Biosensor Detection Methods for Salmonella: a Review

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Abstract. Salmonella is a pathogenic bacterium capable of causing serious food-borne illness, which has caused worldwide concern. It can enter the food supply chain at various stages of production, distribution, and marketing. Due to its high prevalence, it is necessary to develop effective and efficient methods for the identification, detection, and monitoring of Salmonella at an early stage. While traditional detection technologies often have long detection times, complex operation and low sensitivity, emerging biosensor technologies combine novel nanomaterials with signal amplification methods to achieve improved performance. Biosensors show several advantages over traditional assays in terms of specificity, accuracy, and sensitivity and show superiority in rapid response. They are now seen as promising alternative tools since they are accessible and applicable approaches for rapid detection. In recent years, there has been a boom in the development of biosensors for the detection of foodborne Salmonella. This paper focuses on the comparison of new methods for the detection of optical and electrochemical biosensors in recent years, analyzes the different separation strategies and determination methods used, and provides an outlook on the future development of biosensors for the detection of Salmonella.

Keywords: Salmonella, biosensor detection, optical biosensor, electrochemical biosensor, signal amplification, nanomaterials.

1. Introduction

Food contamination caused by all kinds of pathogenic microorganisms and parasites brings a huge loss to the world economy, which greatly arouses people's awareness[1]. According to World Health Organization (WHO), food and water contaminated by pathogenic microorganisms cause 1.8 million deaths per year around the world[2][3]. It is particularly important for the detection of food contamination because most pathogens are small in size, invisible to the naked eye, and have a low infectious dose[4][5][6]. A variety of Salmonella is the predominant cause of the alimentary infection[7][8][9][10]. According to statistics, Salmonella often tops the list of reported cases of bacterial food poisoning in countries around the world each year[11][12]. Therefore, the detection of Salmonella is of essential significance[13][14][15]. In some countries, including the United States of America (USA) and the European Union (EU), kinds of regulations are established to make sure that Salmonella existing in ready-to-eat food could be detected so that consumers will not take them in[16][17][18].

Salmonella belongs to Gram-negative bacteria[19]. There are nearly one thousand types of bacteria strains have been found[20]. Generally, it is transmitted through contaminated meat, eggs, and dairy products[21]. People who consume contaminated food have a high probability of contracting Salmonella, which may cause nausea, vomiting, abdominal pain, diarrhea, and other symptoms[22]. Besides, it could lead to bacteremia, meningitis, and other diseases in severe cases[23]. The traditional ways of detecting Salmonella include polymerase chain reaction (PCR), biochemical identification, and enzyme-linked immune-sorbent assay (ELISA)[24][25]. Most of these methods have disadvantages such as costing too much time, having complicated operation steps, and low sensitivity of the test results[26][27][28]. Therefore, to make full preparation for mass food poisoning incidents, it is greatly necessary to develop technologies supporting rapid and accurate detection[29][30].

Recently, emerging biosensor assays with higher accuracy and easier operation have gradually replaced those traditional methods[31]. Due to the promising applications that they have shown in detecting Salmonella in food products, biosensors are widely used[32]. Nanomaterial-based

biosensors are currently recognized as having superior performance[33]. Nanomaterials with different structures exhibit various properties in terms of mechanics, electricity, and optics, which could be applied to biological signal transduction to improve the sensitivity of detection[34]. Operating portable biosensors for Salmonella detection facilitates the completion of real-time test needs in the field[35]. This paper provides an analysis of the development of isolation detection technologies and biosensors for Salmonella in recent years. It focuses on various types of optical biosensors and electrochemical biosensors. They use different target separation methods, detection methods, and signal amplification methods, and have different detection ranges that can be achieved, each with its advantages. Moreover, it describes the problems that still exist at this stage to support the further breakthrough in Salmonella biosensors.

2. Biosensor

The biosensor is an analytical device that senses biological responses and converts them into electrical signals for detection[36]. It consists of identification elements, physicochemical transducers, and signal amplification instruments[37]. Among them, the identification elements are mainly bio-sensitive materials, including enzymes, antibodies, cells, microorganisms, and nucleic acid[38]. Physicochemical transducers include oxygen electrodes, photosensitive tubes, and field effect tube et al[39]. There are different types of biosensors, which could be classified by biological specificity or the genre of signal transduction.

Biosensors often use antibodies, nucleic acids, and aptamers that specifically detect Salmonella to capture the target strain, while amplifying and type-converting the acquired bio-signals, such as into electrical and optical signals[36]. The correspondence between this signal and the change in Salmonella concentration is then studied for this purpose of detection.

