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Nanomaterial-based biosensors for sensing key foodborne pathogens: Advances from recent decades

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Abstract

Foodborne pathogen contamination has become a severe threat to human health. Traditional methods for foodborne pathogen detection have several disadvantages, including long detection time, low sensitivity, and low selectivity. The emergence of multiple excellent nanomaterials enables the construction of novel biosensors for foodborne pathogen detection. Based on the outstanding properties of nanomaterials, the novel biosensors possess the advantages of sensitivity, specificity, rapidity, accuracy, and simplicity. The present review comprehensively summarizes the advanced biosensors, including electrochemical, colorimetric, fluorescent, and surface enhanced Raman scattering biosensors for sensing key foodborne pathogens in recent decades. Furthermore, several issues are identified for further exploration, and possible directions for the development of biosensors are discussed.

KEYWORDS

biosensor, detection, food safety, nanomaterials, pathogens

Nomenclature: AgNCs, Ag nanoclusters; AgNPs, Ag nanoparticles; AgNWs, Ag nanowires; ATP, adenosine triphosphate; AuNPs, Au nanoparticles; AuNPs, Au nanoflowers; AuNRs, Au nanorods; AuNWs, Au nanowire; CFU, colony forming unit; DLS, dynamic light scattering; *E. coli*, *Escherichia coli*; ECL, electrochemiluminescence; FRET, fluorescence resonance energy transfer; GO, graphene oxide; GOQDs, graphene oxide quantum dots; GQDs, carbon quantum dots; IMBs, immunomagnetic beads; *L. monocytogenes*, *Listeria monocytogenes*; LOD, limit of detection; MNPs, magnetic nanoparticles; MWCNTs, multiwalled carbon nanotubes; NCA, nanoparticle cluster array; PCR, polymerase chain reaction; QDs, quantum dots; RCA, rolling circle amplification; r-GO, reduced graphene oxide; *S. aureus*, *Staphylococcus aureus*; SERS, surface enhanced Raman scattering; SPR, surface plasmon resonance; SWCNTs, single-walled carbon nanotubes; *V. parahaemolyticus*, *Vibrio parahaemolyticus*; Van, vancomycin.

1 | INTRODUCTION

Foodborne illness caused by the pathogenic bacteria poses a severe threat to public health and national economies (Scharff et al., 2016). Despite increased regulation and improved hygiene management systems, microbial contamination of food has caused sporadic cases of illness and disease outbreaks worldwide (Kirk et al., 2015; Scallan et al., 2011). Foodborne pathogen contamination is highly common, even in developed countries. In 2017, according to the World Health Organization in Europe, more than 23 million people got sick from eating contaminated food, and an estimated 4,700 of them lost their lives (World Health Organization, 2017). Hence, foodborne pathogens outbreak remains an important cause of human illness worldwide, and inspection and monitoring of pathogens are of great significance for ensuring the safety of food products, and thus decreasing the occurrence of illness.

With the development of nanotechnology, numerous novel nanomaterials have been synthesized, such as noble metal nanoparticles, magnetic nanoparticles (MNPs), quantum dots (QDs), carbon-based nanomaterials, and nanoarrays (Wu, Xiong, Zhang, Zhang, & Chen, 2014). As shown in Figure 1, according to the shape of the nanoparticles, the noble metal nanoparticles can be divided into spherical nanoparticles, nanorods, multibranched nanoparticles (nanostars and nanoflowers), and so on. The shell-core nanoparticles, compared with bare nanoparticles, exhibit superior performance. For instance, the bare Fe_3O_4 nanoparticles were easily aggregated and showed certain toxicity. When coated Fe_3O_4 nanoparticles with inert silica (such as SiO_2), the coated Fe_3O_4 nanoparticles showed improved stability, reduced toxicity, and could be more easily modified (Jiang et al., 2013). The carbon-based nanomaterials, according to their size and shape, can be divided into carbon quantum dots (GQDs), graphene (graphene oxide [GO] and reduced graphene oxide) and carbon nanotubes (single-walled carbon nanotubes [SWCNTs] and multiwalled carbon nanotubes [MWCNTs]). The fabrication of a regular-nanoarray-based substrate can excite a very strong local electric field. For example, when multiple gold nanorods (AuNRs) formed vertical arrays side by side, the end electric field could be most effectively utilized, and a uniform hot spot distribution and higher overall synergistic performance were realized (Damm et al., 2015).

Nanomaterials have been widely applied in the areas of *in vivo* imaging (Michalet et al., 2005), drug delivery (Tsou, Zhang, Zhu, Syed, & Xu, 2018), cancer treatment (Cheng, Wang, Gong, Liu, & Liu, 2020), catalysis (Zhu et al., 2019), bacteriostasis (Xin et al., 2019), and so on. Meanwhile, nanomaterial-based biosensors have been developed because of their outstanding physical and chemical properties (Chen, Zhou, Li, & Zhu, 2017; Fenzl, Hirsch, & Baeumner, 2016; Singh, Bai, Amalaradjou, & Bhunia, 2018). Due to their size-dependent optical, electronic, and catalytic properties, they have been widely used in electrochemistry (Pumera, 2010), colorimetry (Tan et al., 2017; Yin et al., 2017), fluorescence (Peltomaa et al., 2018), surface enhanced Raman scattering (SERS) (Ando, Fujita, Smith, & Kawata, 2016), and other fields. Different from the traditional bioelements, nanomaterial-modified bioelements can substantially improve the specificity, stability, and sensitivity of biosensors (Velasco-Garcia, 2009). The large surface area of a nanomaterial can immobilize more biomolecules to increase the number of reaction sites (Xu, Huang, Ye, Ying, & Li, 2009). Moreover, the application of nanomaterials to biosensors enables different detection limits depending on the samples to be analyzed and facilitates the tuning of the level of sensitivity according to the requirements. For instance, AuNPs exhibit many interesting properties (Figure 2), including the promotion of the electron transfer after modification on electrode (Liang et al., 2017) and the provision of various ultraviolet-visible (UV-Vis) absorption spectra in its aggregation state (Lou et al., 2012). Moreover, it can enhance or reduce the fluorescence intensity of fluorescent dyes (Samanta, Zhou, Zou, Yan, & Liu, 2014) and can be applied as an SERS substrate to enhance the SERS signal via chemical enhancement and electromagnetic enhancement (Qiu et al., 2018). Therefore, the use of nanomaterials to develop novel biosensors can provide a significant means for their applications in foodborne pathogen detection (Kaittanis, Santra, & Perez, 2010).

Detection and control of pathogens in food are highly important during every step in the farm-to-table continuum. Nanomaterial-based biosensors for foodborne pathogen detection have been used in many investigations. Nanomaterials should be carefully selected according to the desired effects. This review discusses nanomaterial-based biosensors for foodborne pathogen detection that have been developed in recent decades, and focuses mainly on the excellent properties of nanomaterials, which play a pivotal role in improving the detection performance of sensors. In addition, issues for further exploration and possible directions for the development of nanomaterial-based biosensors are discussed.

2 | COMMON FOODBORNE PATHOGENS AND THEIR TRADITIONAL DETECTION METHODS

2.1 | Common foodborne pathogens

Foodborne pathogens are abundant in species due to the wide variety of foods. The most common foodborne pathogens include pathogenic *Escherichia coli* (*E. coli*), *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* (*S. aureus*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), and *Listeria*

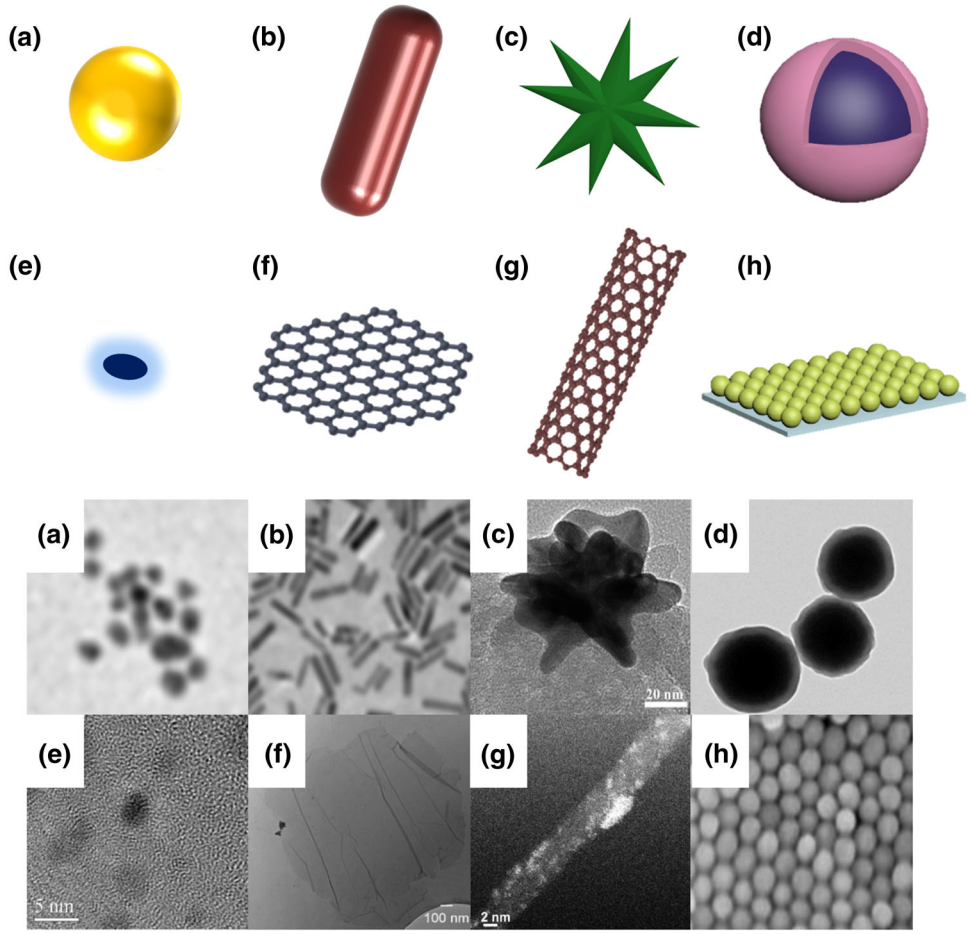

FIGURE 1 Schematic diagrams (top) and transmission electron microscopy figures (bottom) of various nanoparticles. (a) Spherical nanoparticles (Huo et al., 2016); (b) nanorods (Ali et al., 2017); (c) multibranched nanoparticles (Li et al., 2017); (d) shell-core nanoparticles (Huang et al., 2017); (e) quantum dots (Liu et al., 2017); (f) grapheme (Lu, Liu, Ying, & Liu, 2017); (g) carbon nanotubes (Tavakkoli et al., 2017); and (h) nanoarray (Mei & Tang, 2017)

