Zhao, Y. Dong, A label-free immunosensor based on PtPd NCs@ MoS2 nanoenzymes for hepatitis B surface antigen detection, Biosens. Bioelectron. 142 (2019) 111556.
[74] H. Que, D. Zhang, B. Guo, T. Wang, H. Wu, D. Han, Y. Yan, Label-free and ultrasensitive electrochemical biosensor for the detection of EBV-related DNA based on AgDNCs@DNA/AgNCs nanocomposites and lambda exonuclease-assisted target recycling, Biosens. Bioelectron. 143 (2019) 111610.
[75] M. Zhong, L. Yang, H. Yang, C. Cheng, W. Deng, Y. Tan, Q. Xie, S. Yao, An electrochemical immunobiosensor for ultrasensitive detection of *Escherichia coli* O157:H7 using CdS quantum dots-encapsulated metal-organic frameworks as signal-amplifying tags, Biosens. Bioelectron. 126 (2019) 493–500.
[76] C.R. Nemr, S.J. Smith, W. Liu, A.H. Mepham, R.M. Mohamadi, M. Labib, S.O. Kelley, Nanoparticle-mediated capture and electrochemical detection of methicillin-resistant *Staphylococcus aureus*, Anal. Chem. 91 (4) (2019) 2847–2853.
[77] Z. Altintas, M. Akgun, G. Kokturk, Y. Uludag, A fully automated microfluidic-based electrochemical sensor for real-time bacteria detection, Biosens. Bioelectron. 100 (2018) 541–548.
[78] N. Jaiswal, C.M. Pandey, A. Soni, I. Tiwari, M. Rosillo-Lopez, C.G. Salzmann, B.D. Malhotra, G. Sumana, Electrochemical genosensor based on carboxylated graphene for detection of water-borne pathogen, Sensors Actuators B Chem. 275 (2018) 312–321.
[79] S. Shahrokhian, S. Ranjbar, Aptamer immobilization on amino-functionalized metal–organic frameworks: an ultrasensitive platform for the electrochemical diagnostic of *Escherichia coli* O157:H7, Analyst 143 (13) (2018) 3191–3201.
[80] N. Shoaie, M. Forouzandeh, K. Omidfar, Voltammetric determination of the *Escherichia coli* DNA using a screen-printed carbon electrode modified with polyaniline and gold nanoparticles, Microchim. Acta 185 (4) (2018) 217.
[81] J. Xu, C. Zhao, Y. Chau, Y.K. Lee, The synergy of chemical immobilization and electrical orientation of T4 bacteriophage on a micro electrochemical sensor for low-level viable bacteria detection via differential pulse voltammetry, Biosens. Bioelectron. 151 (2020) 111914.
[82] E. Sheikhzadeh, M. Chamsaz, A.P.F. Turner, E.W.H. Jager, V. Beni, Label-free impedimetric biosensor for *Salmonella typhimurium* detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer, Biosens. Bioelectron. 80 (2016) 194–200.
[83] M.R. Hasan, T. Pulingam, J.N. Appaturi, A.N. Zifruddin, S.J. Teh, T.W. Lim, F. Ibrahim, B.F. Leo, K.L. Thong, Carbon nanotube-based aptasensor for sensitive electrochemical detection of whole-cell Salmonella, Anal. Biochem. 554 (2018) 34–43.
[84] D.L. Alexandre, A.M.A. Melo, R.F. Furtado, M.F. Borges, E.A.T. Figueiredo, A. Biswas, H.N. Cheng, C.R. Alves, A rapid and specific biosensor for *Salmonella typhimurium* detection in Milk, Food Bioprocess Technol. 11 (4) (2018) 748–756.
[85] R. Mutreja, M. Jariyal, P. Pathania, A. Sharma, D.K. Sahoo, C.R. Suri, Novel surface antigen based impedimetric immunosensor for detection of *Salmonella typhimurium* in water and juice samples, Biosens. Bioelectron. 85 (2016) 707–713.
[86] L. Wang, X. Huo, W. Qi, Z. Xia, Y. Li, J. Lin, Rapid and sensitive detection of *Salmonella typhimurium* using nickel nanowire bridge for electrochemical impedance amplification, Talanta 211 (2020) 120715.
[87] H. Jiang, D. Jiang, J. Shao, X. Sun, Magnetic molecularly imprinted polymer nanoparticles based electrochemical sensor for the measurement of Gram-negative bacterial quorum signaling molecules (N-acyl-homoserine-lactones), Biosensors and Bioelectronics 75 (2016) 411–419.