TABLE I. Application of Different Optical and Electrochemical Biosensors for Salmonella Detection

Biosensor	Identification components	Test range	Advantages	References
Fluorescent microfluidic biosensor based on magnetic nanoparticle separation, quantum dots labeling, and MnO2 nanoflower amplification	Monoclonal antibody	1.0×102-1.0×107 CFU/ml	high sensitivity; functions of mixing, immune reaction, magnetic separation and QDs release are integrated onto one single microfluidic chip	[44]
Fluorescent biosensor based on quantum dot-labeled streptavidin and poly-L-lysine	Antibody	4.9×101-4.9×107 CFU/ml	high specificity; high sensitivity and the limit of detection is much lower; detection time is lower than those of the other detection methods	[45]

Non-spectroscopic optical biosensor based on a stem-loop DNA probe and retro-reflective signaling	A biotinylated stem-loop DNA probe	0-100nM	excellent selective detection capability; high sensitivity; detect Salmonella without a sophisticated optical instrument; provide a constant background signal	[46]
Acid-responsive and fluorescent microfluidic biosensor using curcumin as a signal reporter and ZnO-Capped mesoporous silica nanoparticles for signal amplification	Polyclonal antibodies (pAbs)	1.1×102-1.1×107 CFU/ml	high sensitivity and stability; rapid and specific detection; excellent mixing efficiency; lower cost	[47]
Colorimetric biosensor based on magnetic grid separation and platinum loaded zeolitic imidazolate Framework-8 nanocatalysts	Antibody	1.3×100-1.3×104 CFU/ml	ultrasensitive; rapid detection; lower limit of detection range; Pt@ZIF-8 nanocatalysts could catalyze H2O2-TMB and amplify biological signals	[49]
Optical biosensor based on porous gold@platinum nanocatalysts and a 3D fluidic chip	Antibody	1.8×101-1.8×107 CFU/ml	high sensitivity and specificity; a shorter detection time; excellent mixing efficiency; Au@PtNCs have high catalytic activity, better stability, and lower cost	[50]
Impedance biosensor for fast detection of Salmonella using 3D magnetic grid separation and urease catalysis	Antibody-conjugated magnetic nanoparticles（MNPs）	1.0×101-1.0×106 CFU/ml	high sensitivity and specificity; detecting without complex manual operations; nice separation efficiency; reduce the operation skills of the technicians	[56]
Impedance biosensor combined with immunomagnetic separation for rapid screening	Antibody-conjugated magnetic nanoparticles (MNPs）	101-105 CFU/ml	high stability; specific separation; efficient concentration; great amplification	[57]

			of impedance signal; the decrease of background noise	
Electrochemical aptasensor using aptamer coated gold interdigitated microelectrode, impedance measurement, and antibody modified nickel nanowires (NiNWs) for target separation and impedance amplification	NiNWs modified with the antibodies	102-106 CFU/ml	more sensitive; have good applicability for the detection of Salmonella; high sensitivity; the impedance signal could be amplified effectively;	[58]
Microfluidic biosensor based on magnetic separation, enzymatic catalysis, and electrochemical impedance analysis	Magnetic nanoparticles (MNPs) modified with the capture antibody	1.6×102-1.6×106 CFU/ml	high sensitivity, practicality and specificity; lower limit of detection range; reduce the need of well-trained technicians; enhance the consistence	[59]
Impedance biosensor based on a Hechtia argentea Lectin	Lectin showing selectivity towards D-mannose	15-2.57×107 CFU/ml	high simplicity and specificity; short analysis time; low limit of detection; lower cost; have the feasibility of miniaturization;	[60]
Electrochemical immunosensor based on Fe3O4@graphene nanocomposite modified glassy carbon electrode	Antibody	2.4×102-2.4×107 CFU/ml	excellent selectivity, stability and repeatability; high sensitivity; a faster detection time; have better detection limits; the cyclic voltammetry response current further increases;	[61]

2.1 Optical Biosesnsors for Salmonella Detection in Food Products

The optical biosensor is a device that amplifies and converts a biological signal into an optical signal and establishes a relationship between it and the concentration of Salmonella based on the specific identification of the target bacteria[40][41]. It enables the monitoring of small signals with the help of optical phenomena such as fluorescence, scattering, and light absorption[41]. In optical biosensors, the antibodies or aptamers are bound to optical materials and then they can capture Salmonella using the specific combination of antigen-antibody. According to the change of some characteristics of optical materials, the concentration of the target microorganisms can be judged and calculated, thus achieving the purpose of detection[42].