monocytogenes (*L. monocytogenes*) (Table 1). *E. coli* are a diverse group of bacteria, and most are harmless. The pathogenic *E. coli* can be divided into six groups, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), and attaching and effacing *E. coli* (A/EEC) (Croxen et al., 2013). *Salmonella* spp. is the leading cause of foodborne diseases in the United States, and it was estimated that over 1 million people in the United States are infected with *Salmonella* each year (Scallan et al., 2011). The DNA homology of *Shigella* spp. is closely related to that of *E. coli*, thus, some of their biochemical characteristics and their reactivities are similar (Bintsis, 2017). All *Shigella* serogroups cause gastrointestinal infection after 12 to 50 hr of incubation (Bintsis, 2017). The presence of *S. aureus* in foodstuffs has been regarded as a public health hazard because it can produce enterotoxin and increase the risk of food poisoning, and the staphylococcal enterotoxins of type A and D are the major causes of outbreaks. *V. parahaemolyticus* is the most common seafood-borne pathogen, which is responsible for the highest incidences of seafood-associated bacterial infections (Mok, Ryu, Kwon, Kim, & Park, 2019). The main virulence genes of *V. parahaemolyticus* include *trh* (which encodes tdh-related hemolysin) and *tdh* (which encodes thermo–stable direct hemolysin) (Kang et al., 2017; Xie, Wu, Zhang, Xu, & Cheng, 2017). In contrast to other foodborne pathogens, *L. monocytogenes* propagates in cold environments and can quickly spread and contaminate food products (Bintsis, 2017).

2.2 | Traditional detection methods

As listed in Table 2, traditional methods for pathogen-detection include traditional culture methods, immunology-based methods (Xi et al., 2019), polymerase chain reaction (PCR) (Bae et al., 2018; Moezi, Kargar, Doosti, & Khoshneviszadeh, 2019), flow cytometry (Wu, Wang, Song, Wang, & Yan, 2016), and adenosine triphosphate (ATP) bioluminescence-based methods (Lee, Park, Im, & Jung, 2008). Traditional culture methods rely on the observation of

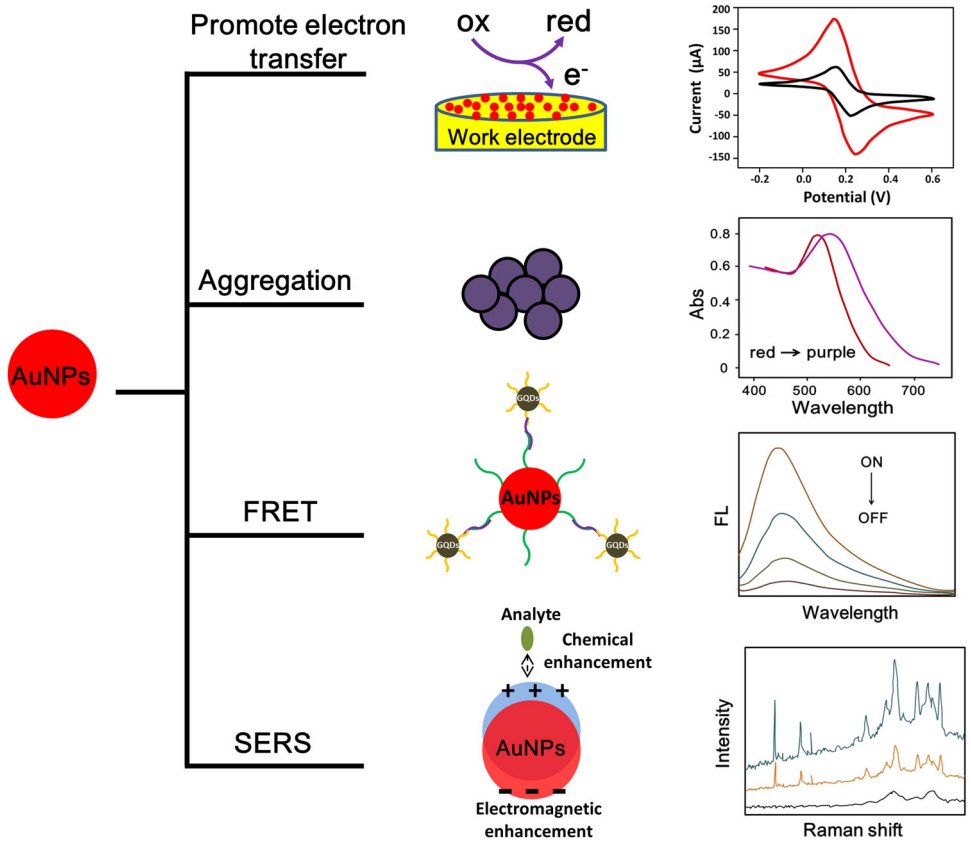

FIGURE 2 Application of AuNPs in the various types of biosensors

TABLE 1 Main characteristics of six common foodborne pathogens

Pathogens	Associated food products	Symptoms	Transmission mode
Escherichia coli	Undercooked ground meat, raw vegetables, raw milk, cheese, fruits, juice, water, and curd	Bloody diarrhea, thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and hemorrhagic colitis	Consumption of food or water contaminated with feces of infected animals or humans
Salmonella spp.	Eggs, meat, poultry, fruits, and vegetables	Diarrhea, fever, vomiting, and abdominal pain	Ingestion of contaminated food or water and direct contact with infected animals
Shigella spp.	Salads, milk, chicken, and shellfish	Gastrointestinal infections, watery diarrhea, fever, fatigue, abdominal cramps, and malaise	Consumption of focally contaminated food or water
Staphylococcus aureus	Pork sausage, ground beef, salmon steaks, ground turkey, oysters, shrimp, cream pies, delicatessen salads, and milk	Vomiting, abdominal cramps, nausea, chills, perspiration, headache, dizziness, general weakness, and muscular cramping	Inadequate cooking and heating, inadequate refrigeration, and poor personal hygiene
Listeria monocytogenes	Uncooked vegetables and meats	Meningitis, gastroenteritis, and septicemia	Consumption of food from infected animals, ingestion of contaminated food or water, and person-to-person contact
Vibrio parahaemolyticus	Sea food, such as fish, shrimp, and shellfish	Stomachache, diarrhea, and vomiting	High consumption of undercooked/raw seafood

TABLE 2 Major advantages and limitations of traditional methods for foodborne pathogen detection

Traditional methods	Major advantages	Major limitations
Culture methods	Low detection limit and high credibility	Long detection time
Immunology-based methods	High accuracy	Low sensitivity
PCR-based methods	High sensitivity	False negatives
Flow cytometry	High sensitivity	Expensive, complex operation
ATP bioluminescence	Rapid	Low accuracy

color change, bacterial colony morphology, and biochemical reactions in a specific medium after bacterial isolation and culture. It has been considered as the golden standard for pathogen detection owing to its low detection limit, high credibility, and simple operation. Nevertheless, because of its long detection time (4 to 9 days for negative results and 14 to 16 days for confirmation of positive results), the application of traditional culture methods is severely limited (Brooks et al., 2004). The antigen-antibody binding-based immunoassay is one of the most successfully employed technologies and has been extensively applied for foodborne pathogen detection (Hochel, Slavickova, Viochna, Skvor, & Steinhauserova, 2007; Magliulo et al., 2007). Although it has a shorter detection time compared to the traditional culture method, it still cannot realize real-time detection. In addition, it has low sensitivity and can be easily interfered by contaminants (Meng & Doyle, 2002). In PCR methods, the target segment of a pathogen can be magnified by several million folds as the DNA template amplification product increases exponentially, which significantly improves the detection sensitivity (Zhou et al., 2017). However, PCR techniques cannot discriminate between viable and nonviable pathogens because DNA is present both in dead and alive cells (Lazcka, Del Campo, & Munoz, 2007). In addition, potential inhibitory compounds in food sample, such as humic acid, may impair PCR amplification and produce false negative results. Flow cytometry can be applied for the sensitive and rapid detection of foodborne pathogens. However, it can only detect liquid samples with relatively simple composition and the instruments are very expensive. The ATP content can reflect the presence and number of living microbes, and the rapid response time (a few minutes) renders ATP bioluminescence methods highly suitable for online hygienic monitoring. Nevertheless, ATP bioluminescence methods have not been widely adopted because of the poor correlation between the ATP and plate count methods (Wu, Wu, Zhang, Li, & Huang, 2011), and the complexity of the samples will also affect the detection results. Meanwhile, the ATP bioluminescence methods are nonspecific because ATP exists in all living cells to provide energy. Hence, the development of novel biosensors for rapid, reliable, sensitive, and low-cost detection of pathogens in food samples is of great significance.

3 | BIOSENSORS

Compared with the conventional methods for foodborne pathogen detection, biosensors exhibit the advantages of higher sensitivity, selectivity, rapidity, and simplicity (Bhunia, 2008; Bhunia, 2014;Lan, Yao, Ping, & Ying, 2017). Two elements of biosensor, including bioreceptor and transducer, are of great importance. The bioreceptor can recognize and combine the target biomolecules, and the transducer can transform the chemical recognition information into a measurable signal. According to the types of signal transducers, biosensors are divided into electrochemical, colorimetric, fluorescent, SERS biosensors, and so on.