[88] S. Mousavi Nodoushan, N. Nasirizadeh, J. Amani, R. Halabian, A.A. Imani Fooladi, An electrochemical aptasensor for staphylococcal enterotoxin B detection based on reduced graphene oxide and gold nano-urchins, Biosens. Bioelectron. 127 (2019) 221–228.
[89] J.S. del Río, M. Svobodova, P. Bustos, P. Conejeros, C.K. O'Sullivan, Electrochemical detection of Piscirickettsia salmonis genomic DNA from salmon samples using solid-phase recombinase polymerase amplification, Anal. Bioanal. Chem. 408 (30) (2016) 8611–8620.
[90] R. Das, A. Dhiman, A. Kapil, V. Bansal, T.K. Sharma, Aptamer-mediated colorimetric and electrochemical detection of *Pseudomonas aeruginosa* utilizing peroxidase-mimic activity of gold NanoZyme, Anal. Bioanal. Chem. 411 (6) (2019) 1229–1238.
[91] J. Lin, R. Wang, P. Jiao, Y. Li, Y. Li, M. Liao, Y. Yu, M. Wang, An impedance immunosensor based on low-cost microelectrodes and specific monoclonal antibodies for rapid detection of avian influenza virus H5N1 in chicken swabs, Biosens. Bioelectron. 67 (2015) 546–552.
[92] H. Haji-Hashemi, P. Norouzi, M.R. Safarnejad, M.R. Ganjali, Label-free electrochemical immunosensor for direct detection of Citrus tristeza virus using modified gold electrode, Sensors Actuators B Chem. 244 (2017) 211–216.
[93] M. Khater, A. de la Escosura-Muñiz, D. Quesada-González, A. Merkoçi, Electrochemical detection of plant virus using gold nanoparticle-modified electrodes, Anal. Chim. Acta 1046 (2019) 123–131.
[94] C. Singhal, M. Khanuja, N. Chaudhary, C.S. Pundir, J. Narang, Detection of chikungunya virus DNA using two-dimensional MoS2 nanosheets based disposable biosensor, Sci. Rep. 8 (1) (2018) 7734.
[95] M.H. Nawaz, A. Hayat, G. Catanante, U. Latif, J.L. Marty, Development of a portable and disposable NS1 based electrochemical immunosensor for early diagnosis of dengue virus, Anal. Chim. Acta 1026 (2018) 1–7.
[96] H.A. Hussein, R.Y.A. Hassan, R.M. El Nashar, S.A. Khalil, S.A. Salem, I.M. El-Sherbiny, Designing and fabrication of new VIP biosensor for the rapid and selective detection of foot-and-mouth disease virus (FMDV), Biosens. Bioelectron. 141 (2019) 111467.
[97] M. Manzano, S. Viezzi, S. Mazerat, R.S. Marks, J. Vidic, Rapid and label-free electrochemical DNA biosensor for detecting hepatitis a virus, Biosens. Bioelectron. 100 (2018) 89–95.
[98] Z. Shakoori, S. Salimian, S. Kharrazi, M. Adabi, R. Saber, Electrochemical DNA biosensor based on gold nanorods for detecting hepatitis B virus, Anal. Bioanal. Chem. 407 (2) (2015) 455–461.
[99] C. Srisomwat, A. Yakoh, N. Chuaypen, P. Tangkijvanich, T. Vilaivan, O. Chailapakul, Amplification-free DNA sensor for the one-step detection of the hepatitis B virus

using an automated paper-based lateral flow electrochemical device, Anal. Chem. (2020) https://doi.org/10.1021/acs.analchem.0c04283.
[100] N. Alizadeh, R. Hallaj, A. Salimi, A highly sensitive electrochemical immunosensor for hepatitis B virus surface antigen detection based on Hemin/G-quadruplex horseradish peroxidase-mimicking DNAzyme-signal amplification, Biosens. Bioelectron. 94 (2017) 184–192.
[101] B. Zribi, E. Roy, A. Pallandre, S. Chebil, M. Koubaa, N. Mejri, H. Magdinier Gomez, C. Sola, H. Korri-Youssoufi, A.M. Haghiri-Gosnet, A microfluidic electrochemical biosensor based on multiwall carbon nanotube/ferrocene for genomic DNA detection of *Mycobacterium tuberculosis* in clinical isolates, Biomicrofluidics 10 (1) (2016), 014115.