Hao et al. developed a sensitive and rapid fluorescent biosensor for Salmonella Typhimurium detection, as shown in Figure 1a. Fluorescent biosensors are characterized by high sensitivity, non-contact detection, and low cost[43]. As is known to us all, because of the wide excitation range, long fluorescent lifetime, and strong fluorescent signal, QDs have been considered an ideal fluorescent probe. Besides, to improve the sensitivity of QDs, they are always combined with other nanomaterials, like manganese dioxide nanoflowers (MnO2 NFs), which are easy-to-synthesize and non-toxic nanomaterials. Scientists used QDs as fluorescent probes and manganese dioxide nanoflowers (MnO2 NFs) as QDs nanocarriers to amplify signals. In the experiment, Salmonella cells were mixed with magnetic nanoparticles modified by monoclonal antibodies and then they were injected with MnO2-QD-pAb NFs into the microfluidic chip to form MNP-bacteria-QD-MnO2 complexes[44]. After that, MnO2 could be dissolved by glutathione (GSH) and release QDs. Finally, the concentration of Salmonella was reflected by the fluorescent intensity of QDs. The whole process was shown in Figure 1b. Following a series of tests, the amount of Salmonella from 1.0×102 to 1.0×107 CFU/mL was linearly related to fluorescent, and the low limit of detection was 43 CFU/mL[44]. High sensitivity was one of the obvious advantages of this biosensor and there were four reasons: (1) The MnO2 NFs with QDs had a high load, causing stronger fluorescent signals; (2) QDs were released into the water by GSH, which reduced the fluorescence absorbed by MnO2; (3) Continuous-flow washing and automatic operations in microfluidic chip had better control of background noise; (4) Complexes were concentrated by the magnetic field in separation chamber, making the fluorescent signals much stronger. And the average recovery rate of target strains was 99.7%. From the results, the biosensor had high sensitivity and it was suitable for the rapid detection of foodborne bacteria including Salmonella typhimurium.

Identification by fluorescence techniques relies on detecting the light signal emitted by the target upon binding to the probe. The higher the concentration of the target, the greater the intensity of the fluorescent signal. Moreover, signal amplification is essential to improve the sensitivity of the biosensor. A new kind of biosensor based on quantum dots was studied by Ding et al. They constructed a fluorescent biosensor for the detection of Salmonella based on a streptavidin (SA) biotin system and polyamine linear polymer poly-L-lysine (PLL)[45]. The details are shown in Figure 1c. Firstly, the target Salmonella Anatum was captured and formed the double-antibody sandwich with paired Salmonella mAb on a black 96-wall plate. Secondly, the SA-biotin-specific bond was applied to immobilize SA on the biotin-modified mAb. After that, they combined the biotin-modified polylysine (BT-PLL) with SA, which would bond again because of the SA-biotin system. Finally, covalent coupling of BT-PLL and water-soluble CdSe/ZnS QD-labeled SA was added to the black 96-wall plate. The strategy to amplify the fluorescent signal in a dendritic manner was achieved due to the overlap of biotin and SA and the covalent coupling of biotinylated PLL[45]. Through the experiment, the linear detection range of the biosensor was from 4.9×103 to 4.9×107 CFU/mL, with a low limit of 4.9×103 CFU/mL. It has demonstrated high sensitivity and accuracy in the detection of Salmonella in milk. The reasons were as follows: (1) SA-biotin-system could amplify the signal effectively; (2) High stability could be ensured after covalent coupling of water-soluble carboxylated QDs and SA, and the nonspecific absorption would reduce; (3)

Researchers used the PLL loaded with many fluorescent labels to form the dendritic structure to amplify a signal. In conclusion, the biosensor was suitable for the detection of Salmonella in milk with short detection time, high accuracy, and specificity.

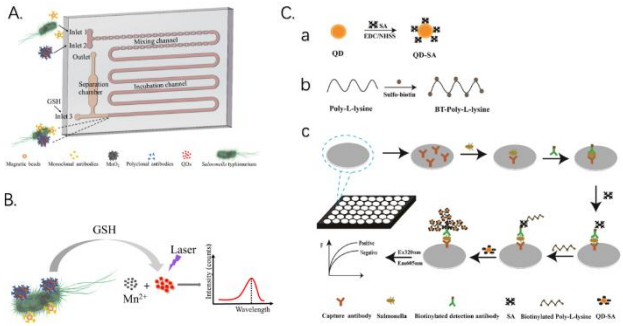

Fig 1 A. The schematic of the microfluidic chip[44]; B. The principle of the fluorescent biosensor for rapid and sensitive detection of Salmonella Typhimurium[44]; C. Detection of Salmonella by fluorescence sensor: (a) the preparation of quantum dot (QD)-streptavidin (SA), (b) the preparation of biotin-modified polylysine (BT-PLL), and (c) the fluorescence detection assay for detection of Salmonella in milk. EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NHSS = N-hydroxysuccinimide sodium salt; Ex = excitation (wavelength); Em = emission (wavelength)[45].