3.1 | Electrochemical biosensors

Electrochemical biosensors can conduct biological or chemical analysis without complex sample preparation and enable analysis with a short detection time and high sensitivity. As shown in Figure 3, an electrochemical biosensor consists of three elements, including target recognition element, signal transduction element, and electrochemical signal output element. Combined with the favorable electronic properties, electrocatalytic activity, and large surface areas of nanoparticles (Geagea, Aubert, Banet, & Sanson, 2015), nanoparticles based on electrochemical biosensors have attracted increased attention for foodborne pathogen detection (Rotariu, Lagarde, Jaffrezic-Renault, & Bala, 2016). Nanoparticles can provide a suitable microenvironment for biomolecule immobilization, promote the electron transfer between immobilized biomolecules and electrode, and increase the electrode surface area for target recognition (Wang, Ping, Ye, Wu, & Ying, 2013). Hence, electrochemical biosensors have the advantages of higher sensitivity, faster response, and easier operation in turbid media compared with traditional methods. According to the monitored electric parameters, electrochemical biosensors can be divided into amperometric biosensors, voltammetric biosensors, potentiometric biosensors, and impedimetric biosensors.

3.1.1 | Amperometric biosensors

The working principle of amperometric biosensors is that the concentration of analyte is linear with respect to the number of transferred electrons (Tomassetti, Serone,

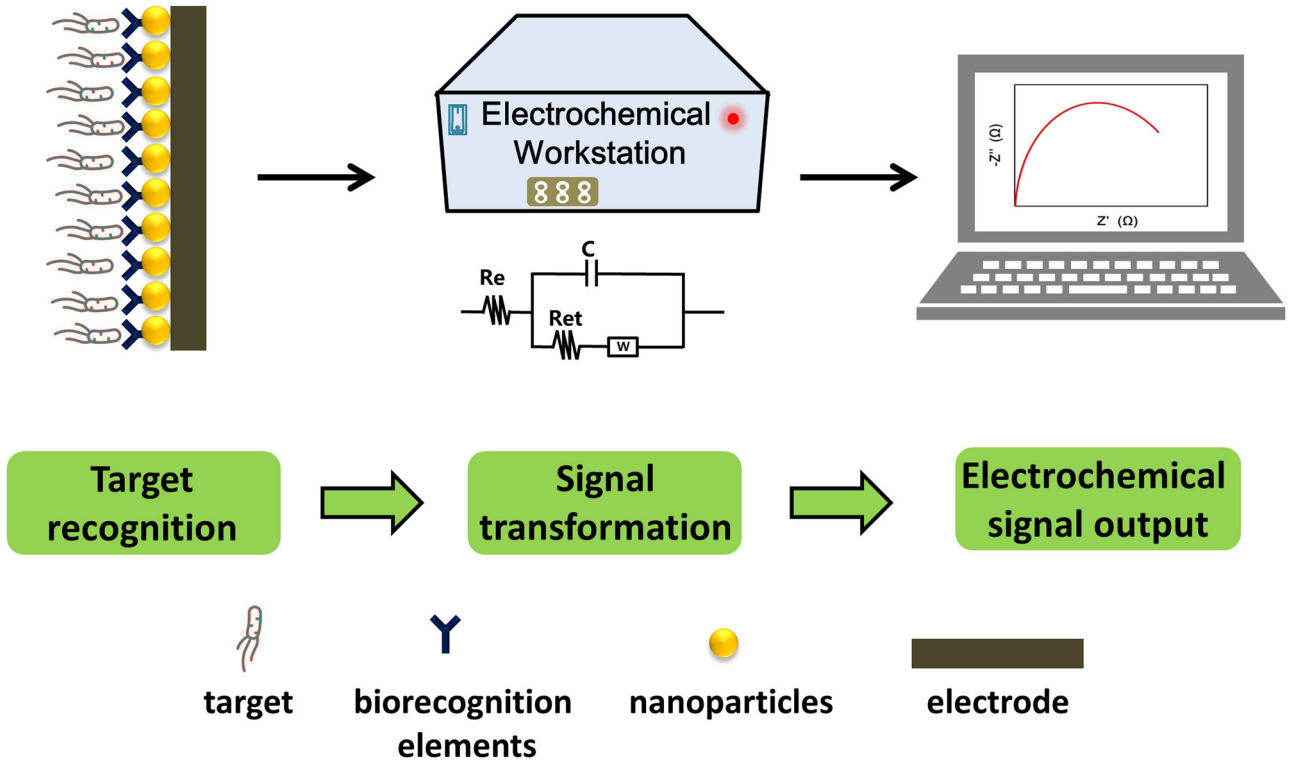

FIGURE 3 Schematic diagram of nanoparticle-based electrochemical biosensors

Angeloni, Campanella, & Mazzone, 2015). Zhu et al. (2013) proposed a simple and sensitive amperometric immunosensor for simultaneously detecting three analytes, including alpha-fetoprotein, carcinoembryonic, and *Streptococcus suis* Serotype 2. The biosensor applied AuNPs, graphene sheets (GS), and 3,4,9,10-perylenetetracarboxylic acid (PTCA) functionalized second antibody as tracer. The first antibodies were immobilized on the surface of electrode and could form sandwich structure with tracer in the presence of target. The linear range was from 0.012 to 50 ng/mL for *Streptococcus suis* Serotype 2 with a limit of detection (LOD) down to 4.2 pg/mL.

Villalonga et al. (2019) developed a core-shell MNPs-based amperometric biosensor for the rapid detection of *Brettanomyces bruxellensis* in wine. Core-shell Fe_3O_4@SiO_2 nanoparticles were modified with antibody for capturing *B. bruxellensis*. The amperometric signal increased with the increased *B. bruxellensis* concentration and the linear range of the amperometric biosensor was from 10 to 10^6 CFU/mL with the LOD down to 5 CFU/mL. Li, Lai, Zhang, and Jin (2011) developed a Fe_2O_3@Au MNPs-based DNA biosensor for amperometric detection of *E. coli*. Fe_2O_3@Au MNPs were applied as the DNA supporters and magnetic separation media. The LOD of the biosensor was down to 5 CFU/mL using the signal amplification strategy.

3.1.2 | Voltammetric biosensors

Voltammetric biosensors monitor the changes in current caused by the oxidation or reduction reaction of the electrochemically active analytes, which can be analyzed by varying potential in the electrochemical system (Chillawar, Tadi, & Motghare, 2015). As shown in Figure 4a, Shoaie, Forouzandeh, and Omidfar (2018) developed a voltammetric biosensor for fast detection of *E. coli* based on a polyaniline and AuNPs-modified screen-printed carbon electrode with an LOD down to 4 CFU/mL. The low LOD of the biosensor was attributed to the application of polyaniline and AuNPs, which could significantly increase the conductivity and surface area for biomolecules immobilization. Similarly, Zhu, Zhao, and Dou (2018) also utilized the unique electrochemical properties, small dimensions, and high surface-to-volume ratios of AuNPs for the fabrication of a voltammetric sandwich biosensor. The biosensor was successfully applied to *Cronobacter sakazakii* detection in spiked infant milk. Zhu et al. (2014) developed a novel amperometric biosensor based on the rolling circle amplification (RCA) strategy for *Salmonella* detection in milk samples. The target DNA of *Salmonella* was first captured on the surface of electrode by a DNA-AuNPs probe. After a series of amplification reactions, the DNA–AuNPs recognized RCA product and produced an enzymatic amperometric signal. The linear

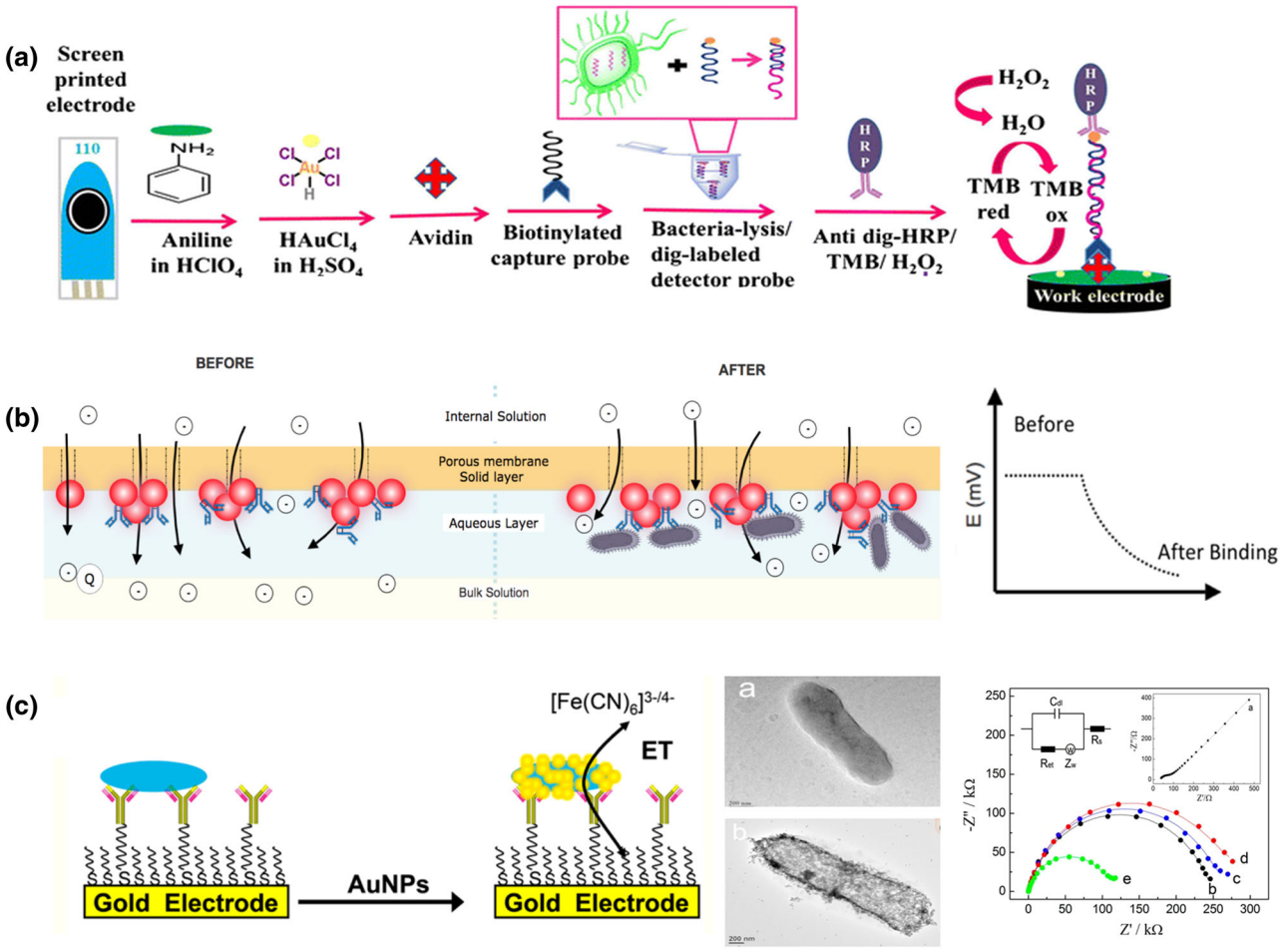

FIGURE 4 Schematic representations of electrochemical biosensors. (a) A polyaniline and AuNPs-modified screen-printed carbon electrode for voltammetric determination of the *E. coli* DNA. (b) *In situ* formation of AuNPs in a polymer inclusion membrane for label-free detection of *Salmonella* in a potentiometric immunosensor. (c) AuNPs-based impedimetric immunosensor for *E. coli* O157:H7 detection

range of the proposed biosensor for target DNA detection was from 10 aM to 10 pM and the LOD was down to 6.76 aM. Nze et al. (2019) developed a technique for separating and electrochemically detecting *E. coli* in ground meat. They combined hydrodynamic cavitation and antibody-coated magnetic beads for immunomagnetic separation of samples, which substantially amplified the detection capabilities of the biosensors.