[102] K. Ghanbari, M. Roushani, A nanohybrid probe based on double recognition of an aptamer MIP grafted onto a MWCNTs-chit nanocomposite for sensing hepatitis C virus core antigen, Sensors Actuators B Chem. 258 (2018) 1066–1071.
[103] R. Singh, S. Hong, J. Jang, Label-free detection of influenza viruses using a reduced Graphene oxide-based electrochemical Immunosensor integrated with a microfluidic platform, Sci. Rep. 7 (1) (2017) 42771.
[104] S. Devarakonda, R. Singh, J. Bhardwaj, J. Jang, Cost-effective and handmade paper-based immunosensing device for electrochemical detection of influenza virus, Sensors (Basel) 17 (11) (2017) 2597.
[105] J. Lum, R. Wang, B. Hargis, S. Tung, W. Bottje, H. Lu, Y. Li, An impedance Aptasensor with microfluidic chips for specific detection of H5N1 avian influenza virus, Sensors (Basel) 15 (8) (2015) 18565–18578.
[106] Y.H. Hou, J.J. Wang, Y.Z. Jiang, C. Lv, L. Xia, S.L. Hong, M. Lin, Y. Lin, Z.L. Zhang, D.W. Pang, A colorimetric and electrochemical immunosensor for point-of-care detection of enterovirus 71, Biosens. Bioelectron. 99 (2018) 186–192.
[107] D. Cheng, Y. Zhang, D. Wen, Z. Guo, H. Yang, Y. Liu, J. Kong, Hairpin probes based click polymerization for label-free electrochemical detection of human T-lymphotropic virus types II, Anal. Chim. Acta 1059 (2019) 86–93.
[108] C. Bai, Z. Lu, H. Jiang, Z. Yang, X. Liu, H. Ding, H. Li, J. Dong, A. Huang, T. Fang, Y. Jiang, L. Zhu, X. Lou, S. Li, N. Shao, Aptamer selection and application in multivalent binding-based electrical impedance detection of inactivated H1N1 virus, Biosens. Bioelectron. 110 (2018) 162–167.
[109] L.A. Layqah, S. Eissa, An electrochemical immunosensor for the corona virus associated with the Middle East respiratory syndrome using an array of gold nanoparticle-modified carbon electrodes, Microchim. Acta 186 (4) (2019) 224.
[110] J. Huang, Z. Xie, Y. Huang, L. Xie, S. Luo, Q. Fan, T. Zeng, Y. Zhang, S. Wang, M. Zhang, Z. Xie, X. Deng, Electrochemical immunosensor with Cu(I)/Cu(II)-chitosan-graphene nanocomposite-based signal amplification for the detection of newcastle disease virus, Sci. Rep. 10 (1) (2020) 13869.
[111] N. Wang, M. Kitajima, K. Mani, E. Kanhere, A.J. Whittle, M.S. Triantafyllou, J. Miao, Miniaturized electrochemical sensor modified with aptamers for rapid norovirus detection, 2016 IEEE 11th Annual International Conference on Nano/Micro Engineered and Molecular Systems (NEMS) 2016, pp. 587–590.
[112] W. Bialobrzeska, D. Firganek, M. Czerkies, T. Lipniacki, M. Skwarecka, K. Dziabowska, Z. Cebula, N. Malinowska, D. Bigus, E. Biega, K. Pyrc, K. Pala, S. Zoledowska, D. Nidzworski, Electrochemical Immunosensors based on screen-printed gold and glassy carbon electrodes: comparison of performance for respiratory syncytial virus detection, Biosensors 10 (11) (2020).
[113] D. Lin, T. Tang, D. Jed Harrison, W.E. Lee, A.B. Jemere, A regenerating ultrasensitive electrochemical impedance immunosensor for the detection of adenovirus, Biosens. Bioelectron. 68 (2015) 129–134.
[114] H.A.M. Faria, V. Zucolotto, Label-free electrochemical DNA biosensor for zika virus identification, Biosens. Bioelectron. 131 (2019) 149–155.
[115] C. Tancharoen, W. Sukjee, C. Thepparit, T. Jaimipuk, P. Auewarakul, A. Thitithanyanont, C. Sangma, Electrochemical biosensor based on surface imprinting for Zika virus detection in serum, ACS Sens. 4 (1) (2019) 69–75.