Although fluorescence-based biosensor has been widely used in the detection of foodborne microorganisms, it still suffers from the problem of requiring complex optics such as filters and specific light sources. To overcome these drawbacks, a kind of non-spectroscopic optical biosensor based on a stem-loop DNA probe and retro-reflective signaling was developed by Kim et al. They chose the retroreflective Janus microparticle (RJP) to be the signaling probe, which could be observed through visible light without using a filter. Due to its characteristics, this biosensor used a less complicated optic system. In the study, a biotinylated stem-loop DNA was applied to capture the target DNA, and a streptavidin-conjugated RJP (SA-RJP) was used as the detection molecule, to make sure the RJPs could play the role of a probe[46]. The biotinylated stem-loop DNA probe was stretched when target gene DNA appeared on its immobilized sensing surface, thus exposing the biotin to react with SA-RJP, as shown in Figure 2a. The amount of exposed biotin increased with the amount of the target, and thus the number of RJPs on the surface of biosensors could be observed to increase with the concentration of target DNA[46]. Scientists calculated the amount of Salmonella with the help of the number of RJPs observed. The experimental results showed that the biosensor could analyze Salmonella in the concentration of 0 to 100 nM, with a low detection limit of 2.48 pM. Compared with other fluorescent biosensors, this biosensor detection system was simpler and could use common equipment such as a CMOS camera and a white LED to count RJPs. It enabled highly sensitive detection of target Salmonella. Besides, it was highly selective for the target bacteria, and the researchers verified this property using oligonucleotides with mismatched sequences. Based on the above results, it could be concluded that this biosensor system was a promising platform for the detection of foodborne Salmonella.

Besides, other types of optical biosensors for Salmonella were developed by researchers. Different from biosensors depending on single-modality signal output, a biosensor based on dual-model detection was created by Huang et al. and it could be used in fluorescent and colorimetric conditions. Scientists used curcumin (CUR) which can be extracted from ginger family plants. It is a natural compound with poor solubility. When CUR dissolves in an alkaline or acidic solution, it shows fluorescence characteristics and excellent intrinsic allochroic effect making it become an ideal material for a dual-signal reporter[47]. Until then, sodium molecule (NaOH) was the only chemical reagent studied as a signal molecule to release CUR[48]. In recent years, researchers have begun to investigate new materials, like mesoporous silica nanoparticles (MSNs) with features including large surface area, terrific biocompatibility, and high physical and chemical

stability. Huang et al. developed an acid-responsive microfluidic biosensor that used CUR as the signal reporter and MSNs capped with ZnO as the signal amplifier. The specific principles are shown in Figure 2b. This kind of biosensor could achieve the goal of detecting Salmonella in the fluorescent and colorimetric modal[47]. Firstly, MSNs were mixed with CUR to form MSN@CUR nanoparticles (MC NPs) and then those particles were incubated with ZnO to obtain MSN@CUR@ZnO nanoparticles (MCZ NPs), which could prevent CUR from early release. After that, the complexes would be modified by polyclonal antibodies (pAbs) against Salmonella Typhimurium so MSN@CUR@ZnO@pAbs formed. Then, MNP-bacteria-MCZP complexes could be gotten in a microfluidic chip, where Salmonella typhimurium cells, MNPs, and MCZP NPs were conjugated. Finally, scientists introduced acetic acid (HAc) to release CUR. The concentration of Salmonella could be reflected by both the fluorescence and color changes. The detection range of the biosensor was from 102 to 107 CFU/mL, with the lower detection limit of fluorescent measurement calculated to be 40 CFU/mL and of colorimetric measurement calculated to be 63 CFU/mL[47]. In the spiked chicken samples, the mean recovery of Salmonella was about 104%. Compared to the traditional single signal biosensor, this kind of biosensor showed several advantages: (1) CUR was cheaper and more facile than other florescent dyes, which reduced costs; (2) High sensitivity due to strong signal and absorbance and the detectable fluorescent intensity of CUR at the concentration low as 1 μM; (3) CUR had a stable absorbance and fluorescence in HAc solution, which improved stability of this biosensor; (4) The premature release of CUR was prevented because ZnO NPs was loaded on MSNs firmly to cap the pores. Therefore, this biosensor was a promising platform, achieving specific, rapid, and sensitive detection of Salmonella typhimurium.