The DNA concentration of pathogen can reflect the pathogen concentration and its amplification is of great importance for the trace detection of pathogens in foodstuffs (Zhao, Chen, Li, Wang, & Fan, 2015). Based on the target recycling amplification strategy, Guo et al. (2017) developed a rapid and highly sensitive dual-target electrochemical biosensor for simultaneously detecting *E. coli* O157:H7 rfbE gene and *Salmonella enterica* serovar Typhimurium gyrB gene. The phi29 polymerase-assisted target recycling strategy significantly amplified current signals. Chen et al. (2017) developed a voltammetric biosensor for *Mycobacterium tuberculosis* DNA detection. The redox nanoprobe, which was based on AuNPs modified polyaniline-reduced graphene oxide (PANI-rGO) composite, showed a favorable electrochemical activity for the generation and enlargement of the voltammetric signal. The detection limit of biosensors could be improved further if nanoparticles integrated with DNA amplification strategy.

In addition to metal nanoparticles, carbon-based nanomaterials, such as GO and MWCNTs, have also been widely applied in electrochemical biosensor because of their large surface area and excellent electron transfer properties. In contrast to AuNPs, carbon nanotubes are insoluble and always modified with carboxylic or amino groups, or combined with other nanomaterials to improve their solubility (Kucherenko, Soldatkin, Kucherenko, Soldatkina, & Dzyadevych, 2019). Xie et al. (2016) described a "diffusion-to-surface" ratiometric graphene electrosensor for the detection of Gram-negative bacteria expressed mannose-binding proteins with extremely high selectivity. Combined with the advantages of chitosan (CS), MWCNTs, and AuNPs, Sun et al. (2015) developed a CS–MWCNTs/AuNPs composite-based DNA

electrochemical biosensor for *S. aureus* gene sequence detection. The CS–MWCNTs/AuNPs composite increased the number of single-strand DNA (ssDNA) adsorbed on the electrode surface and accelerated the electrochemical response of methylene blue. Muniandy et al. (2019) developed an rGO-TiO_2 nanocomposite-based electrochemical biosensor for *S. enterica* detection. Incorporating GO with TiO_2 could substantially increase the overall surface area and enhance the electronic properties of electrode. The bacterial cells were absorbed on the electrode interface and inhibited electron transfer, and thus decreased the differential pulse voltammetry (DPV) signal. The proposed biosensor exhibited a wide detection range (10 to 10^8 CFU/mL) and a low detection limit (10 CFU/mL).

3.1.3 | Potentiometric biosensors

Potentiometric biosensors utilize a high voltmeter to detect the electrical potential difference between the working electrode and reference electrode. Zelada-Guillen, Bhosale, Riu, and Rius (2010) developed a real-time potentiometric biosensor for *E. coli* O157:H7 detection in complex samples. They used SWCNTs as ion-to-electron transducers and aptamers as biorecognition elements, which showed great sensitivity and selectivity. Similarly, Zelada-Guillen, Sebastian-Avila, Blondeau, Riu, and Rius (2012) also applied SWCNTs as ion-to-electron potentiometric transducer and aptamers for *S. aureus* recognition. They found that covalent interaction between the aptamers and SWCNTs could greatly improve the LOD of the biosensor compared to the noncovalent adsorption. Carbon nanotube has also been applied in another potentiometric aptasensor for ultrasensitive detection of living bacteria (Zelada-Guillen, Riu, Duzgun, & Rius, 2009). The reported SWCNTs-based potentiometric sensor could be used to detect pathogen at nearly real time (a few seconds), which made the pathogen detection as easy as measuring the pH value. Meanwhile, the developed biosensor could accurately detect the bacteria with a dynamic range from 0.2 to 10^3 CFU/mL.

Silva et al. (2019) applied an AuNPs polymer inclusion membrane (AuNPs-PIM) as sensing platform for antibody immobilization and signal-output amplification for *S. enterica* serovar Typhimurium detection (Figure 4b). The AuNPs-PIM-based potentiometric immunosensor had a low detection limit of 6 cells/mL and could be successfully performed in commercial apple juice samples. Shaibani et al. (2018) integrated electrochemical sensor with optical method, and proposed a nanofiber-light addressable potentiometric sensor for *E. coli* detection. The developed sensor could selectively detect *E. coli* in orange juice samples within 1 hr.

3.1.4 | Impedimetric biosensors

Impedimetric biosensors have also been proved effective for pathogen detection. Those biosensors analyze the impedimetric signal of bacteria when they are associated with or attached to the sensing surface. Capture of pathogens at the electrodes can increase interface impedance. The use of nanomaterial tags can offer several benefits in terms of simplicity, cost, and time. Lin, Pillai, Lee, and Jemere (2019) compared two electrochemical impedimetric biosensors and demonstrated that AuNPs-modified electrode had a better performance than planar electrode. They found that self-assembled AuNPs and protein G could increase the active surface area by 2.2 times. Moreover, the AuNPs-based impedimetric biosensor had a wider range and lower LOD compared to planar gold electrode biosensor. As shown in Figure 4c, Wan et al. (2016) developed an "ON-OFF" impedimetric immunosensor for *E. coli* O157:H7 detection. In the presence of *E. coli* O157:H7, the AuNPs effectively attached to the pathogens through strong interaction and changed the impedimetric signals. The biosensor showed high sensitivity and specificity. Ranjbar, Shahrokhian, and Nurmohammadi (2018) proposed a nanoporous gold-based impedimetric biosensor for sensitive and selective detection of *S. enterica* serovar Typhimurium in egg samples. The linear dynamic range of the biosensor was from 6.5×10^2 to 6.5×10^8 CFU/mL and the LOD was as low as 1 CFU/mL.

As DNA-based biosensors have advanced in the detection of pathogens, Lee et al. (2018) developed a novel impedimetric biosensor for *S. aureus* DNA detection. They used a Si_3N_4 deposited nanogap electrode chip to detect the impedimetric signal of bulk solution of PCR products. The nanogap impedimetric sensor was able to rapidly monitor *S. aureus* DNA at concentrations of as low as 260 pM, thereby outperforming the microgap electrode sensor by sevenfold. Tak, Gupta, and Tomar (2014) developed an impedimetric electrochemical DNA biosensor based on the flower-like ZnO nanomaterials for the bacteria detection. The novel ZnO nanostructured bioelectrode exhibited a broad response range (5 to 240 ng/μL), a low detection limit (5 ng/μL), and high stability.

3.2 | Colorimetric biosensors

Nanoparticle-based colorimetric biosensors have received significant attention because of their simplicity and versatility (Zhao, Brook, & Li, 2010). The absorbed light frequency depends on the shape, size, constituent, and aggregation state of the nanoparticles. Noble metal nanoparticles present an extremely high molar extinction coefficient. For example, the extinction coefficient of 13 nm AuNPs (2.7×10^8 M^{-1} cm^{-1}) is three orders magnitude larger than that of common dyes. Thus, the sensitivity of AuNPs-based colorimetric biosensors is much higher than those of common dyes according to the Beer–Lambert law (Apyari, Dmitrienko, Arkhipova, Atnagulov, & Zolotov, 2012). The most common principle for colorimetric foodborne pathogen detection is based on the change of plasma coupling between nanoparticles

(Bui, Ahmed, & Abbas, 2015). As shown in Figure 2, the monodisperse AuNPs exhibited a red color in solution. However, the AuNPs aggregated after the addition of target, which presented as a purple color and resulted in a red shift in UV-Vis spectrum (Lee, Han, & Mirkin, 2007; Marie-Christine & Didier, 2004; Rosi & Mirkin, 2010). A mercaptoethylamine-modified AuNPs (MEA-AuNPs) sensor was developed for the assay of *E. coli* O157:H7 (Su et al., 2012). MEA could not only conjugate to AuNPs easily through S-Au bond, but also recognize *E. coli* O157:H7 through the electrostatic interactions. Thus, in the presence of *E. coli* O157:H7, the monodisperse MEA-AuNPs aggregated and the color changed from red to blue. The absorption ratio ($A_{625\ nm}/A_{520\ nm}$) of the MEA-AuNPs was linearly correlated with *E. coli* O157:H7 concentration ranged from 2.91×10^8 to 16×10^8 CFU/mL. This biosensor was extremely rapid (less than 5 min) and the color change could be observed even by the naked eyes. However, this method relied on the electrostatic interactions between *E. coli* and MEA-AuNPs, and thus it had poor specificity.