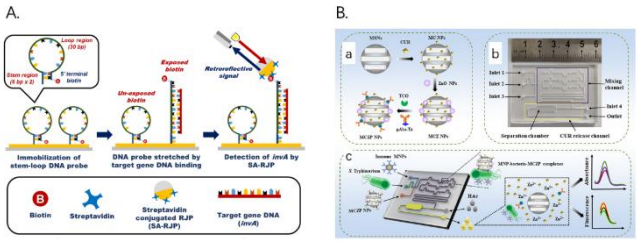

Fig 2 A. Schematic illustration of the developed Salmonella target gene DNA (invA) system detection using retroreflective Janus microparticles (RJPs). In the presence of the target gene DNA (invA), the stem- loop DNA probe is stretched, exposing biotin. Then, the exposed biotin can be detected using streptavidin- conjugated RJPs. The inset shows the stem- loop DNA probe, which is composed of a loop- region (30 bp, stem- region (6 bp × 2), an amine group at the 3′ end, and biotin at the 5′ end. The sequence of the loop region is complementary to the target gene DNA[46]; B. Schematic of the proposed microfluidic biosensor for rapid and sensitive detection of S. Typhimurium[47].

A kind of colorimetric biosensor, which could make an ultrasensitive detection of Salmonella typhimurium, was reported to be created by Wang et al. It used the magnetic grid separation column to separate the target bacteria from a large amount of sample effectively and platinum-loaded zeolitic imidazolate framework-8(PT@ZIF-8) nanocatalysts to amplify the biological signal[49], as shown in Figure 3a. Some scientists studied the forming of magnetic particle chains and applied them for cell sorting, separation, and pre-enrichment. Combining particle chains with magnetic flow separation technology promises to enable the rapid isolation of the target bacteria that we need from a large number of samples. To improve the sensitivity of biosensors, signal amplification is a proven strategy. The most commonly used material for signal amplification is an enzyme, but due to its high cost, low stability, and poor environmental tolerance, researchers are searching for signal amplification materials with more excellent properties. Nanocatalysts have attracted much attention in recent years due to their low cost, good stability, and adjustable catalytic activity. Among nanomaterials, platinum nanoparticles are regarded as one of the promising materials for a wide range of applications because of their catalytic activity, excellent biocompatibility, and chemical

stability. Besides, novel nanocarriers, such as zeolitic imidazolate framework-8(ZIF-8), are used as loadings of Pt nanoparticles to enhance their catalytic response for the purpose of improving the sensitivity of biosensors[49]. In this study, Salmonella in the sample was first separated using a magnetic grid separation column with an immunomagnetic particle chain, and then conjugated with immune Pt@ZIF-8 nanocatalysts to mimic peroxidase for catalysis of hydrogen peroxide-3,3',5,5'-tetramethylbenzidine. Finally, the amount of target bacteria was determined by measuring the characteristic wavelength at 450 nm. This biosensor could detect Salmonella within the concentration from 101 to 104 CFU/mL, with a low detection limit of 11 CFU/mL. Compared with others, this biosensor had the advantages of shorter detection time and higher sensitivity because of the effective catalytic effect of Pt@ZIF-8 and the excellent separation of magnetic grid separation column. Moreover, it had good specificity for Salmonella typhimurium. All of these advantages ensured the superior practicality of the detection of target bacteria.

In terms of foodborne bacteria detection, rapid screening and isolation of target bacteria have always been a great challenge. A large amount of research has been carried out in the study of signal enhancement materials and methods. Porous gold@platinum nanocatalysts(Au@PtNCs) have got more and more attention because of their nice molecular accessibility, high specific surface area, and quantities of catalytic active sites[50]. Besides, they are proven to have a similar ability to process enzymatic activity, which meant Au@PtNCs can replace natural enzymes in the application. In the development of microfluidic biosensors, the mixing efficiency of the key component, the micromixer, has a large impact on the binding of enzymes and substrates and the immune response to antigens and antibodies. The 3D structure for passive mixing has aroused people's awareness due to its nonexternal power requirement and high mixing efficiency. Here, Zheng et al. have developed a biosensor combining porous gold@platinum nanocatalysts with a micromixer of passive 3D structure for the detection of Salmonella in food[50], as shown in Figure 3b. Firstly, immunomagnetic nanoparticles and the passive 3D micromixer were used to separate the target. Then, they used the immune Au@PtNCs to label target cells and catalyze hydrogen peroxide-3,3',5,5'-tetramethylbenzidine. Finally, the number of bacteria could be calculated by measuring the absorbance at 652 nm. This kind of biosensor detected the concentration of Salmonella ranging from 1.8×101 to 1.8×105 CFU/mL with a low detection limit of 17 CFU/mL. It had the potential to achieve a high sensitivity mainly due to the three aspects as follows: (1) The microfluidic chips with a passive 3D structure made the immune response between the antibody and the target bacteria more efficient; (2) Au@PtNCs had a low cost, high stability and better catalytic activity, which enabled them to support effective amplification of biological signals; (3) The immune magnetic nanoparticles were applied to do the specific separation and enrichment of Salmonella. Overall, the biosensor ensured detection in a short period and had the potential to enable the detection of other target bacteria by changing the type of antibody.