In order to improve the sensitivity and specificity of AuNPs-based colorimetric biosensors, Xu et al. (2017) developed a novel colorimetric biosensor for the rapid detection of *E. coli* O157:H7 in milk samples that was based on the specificity of antibody and transformation of dimer/tetramer of concanavalin A (Con A) under various pH conditions. The large number of binding sites of bacteria for Con A and the strong ability of Con A to interact with AuNPs resulted in the aggregation of AuNPs, thereby providing a simple and sensitive detection platform for *E. coli* with a detection limit down to 41 CFU/mL. Similarly, a more recent article reported a plasmonic enhanced lateral flow sensor (pLFS) concept with an extremely enhanced colorimetric signal by using liposome encapsulating reagent to trigger the aggregation of AuNPs (Ren, Ballou, FitzGerald, & Irudayaraj, 2019). The proposed pLFS was demonstrated over 1000-fold improvement in *E. coli* O157:H7 detection sensitivity compared with conventional lateral flow immunoassays. Zheng et al. (2019) reported a novel biosensor that utilized AuNPs to determine the concentration of *E. coli* O157:H7 and monitored color change of the AuNPs using a smart phone imaging application. This biosensor also exhibited satisfactory sensitivity and specificity for the detection of *E. coli* O157:H7 in chicken samples whose detection limit was 50 CFU/mL. Similarly, Quintela, de los Reyes, Lin, and Wu (2019) proposed a colorimetric biosensor for a variety of *Salmonella* spp. detection. In the presence of *Salmonella* spp. DNA, the oligonucleotide-modified AuNPs could undergo a highly stable DNA sandwich hybridization and showed no aggregation even in high salt solution. The biosensor could simultaneously screen 19 viable *Salmonella* spp. strains with 100% specificity and exhibited a superior detection limit of 10 CFU/mL.

Compared to spherical AuNPs, AuNRs have a higher extinction coefficient (up to $6.0 \times 10^9\ M^{-1}\ cm^{-1}$) in the longitudinal plasmon band. Moreover, a small change in the refractive index close to AuNRs will cause a strong variation of the longitudinal plasmon resonance absorption, which increases their sensitivity for sensing the specified target binding event. Eum, Yeom, Kwon, Kim, and Kang (2010) developed a sandwich biosensor for *E. coli* O157:H7 detection, which applied antibody-modified AuNRs for bacteria recognition. The AuNRs-labeled antibody improved the sensitivity of the biosensor by 3.8 times compared to the non-AuNRs-labeled antibody. Alamer, Eissa, Chinnappan, and Zourob (2018) applied nanobeads and proposed a colorimetric immune-biosensor for the rapid detection of four pathogens, including *S. enterica* serovar Typhimurium, *S. enterica* serovar Enteritidis, *S. aureus*, and *Campylobacter jejuni*. Nanobeads of various colors were conjugated with antibodies for the corresponding pathogens. The biosensor required only a cotton swab immersed in nanobeads to conduct the entire analysis process, which was rapid, simple, and of low cost.

In addition to their indicator function, nanomaterials can play other important roles in colorimetric-based foodborne pathogen biosensors. For instance, AuNPs could be easily modified with enzymes or catalysts to increase the sensitivity of chromogenic reaction. Su et al. (2013) developed a rapid and novel colorimetric method for *E. coli* O157:H7 detection with a detection limit down to 7 CFU/mL. AuNPs were utilized as templates to guide the growth of Pt. These synthesized Au@Pt nanoparticles were modified with 4-mercaptophenylboronic acid and adsorbed on the surface of *E. coli* O157:H7, and thus they could catalyze the oxidation of the peroxidase substrate 3,3,5,5-tetramethylbenzidine by hydrogen peroxide. The absorbance at 652 nm was proportional to the *E. coli* O157:H7 concentration ranging from 7 to 6×10^6 CFU/mL. In another study (Ren, Liu, & Irudayaraj, 2017), a net fishing enrichment strategy for simple, fast, and selective colorimetric detection of *E. coli* O157:H7 was developed. AuNPs were employed as a platform modified with antibodies and horseradish peroxidase (HRP) to improve the signal for *E. coli* detection, and the detection limit of the method was as low as 100 CFU/mL.

3.3 | Fluorescent biosensors

With the development of novel fluorescent materials, the fluorescent biosensor has emerged as one of the most powerful tools with wide applications in the area of foodborne pathogen detection. As a new type of fluorescent nanolabel, QDs have become more and more popular because of their superior optical properties, including high quantum yield, less photo bleaching, and a wide excitation spectrum. As

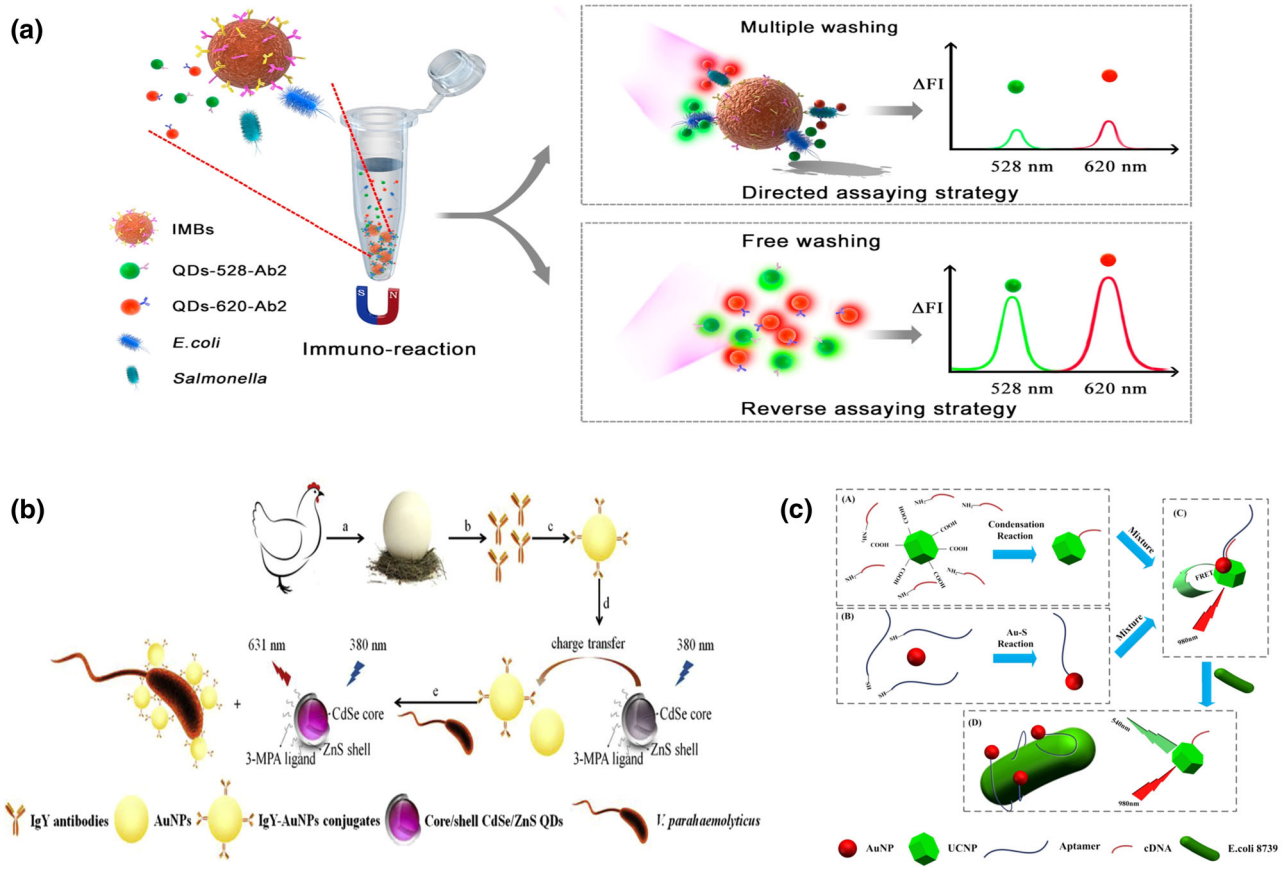

FIGURE 5 Schematic representations of fluorescent biosensors. (a) A QD-mediated strategy for one-step detection of *E. coli* and *Salmonella*. (b) A CdSe/ZnS QD and AuNPs-based turn on fluorescent biosensor for *V. parahaemolyticus* detection. (c) A UCNP-based FRET aptasensor for rapid and ultrasensitive *E. coli* 8739 detection

shown in Figure 5a, Yin et al. (2016) developed a rapid and multiplexed immunosensor based on QDs and immunomagnetic beads (IMBs) for the one-step simultaneous detection of *E. coli* O157: H7 and *Salmonella* pathogens. The proposed QDs-based strategy prevented interference from light that was scattered from the IMBs and simplified the washing steps. Similarly, Su et al. (2004) used QDs as a fluorescence marker, which were coupled with immunomagnetic separation, for *E. coli* O157:H7 detection with the LOD 100 times lower than that of fluorescein isothiocyanate (FITC)-based assay.

In addition to the simple fluorescent labeling biosensors, most of the fluorescent biosensors are based on the fluorescence intensity change that occurs after the system encounters the target. Liu et al. (2017) proposed a simple but robust fluorescence OFF-ON biosensor for detecting *V. parahaemolyticus* in complex food samples and realized higher sensitivity and selectivity (Figure 5b). The fluorescence of CdSe/ZnS QDs was first quenched by AuNPs through a charge-transfer quenching strategy and then recovered in the presence of *V. parahaemolyticus*. Sahoo, Sharma, Chattopadhyay, and Ghosh (2012) developed a fluorescent paracetamol dimer–Au nanoparticle composite for rapid and simple detection of bacteria, which was based on the high surface free energy of AuNPs for attachment to the cell walls of bacteria that caused a fluorescence intensity change. Elahi, Kamali, Baghersad, and Amini (2019) developed a fluorescent nanobiosensor based on the florescence probe-modified AuNPs and DNA-modified MNPs for the sensitive detection of *Shigella* spp. multi-Spa genes. In the presence of target DNA, the AuNPs, target DNA and MNPs formed a sandwich complex, which was isolated by a magnet. The florescent DNA of AuNPs was finally released and the florescence intensity increased with the concentration of target DNA. The linear range of the biosensor was from 2.3×10^2 to 2.3×10^7 CFU/mL, and the detection limit was as low as 10^2 CFU/mL. It has been reported that thiocholine (TCh) could significantly enhance the fluorescence of DNA Ag nanoclusters (AgNCs) (Zhang, Cai, Qi, Lu, & Qian, 2013). Based on this, Zheng, Qi, and Zhang (2018) proposed a novel DNA-AgNCs-based fluorescent biosensor for *E. coli* detection. In the presence of target bacteria, DNAzyme on the surface of MNPs released acetylcholinesterase and thus produced Tch to enhance the fluorescence of DNA-AgNCs. The biosensor had a low LOD of 60 CFU/mL and a broad linear range from 10^2 to 10^7 CFU/mL.