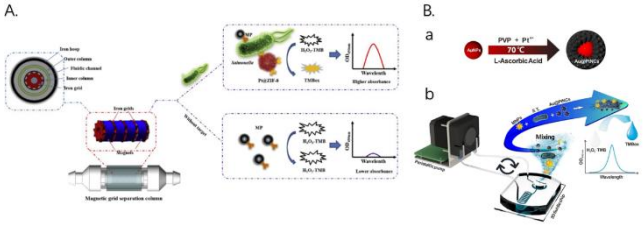

Fig 3 A. Schematic of the proposed colorimetric biosensor for ultrasensitive detection of Salmonella from large volume of sample[49]; B. a. Schematic for the Synthesis of Porous Gold@Platinum Nanocatalysts; b. Schematic of the Optical Biosensor for Detection of Salmonella typhimurium[50].

2.2 Electrochemical Biosesnsors for Salmonella Detection in Food Products

The signal conversion method of this type of biosensor is electrochemical. The electrochemical biosensor has received a lot of attention from researchers due to its portability, high versatility, and

sensitivity[51][52]. It uses materials such as nucleic acids, antigens, antibodies, and aptamers to specifically capture the target of detection[53]. Then, the acquired biological signals are converted into chemical signals that can be monitored by resistors, etc. for measurement. By establishing the correspondence between the intensity of chemical signals and the concentration of the target, the desired data can be calculated[54]. Most of the electrochemical biosensors can achieve quantitative measurement of Salmonella with short detection time, high specificity, and sensitivity, which are expected to be further optimized and improved to enhance the performance[55].

In the process of food bacteriological detection, complex food samples can cause large interference in the detection process, thus achieving efficient isolation and enrichment of the target strains is the key to improving the accuracy of the detection. Hou et al. developed a biosensor for the specific detection of Salmonella based on the results of a previous study in which researchers used magnetic nanoparticles(MNPs) for bacterial isolation. In the study, a kind of self-assembled MNP chain was applied for continuous-flow separation of Salmonella from the sample, and the signal created was labeled and amplified by gold nanoparticles(GNPs) coated by urease, and then researchers made the sensitive detection of catalysate using linear scan voltammetry[56]. The structure of the biosensor and the principle and structure of the magnetic separation part is shown in Figure 4. Firstly, ring iron gears and mutually repelling cylindrical magnets were used to control MNPs which were coated by anti-Salmonella monoclonal antibody. Then, bacteria-MNP complexes formed on the chains after the sample was continuous-flow into the channel and the specific separation finished. Enzymatic bacteria could form after being labeled by urease coater GMPs and anti-Salmonella polyclonal antibodies. Next, urease on the enzymatic bacteria drew and catalyzed urea after removing the residual GNPs, and ammonium carbonate, products of the catalytic process, appeared. Finally, the scientists transferred the catalysate to a microfluidic chip with a thin-film Ag/AgCl reference electrode array to make the detection using linear scan voltammetric measurement. This kind of biosensor detected the concentration of Salmonella ranging from 1.0×101 to 1.0×106 CFU/mL, with a low detection limit of 101 CFU/mL[56]. The biosensor achieved an average recovery rate of 104.3% for Salmonella in the sample. Combining all these points, this electrochemical biosensor based on self-assembled magnetic nanoparticle chains and enzyme catalysate had a relatively excellent performance. It had the advantages of fast detection speed, high sensitivity, and semi-automatic operation. It was also useful for the detection of other types of pathogens by changing the kind of antibody.

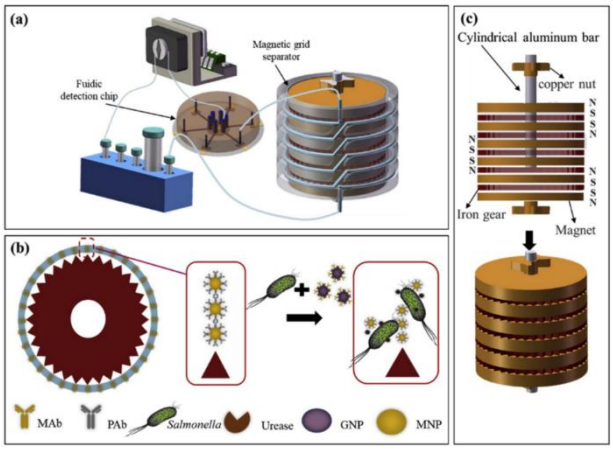

Fig 4 (a) Structure diagram of the electrochemical biosensor; (b) Principle of magnetic grid separation and gold nanoparticle labeling; (c) Structure diagram of the magnetic grid separator[56].