Moreover, the principle of fluorescence resonance energy transfer (FRET) can be exploited to design a molecular switch in the biosensor (Park, Jeong, Yi, Lee, & Shin, 2019). In the FRET system, the fluorescence of the donor can be quenched by the acceptor, and the quenching efficiency is dependent on the distance between the donor and the acceptor. Hence, FRET is an effective mean to monitor particle interaction or molecular recognition within the range of 1 to 10 nm (Rüdiger, Marco, Rosario, & Tullio, 2003; Sapsford, Berti, & Medintz, 2006). Nanoparticles are regarded as excellent fluorescence quenchers in the FRET system because of their extremely high molar extinction coefficients and broad energy bandwidth (Jain, El-Sayed, & El-Sayed, 2007; Jung, Cheon, Liu, Lee, & Seo, 2010). Jin et al. (2017) developed a novel OFF-ON upconversion nanoparticles (donor) and AuNPs (acceptor)-based FRET biosensor for *E. coli* ATCC 8739 detection, whose detection ranged from 5 to 10^6 CFU/mL and the detection limit down to 3 CFU/mL (Figure 5c). The low detection limit of the biosensor originated from the excellent photostability and low background fluorescence intensity of upconversion nanoparticles, and high quenching efficiency of AuNPs. Wang, Zhao, O'Donoghue, and Tan (2007) applied multicolor FRET NPs for multiplexed bacteria monitoring, and reported that the method was fast, accurate, sensitive, and selective. Meanwhile, this FRET-based system could be used for simultaneously detecting multiple bacteria, including *E. coli*, *S. enterica* serovar Typhimurium, and *S. aureus*. QDs have also been utilized for FRET-based biosensors as donors for pathogen detection. Shi et al. (2015) developed a biosensor for *S. aureus* DNA detection based on the FRET between GQDs and AuNPs, which exhibited high sensitivity and specificity. The fluorescence quenching efficiency of AuNPs could reach approximately 87% with 100 nM target DNA of pathogen, and the LOD of the FRET biosensor was as low as 1 nM.

In addition to AuNPs, GO has also been reported as an efficient energy acceptor in FRET. Moreover, GO can noncovalently interact with nucleotide bases of ssDNA via π–π stacking interactions, and thus it has been widely applied for DNA analysis (He et al., 2010). However, GO has poor water solubility. Graphene oxide quantum dots (GOQDs), which are nanometer-sized fragments of GO, have excellent water solubility and can overcome the drawback of GO (Gao, Zhong, Gao, & Jia, 2018). Gao et al. (2018) engineered a fluorescent aptasensor for rapidly detecting *Pseudomonas aeruginosa* based on the FRET between GOQDs and 5-carboxyfluorescein-labeling complementary DNA (FAM-cDNA). The fluorescence of FAM-cDNA was first quenched by GOQDs. After the addition of target, the FAM-cDNA was separated from GOQDs and the fluorescence was turned on. The linear dynamic range of the fluorescent biosensor was from 1.28×10^3 to 2.0×10^7 CFU/mL and the LOD reached 100 CFU/mL.

3.4 | SERS biosensors

SERS challenges present fluorescent assays and has been widely applied to the sensitive detection of foodborne pathogens due to its attractive properties, including ultrasensitivity, multiplexing ability, high speed, and comparatively low cost. SERS-based biosensors can be used for analyzing complex matrices, including aqueous-rich samples, since water does not affect SERS signal. Fabrication of nanoparticle substrates is very important for SERS performance, which can enhance the Raman signals by 10^{14} times attributed to the local electromagnetic field enhancement (Kneipp et al., 1997; Nie & Emory, 1997) and chemical enhancement. The SERS enhancement depends critically on the size, shape, characteristics, and surface state of the metal nanoparticles. For instance, different from spherical AuNPs, AuNFs have more "hot spots" as substrate to enhance SERS signal (Luong et al., 2019). Compared to AuNRs, silver-coated AuNRs exhibited better ability to enhance SERS signal because of the electron transfer between gold core and silver shell in the nanostructure of bimetallic rod-shape (Xu, Tang, Chase, Sparks, & Rabolt, 2018). As shown in Figure 6, current SERS-based biosensors for pathogen bacteria detection can be divided into two major categories: label-free biosensors (direct) and label-based biosensors (indirect). The label-free biosensors are more straightforward since they directly acquire the intrinsic SERS spectrum of the target pathogen. Conversely, the SERS signals of label-based biosensors are from extrinsic Raman reporters or molecular.

3.4.1 | Label-free biosensors

Based on the mutual interaction between bacteria and SERS substrate, the label-free method can directly acquire the intrinsic Raman fingerprint without using a secondary label dye. For example, dipicolinic acid is a typical biomarker of spore-forming bacteria, and it can be quantified at the peak of 1,001 cm^{-1} due to its ring breathing vibration (Zhang, Shah, & Van Duyne, 2006). Meanwhile, the bacterial cell wall exhibits peaks at 624, 652, 735, 955, 1,330, and 1,456 cm^{-1}. Yan et al. (2009) fabricated a nanoparticle cluster array (NCA) to rapidly discriminate spectra of three tested bacteria species, namely, *E. coli*, *S. aureus*, and *Bacillus cereus*, which provided strong and reproducible SERS signals. They revealed that the spectral and field localization properties of NCAs-based SERS substrates could be controlled systematically by changing the number of nanoparticles in the clusters and edge-to-edge separation. Qiu et al. (2016) developed a columnar nanoarray based on Au@Ag nanorods, which could efficiently amplify the weak SERS signals of Gram-positive bacteria, such as *Staphylococcus xylosus*, *L. monocytogenes*, and *Enterococcus faecium*, and thus could sensitively discriminate the food samples containing mixtures of bacteria. Juneja et al. (2019) developed a gold nanoflower

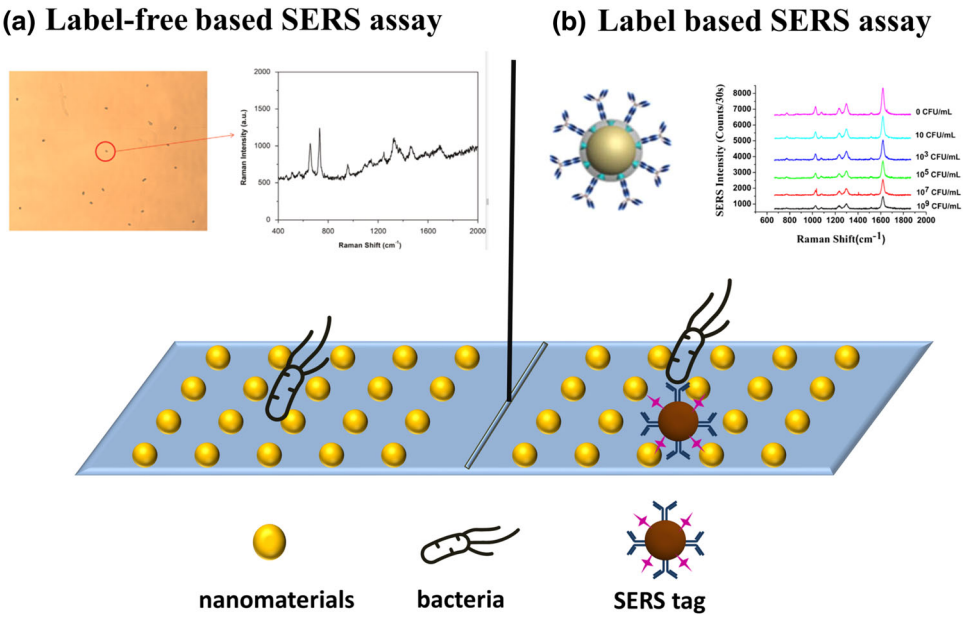

FIGURE 6 Schematic illustration of label-free SERS biosensors and label-based SERS biosensors for foodborne pathogen detection. (a) Label-free SERS biosensors (Yang, Zhou, Haisch, Niessner, & Ying, 2016). (b) Label-based SERS biosensors (Zhu et al., 2018)

(AuNFs)-based SERS substrate for *S. aureus* detection with an LOD of 10^3 CFU/mL. The biosynthetic 3D anisotropic AuNFs served as superior SERS enhancers and significantly improved the signal strength. Liu et al. (2011) developed a vancomycin (Van)-coated AgNPs array for label-free bacteria detection. The Van-coated SERS substrate could increase the capture ability for bacteria by approximately 1,000-fold, thereby resulting in high sensitivity and selectivity.

However, the bare metallic nanoparticles can easily absorb interfering molecules in sample solution due to their high surface energy. Hence, metal nanoparticles are always coated with a silica layer, liposomes, or polymers to improve the stability and reproducibility of SERS signals. Feng et al. (2013) prepared a series of Au-core silica-shell nanoparticles in different sizes, shapes, and shell thicknesses, which rendered SERS universally applicable to the surfaces without the interference of morphology and composition. The prepared SERS substrate decreased the detection time to 2 hr when applied to living bacteria detection.

In contrast to nanostructured metal surfaces, the metallic colloids (such as AuNPs and AgNPs) are simply synthesized and have been widely used in SERS bioanalysis. The amplified Raman signal mainly depends on the number of nanoparticles interact with pathogens and the distance between the nanoparticles and pathogens. Preciado-Flores et al. (2011) synthetized silver nanoparticles (AgNPs) and silver nanowires (AgNWs) to enhance SERS signal for *Shewanella oneidensis* detection. The AgNWs and AgNPs could attach to the bacteria through surface affinities and geometric considerations, thereby creating many SERS "hot spots" for signal amplification. Zhou et al. (2014) reported an assay, which could *in situ* synthesize AgNPs on the surface of cell wall (Bacteria@AgNP) for bacteria detection. The results showed that this was a more efficient, more rapid, and simpler method to deposit AgNPs on the bacterial cell wall for SERS detection compared to traditional mixed colloid−bacterial suspension.