Wang et al. made an improvement to an existing method of electrochemical biosensors. Based on the previous research method of immunomagnetic separation and impedance-based biosensor, they experimented with and compared several different separation strategies for Salmonella and selected the most optimal one to enhance the detection efficiency of the biosensor from the following two aspects[57]: (1) The scientists used three methods to isolate and combine target

strains from samples to form magnetic bacteria through modified capillaries and immunomagnetic nanoparticles. The experimental results showed that the target was captured and enriched in the test tube-the separation in the capillary was the most excellent and had an ideal efficiency; (2) The magnetic bacteria labeled by immune gold nanoparticles coated with urease were injected into the capillary. After catalyzing the urea, they chose different electrodes to measure the impedance of the catalysate. By comparison, the screen-printed electrode was found to be the most suitable with higher sensitivity and stability. In the study, the researchers first used MNPs to specifically isolate and capture Salmonella and then highly concentrated them in a small volume of phosphate-buffered saline(PBS) to form the magnetic bacteria. Then, the urease and the anti-Salmonella detection antibodies were used to modify the GNPs, which were then applied to label the magnetic bacteria and from the enzymatic bacteria. After that, those complexes were injected into the modified coaxial capillary. Finally, urea was catalysated by the enzymatic bacteria to form ammonium carbonate, which could decrease the impedance of the mixture. And the changes would be detected and analyzed by the impedance analyzer and an App on the smartphone, from which we could get the concentration of Salmonella[57]. The whole process is shown in Figure 5. This biosensor was easy to operate and measured the concentration of the target bacteria with a minimum of 102 CFU/mL in less than two hours. This improved assay had a wide application in Salmonella detection in the food supply chain.

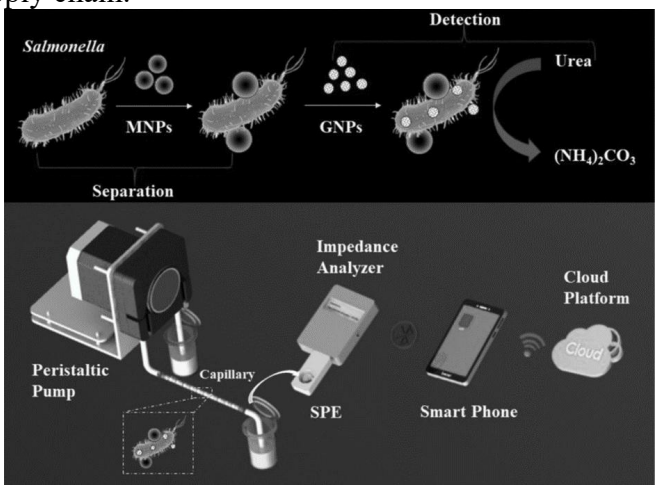

Fig 5 Scheme of the biosensor-based strategy for rapid screening of Salmonella in poultry supply chains. The target salmonella is first separated by the immune magnetic nanoparticles (MNPs), then labeled by the gold nanoparticles (GNPs) with urease, and finally used to catalyze urea. Thecatalysate is measured by the impedance analyzer with the electrode to obtain the result. The result is first transmitted via Bluetooth to the smart-phone, then analyzed by the App, and finally uploaded onto the cloud platform for risk assessment. Abbreviation: SPE, screen-printed electrode[57].

The impedance biosensor can detect the impedance change of the electrochemical transducer after being perturbed by sinusoidal voltage and has the advantages of low cost and short detection time. Interdigitated microelectrodes(IMEs) are often used as sensors for impedance biosensors because of their small size, fast reaction kinetics, and high signal-to-noise ratio, in order to accurately measure the impedance changes on the electrode surface caused by biological reactions. However, due to the small impedance change caused by the reaction of the antibody with the target bacteria at the electrode, many nanomaterials are incorporated into the sensor and used for the amplification of the biosignal. Wang et al. used an impedance biosensor, based on nickel nanowires(NiNWs) to achieve signal amplification through microelectrode as conductive bridges[58], as shown in Figure 6a. In the study, streptavidin was applied to fix the gold interdigitated microelectrode firstly, followed by conjugating with the biotinylated aptamers via streptavidinbiotin binding and blocking with BSA to avoid non-specific reaction[58]. Then, the NiNWs modified with anti-Salmonella typhimurium antibodies were used to separate the target