3.4.2 | Label-based biosensors

Although discarding the fingerprint Raman spectra of bacteria, label-based biosensors have a higher sensitivity than label-free biosensors. Their Raman signals are uncomplicated and can be analyzed effectively (Zong et al., 2018). Metal nanoparticles can be modified with target recognition elements (such as antibodies, peptides, and aptamers) and Raman reporting molecules (such as 4-ATP and 4-MBA) to form SERS tag. Wang et al. (2015) presented a highly effective and multifunctional SERS chip for capturing, sensing, and inactivating bacteria. Based on the high SERS enhancement (enhancement factor: 1.3×10^7) of AgNPs decorated silicon wafer, the detection limit of the sensor was as low as 100 cells/mL. Rodriguez-Lorenzo et al. (2019) combined SERS spectroscopy with microfluidics to detect and discriminate *L. monocytogenes* in an automated and fast manner. They used gold nanostars as SERS tags to report the presence of *L. monocytogenes*. The SERS signal could be detected in continuous flow after the SERS tags recognized *L. monocytogenes* in just 100 s. Diaz-Amaya,

Lin, Deering, and Stanciu (2019) proposed an aptamer-based SERS biosensor for sensitive and specific detection of *E. coli* O157:H7 in the presence of *L. monocytogenes*, *S. aureus*, and *S. enterica* serovar Typhimurium. Aptamer for *E. coli* O157:H7 was covalently conjugated to AuNPs as capture biomolecules for whole cell detection. The SERS biosensor could be applied to detect and quantify *E. coli* O157:H7 with LOD down to 10^2 CFU/mL in ground beef samples within 20 min.

The SERS tag in label-based SERS biosensors can also act as an internal standard to correct the intensities of SERS signal. Thus, label-based SERS biosensors have a higher reproducibility compared to label-free SERS biosensors. Recently, label-based SERS biosensors have been widely explored for simultaneous detection of multiple pathogens. For example, Gracie et al. (2014) reported a new quantitative biosensor for the detection of *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*, which relied on the lambda exonuclease and SERS active dye (cy3). Their new assay format first detected all three pathogens simultaneously and produced consistent and accurate results. Yuan et al. (2018) proposed a novel biosensor based on 4-MPBA-modified Au@Ag-GO nanocomposites (as SERS tag) and antimicrobial peptide-functionalized MNPs (for capturing bacteria). The biosensor could be applied to detect three bacterial pathogens, namely, *E. coli*, *P. aeruginosa*, and *S. aureus* with the detection limit as low as 10 CFU/mL.

As one of the most classical assays, "sandwich-type" assay can be combined with SERS technology for foodborne pathogen detection. Taking advantages of well-defined geometries and excellent physicochemical properties of AuNPs and AuNWs, Kang, Yoo, Yoon, Lee, and Kim (2010) developed an AuNPs-on-wire-based SERS sensor for pathogen DNA detection with a detection limit of 10 pM. In the presence of target DNA, the Au particle-on-wire formed according to the sandwich hybridization of probe-target-reporter DNA, thus creating SERS hot spots between AuNWs and AuNPs to enhance the SERS signal of Cy5. Zhang et al. (2015) developed an SERS aptasensor for simultaneously detecting of *S. enterica* serovar Typhimurium and *S. aureus*. The sandwich structure consisted of Raman molecules and aptamer-modified AuNPs (signal probe) and aptamer-modified Fe_3O_4 magnetic AuNPs (capture probe). The SERS signal of MBA at 1,582 cm^{-1} was used for *S. enterica* serovar Typhimurium detection with the LOD of 15 CFU/mL and the SERS signal of DNTB at 1,333 cm^{-1} was used for *S. aureus* detection with the LOD of 35 CFU/mL.

3.5 | Other biosensors

Electrochemiluminescence (ECL) biosensors combined electrochemical and photoluminescence analysis, and exhibited the advantages of high sensitivity and selectivity for foodborne pathogen detection. Most recently, Liu et al. (2019) developed a $Ru(bpy)_3^{2+}$/ZnO nanorod array-based ECL biosensor for *E. coli* O157:H7 detection. The antibody could distinguish *E. coli* O157:H7 and form a nonconductive biocomplex to reduce ECL signal. The linear range of the biosensor was from 2 to 10^5 CFU/mL with the LOD of 143 CFU/mL. Hao et al. (2017) fabricated an ECL aptasensor for supersensitive detection of *E. coli*. They first applied 3D nitrogen doped graphene hydrogel and AgBr nanoparticles for signal amplification, and the LOD of the aptasensor was as low as 0.17 CFU/mL.

Surface plasmon resonance (SPR)-based biosensors have been widely applied for foodborne pathogen detection. SPR is an interfacial oscillation (resonance oscillation of conducting electrons) between positive and negative permittivity materials under the activation of incident light (Rubab, Shahbaz, Olaimat, & Oh, 2018). Melaine, Saad, Faucher, and Tabrizian (2017) developed a biosensing strategy for simultaneous SPR imaging of *P. aeruginosa*, *S. enterica* serovar Typhimurium, and *Legionella pneumophila* (*L. pneumophila*) based on the DNA probe sandwich strategy. The DNA-modified AuNPs were hybridized with 16S rRNA extracted from the pathogens at the interface and substantially enhanced the SPR signal. The dynamic range of the SPR biosensor was from 0.01 to 100 ng/mL, and the LOD was down to 10 pg/mL. Zhou et al. (2018) proposed an AgNPs-rGO-based SPR biosensor for *E. coli* O157:H7 detection without complex DNA extraction. The AgNPs-rGO composites were bounded to the surface of fiber probes to greatly enhance the sensitivity of the biosensor. Meanwhile, they applied magainin I as a recognition element to directly adsorb *E. coli* O157:H7 cells. The linear range of the biosensor was from 1.0×10^3 to 5.0×10^7 CFU/mL with the LOD of 5.0×10^2 CFU/mL.

Au nanoflowers (AuNFs) have stronger light scattering capabilities than spherical AuNPs. Zhan et al. (2019) first reported an AuNFs-based dynamic light scattering (DLS) biosensor for *E. coli* O157:H7 detection in milk samples. They systematically evaluated several important parameters influencing the sensitivity of the DLS immunosensor, including the size and concentration of AuNFs, the number of antibodies modified on the surfaces of AuNFs, and the immunoreaction time between the AuNFs and *E. coli* O157:H7. Under the optimal conditions, the biosensor could ultrasensitively detect *E .coli* O157:H7 with the LOD down to 2.7 CFU/mL.

4 | TRENDS AND CHALLENGES IN NANOMATERIAL-BASED BIOSENSORS

As listed in Table 3, many impressive laboratory-based studies have been conducted for foodborne pathogen detection,

TABLE 3 Nanomaterial-based biosensors for foodborne pathogen detection

Type	Target pathogen	Nanomaterials	LOD	Linear range	References
Electrochemical biosensors	*Streptococcus suis Serotype 2*	AuNPs and GS	4.2 pg/mL	0.012–50 ng/mL	Zhu et al. (2013)
	Brettanomyces bruxellensis	Fe_3O_4@SiO_2 NPs	5 CFU/mL	10–10^6 CFU/mL	Villalonga et al. (2019)
	E. coli	Fe_2O_3@Au MNPs	5 CFU/mL	10^3–5×10^5 CFU/mL	Li et al. (2011)
	E. coli	PANI/AuNP composite	4 CFU/mL	10^2–10^8 CFU/mL	Shoaie et al. (2018)
	C. sakazakii	RGO/thionine/AuNPs nanocomposite	10^4 CFU/mL	8.8×10^4–8.8×10^8 CFU/mL	Zhu et al. (2018)
	M. tuberculosis	AuNPs and PANI-rGO	50 fM	0.1– 10^4 pM	Chen et al. (2017)
	S. aureus	CS–MWCNTs/AuNPs composite	0.33 fM	10^{-15}–10^{-8} M	Sun et al. (2015)
	Salmonella enterica	rGO and TiO_2 nanoparticles	10 CFU/mL	10–10^8 CFU/mL	Muniandy et al. (2019)
	E. coli O157:H7	SWCNT	4 CFU/mL	4–10^4 CFU/mL	Zelada-Guillen et al. (2010)
	S. aureus	SWCNT	8×10^2 CFU/mL	8×10^2–1×10^8 CFU/mL	Zelada-Guillen et al. (2012)
	S. enterica serovar Typhimurium	SWCNT	0.2 CFU/mL	0.2–1×10^3 CFU/mL	Zelada-Guillen et al. (2009)
	S. enterica serovar Typhimurium	AuNPs polymer inclusion membrane	6 cells/mL	13–1.3×10^6 cells/mL	Silva et al. (2019)
	E. coli	Nanofiber	10^2 CFU/mL	10^2–10^6 CFU/mL	Shaibani et al. (2018)
	E. coli	AuNPs	10^2 CFU/mL	300–1×10^5 CFU/mL	Wan et al. (2016)
	S. enterica serovar Typhimurium	Nanoporous gold	1 CFU/mL	6.5×10^2–6.5×10^8 CFU/mL	Ranjbar et al. (2018)
	E. coli O157:H7	AuNPs	48 CFU/mL	48–1×10^7 CFU/mL	Lin et al. (2019)
	S. aureus	Nanogap electrode	260 fM	2.6×10^2–2.6×10^5 fM	Lee et al. (2018)
Colorimetric biosensor	*E. coli* O157:H7	AuNPs	NO	2.91×10^8–16×10^8 CFU/mL	Su et al. (2012)
	E. coli O157:H7	AuNPS and MNPs	41 CFU/mL	10^3–10^6 CFU/mL	Xu et al. (2017)
	E. coli O157:H7	AuNPs	10^2 CFU/ml	1×10^2–6×10^2 CFU/mL	Ren et al. (2019)
	E. coli O157:H7	AuNPS and MNPs	50 CFU/mL	5–5×10^4 CFU/mL	Zheng et al. (2019)
	Salmonella spp.	AuNPs	10 CFU/mL	10–10^8 CFU/mL	Quintela et al. (2019)
	S. enterica serovar Typhimurium, *S. enterica* serovar Enteritidis, *S. aureus*, and *Campylobacter jejuni*	Nanobreads	10 CFU/mL	10–10^8 CFU/mL	Alamer et al. (2018)
	E. coli	Au@Pt NPs	7 CFU/mL	7–6×10^6 CFU/mL	Su et al. (2013)
	E. coli O157:H7	AuNPs	10^2 CFU/mL	10^2–10^5 CFU/mL	Ren et al. (2017)
Fluorescent biosensor	*E. coli* O157: H7 and *Salmonella*	QD and IMBs	720 CFU/mL (*Salmonella*) 1.5×10^3 CFU/mL (*E. coli*)	5×10^3–5×10^6 CFU/mL (*Salmonella*) 10^4–10^7 CFU/mL (*E. coli*)	Yin et al. (2016)

(Continues)

TABLE 3 (Continued)

Type	Target pathogen	Nanomaterials	LOD	Linear range	References
	E. coli O157:H7	QDs and MBs	10^3 CFU/mL	10^3–10^7 CFU/Ml	Su and Li (2004)
	V. parahaemolyticus	CdSe/ZnS QDs and AuNPs	10 CFU/mL	10–10^4 CFU/mL	Liu et al. (2017)
	P. acidilacticiCFR K7	PD–AuNPs composite	10^2 CFU/mL	10^2–10^6 CFU/mL	Sahoo et al. (2012)
	Shigella spp.	AuNPs and MNPs	10^2 CFU/mL	2.3×10^2–2.3×10^7 CFU/mL	Elahi et al. (2019)
	E. coli	MNPs and AgNCs	60 CFU/mL	10^2–10^7 CFU/mL	Zheng et al. (2018)
	E. coli	AuNPs and UCNPs	3 CFU/mL	5–10^6 CFU/mL	Jin et al. (2017)
	S. aureus	GQDs and AuNPs	1 nM	NO	Shi et al. (2015)
	Pseudomonas aeruginosa	GOQDs	10^2 CFU/mL	1.28×10^3–2.0×10^7 CFU/mL	Gao et al. (2018)
SERS biosensor	*S. aureus*	AuNFs	10^3 CFU/mL	10^3–10^8 CFU/mL	Juneja and Bhattacharya (2019)
	S. oneidensis	AgNPs and AgNWs	3.7×10^5 cells/mL	NO	Preciado-Flores et al. (2011)
	E. coli and *S. epidermidis*	AgNPs	2.5×10^2 cells/mL	2.5×10^2–1×10^8 cell/mL	Zhou et al. (2014)
	E. coli O157:H7	AuNPs	10^2 CFU/mL	10^2–10^6 CFU/mL	Diaz-Amaya et al. (2019)
	E. coli and *S. aureus*	AgNPs@Si	100 cells/mL	10^2–10^7 cells/mL	Wang et al. (2015)
	E. coli, *S. aureus*, and *P. aeruginosa*	Fe_3O_4NPs, Au@Ag-GO nanocomposites	10 CFU/mL	10–10^6 CFU/mL	Yuan et al. (2018)
	E. faecium, S. aureus, S. maltophilia, and *V. vulnificus*	AuNPs and AuNWs	10 pM	10^{-11}–10^{-8} M	Kang et al. (2010)
	S. enterica serovar Typhimurium and *S. aureus*	AuNPs and MGNPs	35 CFU/mL (*S. aureus*) and 15 CFU/mL (*S. enterica* serovar Typhimurium)	10^2–10^7 CFU/mL	Zhang et al. (2015)
ECL biosensors	*E. coli* O157:H7	$Ru(bpy)_3^{2+}$/ZnO nanorods	143 CFU/mL	2×10^2–10^5 CFU/mL	Liu et al. (2019)
	E. coli	AgBr NPs	0.17 CFU/mL	0.5–500 CFU/mL	Hao et al. (2017)
SPR biosensors	*P. aeruginosa, S. enterica* serovar Typhimurium, and *L. pneumophila*	AuNPs	10 pg/mL	0.01–100 ng/mL	Melaine et al. (2017)
	E. coli O157:H7	AgNPs-rGO	5.0×10^2 CFU/mL	1.0×10^3–5.0×10^7 CFU/mL	Zhou et al. (2018)
DLS biosensor	*E. coli* O157:H7	AuNFs	2.7 CFU/mL	6–6×10^4 CFU/mL	Zhan et al. (2019)

and the investigated methods are simple, efficient, rapid, sensitive, and selective. Figure 7a shows that the number of published papers increased rapidly in the last 10 years. Most papers focused on the detection of *S. aureus* and *E. coli* pathogens, while relatively few papers on *V. parahaemolyticus* and *S. aureus* detection were published (Figure 7b).

Unlike the successful commercialization of glucose meters, the manufacture of commercial nanomaterial-based biosensors for bacterial detection has lagged behind the research output (Luong, Male, & Glennon, 2008). Rarely are they handheld and user-friendly. Before the nanomaterial-based biosensors arrive to the market, one of the most crucial considerations is the economic benefit evaluation. However, most of the published studies failed to consider the cost-effectiveness of their proposed biosensors, thereby hindering the assessment of their practical feasibility in the market. Moreover, four key issues must be discussed before the nanobiosensors get to market, including the stabilities

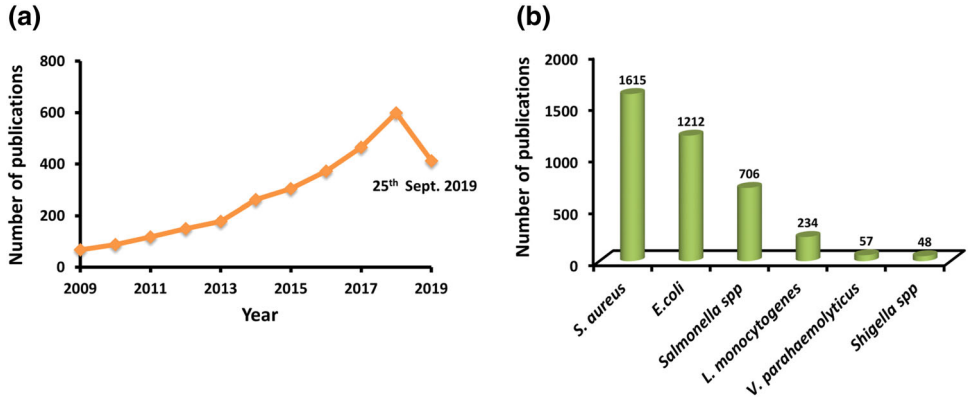

FIGURE 7 (a) Trends of the number of publications on nanobiosensors-based detection of foodborne pathogens. (b) The numbers of publications on the detection of various types of pathogens in the last 10 years

of nanoparticles in complex food samples, the affinities of biorecognition elements during immobilization process and storage, and the miniaturization of detector and multiplexing analysis.

Real food samples are highly complex and differ substantially in terms of their properties. Some are water-soluble, while others are oil-soluble; some are acidic, while others are alkaline. Meanwhile, the nanoparticles can easily aggregate due to their high surface energy. For example, AuNPs can be easily aggregated at high ionic strength. Although many researchers have reported the application of their biosensors in pathogen detection in real food samples, few have discussed the application scope of their methods. In bringing a laboratory-based method to market, the individual suitability of nanomaterial-based biosensor for a food sample should be assessed carefully. Moreover, in most instances, foodborne pathogens are present in very small amounts (<100 CFU/g) among the millions of other bacteria, hence, they are more difficult to detect (Velusamy, Arshak, Korostynska, Oliwa, & Adley, 2010).

The biorecognition element is one of the key parts of a biosensor. The most widely used biorecognition elements are antibodies (Li et al., 2019; Zhang & Miller, 2019) and aptamers (Paniel & Noguer, 2019; Zhan et al., 2020). As reported in many articles, antibodies and aptamers exhibited numerous advantages. However, their affinities will be reduced because of the immobilization process and interference of other substances in food samples. Moreover, the binding efficiency of antibodies and aptamers decreases with the extension of storage time; thus, their shelf lives are not long at room temperature. Hence, the development of novel biorecognition elements is of great significance for the practical application of biosensors. Recently, alternatives to antibodies and aptamers, including reporter phages (Zhang et al., 2016), lectins (Zhu, Ng, Wang, Ho, & Ding, 2006), antimicrobial peptides (Wu et al., 2017; Xiang, Lyu, Zhu, Bhunia, & Narsimhan, 2017), and host receptors (Koo, Aroonnual, & Bhunia, 2011), have been regarded as suitable candidates. Molecular imprinting strategy has satisfactory prospects for bacterial capture applications, which can bind to targets through three-dimensional binding sites (Wu et al., 2019). The three-dimensional binding sites are complementary to the shape, size, and orientation of targets, and therefore, this approach is highly specific (Birnbaumer et al., 2009).

Another important element of a biosensor is the detection module. However, a substantial majority of the optical and electrochemical detectors are bulky and expensive, which hinder the manufacture of small device. Nowadays, research has concentrated on the portable detection systems for optical and electrochemical sensing. For example, Scognamiglio et al. (2012) proposed a portable multipurpose biosensor combined both optical and electrochemical transduction systems, which could be well applied in practical detection. However, for foodborne pathogens, few such systems have been reported.

The multiplexing detection of several pathogens in a single sample has become a hotspot since the real food sample contains more than one kind of pathogen. However, although various studies have reported that their biosensors could detect a variety of bacteria at the same time, few of these biosensors were commercially manufactured. Moreover, difficulties are encountered in distinguishing live or dead bacteria in the food samples.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

The present review comprehensively summarizes the excellent properties of nanomaterials and their application in biosensors for foodborne pathogen detection. In addition, it identifies the advantages of nanomaterial-based biosensors, including high sensitivity and specificity, simple operation, and rapid detection compared to traditional methods. Despite

the progress and advances in laboratory-based research on foodborne pathogen detection, challenges remain in practical applications. In the future, additional excellent nanomaterial-based biosensors will be proposed, and new solutions will emerge from collaborative endeavors of scientists in the fields of nanotechnology and food science. If the sensitive, rapid, and portable nanomaterial-based biosensors for foodborne pathogens detection widely applied in practice, the uncontaminated foodstuffs would be released within several hours or even several minutes, thereby substantially promoting the development of food market. Hence, further studies need to be conducted on the feasibility and wide practical applicability of biosensors for detecting foodborne pathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ruyuan Zhang prepared the topic and wrote the manuscript, Tarun Belwal was responsible for the structure and revision of the manuscript, Li Li helped to collect data from the published literature and provided advice on the writing, Xingyu Lin and Yanqun Xu helped to collect data from the published literature and provided advice on the tables and figures, and Zisheng Luo corrected, revised, and finalized the manuscript.

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