Salmonella magnetically, forming the bacteria-NiNWs complexes, which would form aptamer-bacteria-NiNW complexes by incubating on the microelectrode. After that, NiNWs were controlled by an external arc magnetic field and formed conductive NiNW bridges. Scientists measured the enhanced impedance change and concluded the concentration of the target bacteria. This kind of biosensor could detect Salmonella ranging from 102 to 106 CFU/mL in 2 hours, with a low limit of 80 CFU/mL. Experiments have proved that those nickel nanowires had an ideal signal amplification effect and could improve the detection sensitivity of biosensors without increasing the time of detection[58]. It could play an excellent role in detecting the target bacteria subsequently.

Faster testing is significant for the detection of pathogens in food. A microfluidic biosensor based on magnetic separation, enzymatic catalysis, and electrochemical impedance analysis was developed by Liu et al. in order to make sensitive and rapid detection of Salmonella typhimurium. Researchers used the click chemical reaction between trans-cyclooctene-PEG4-NHS ester(TCO) and tetrazine-sulfo-NHS ester(Tz) to synthesize the immune probes[59]. In the study, MNPs modified with capture antibodies, the bacteria sample, and enzymatic probes modified with glucose oxidase(GOx) and detection antibodies were first injected into the microfluidic chip. After mixing and incubating for some time, the MNP-bacteria-probe sandwich complexes formed. Then, they injected the glucose with high impedance, which could be catalyzed into gluconic acid with low impedance and hydrogen peroxide with high impedance by the GOx. Finally, the change of impedance could be measured by the electrochemical impedance analyzer and low-cost interdigitated microelectrode and the amount of Salmonella in the sample could be determined. The synthesis of the immune enzymatic probes, the structure of the microfluidic chip, and the principle of the impedance biosensor are shown in Figure 6b. This kind of biosensor could detect the concentration of Salmonella ranging from 1.6×102 to 1.6×106 CFU/mL in 1 hour under optimal conditions, with a low limit of 73 CFU/mL[59]. The examination of chicken samples with Salmonella added showed that the biosensor had high sensitivity and practicality for the following reasons: (1) The target bacteria reacted effectively with immune enzymatic probes forming after the efficient mixing and incubation in the microfluidic chip and MNPs; (2) Immune enzymatic probes with GOx amplified the impedance signals significantly; (3) The microelectrode could measure the impedance sensitively. Functions of mixing, separation, and catalysis were concentrated on a single microfluidic chip and could perform automatically. It had a strong potential for the future of foodborne bacteria detection.

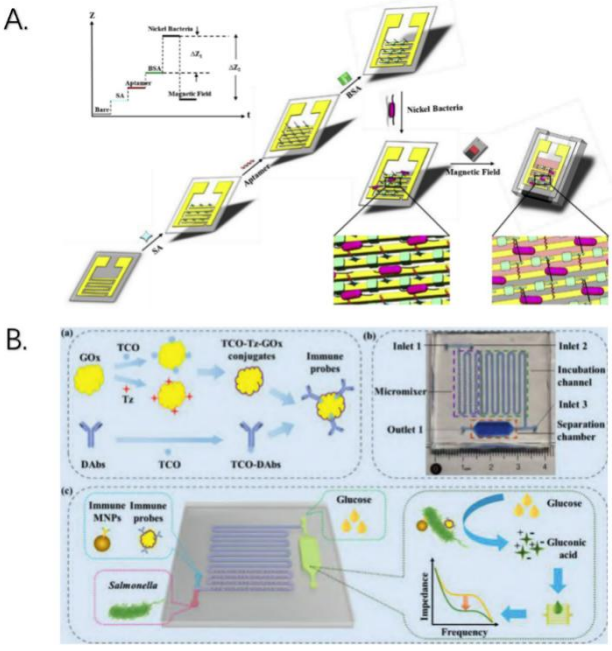

Fig 6 A. Schematic of the electrochemical aptasensor for rapid and sensitive detection of Salmonella Typhimurium[58]. B. (a) The synthesis of the immune enzymatic probes; (b) The

structure of the microfluidic chip; (c) The mechanism of the impedance biosensor for rapid and sensitive detection of Salmonella typhimurium[59].

Lectin is a selective recognition element that enables the detection of pathogens. The process of obtaining lectins is not cumbersome. Since lectins are able to bind to certain substances on the surface of viruses or bacteria, they are often used to capture and detect target pathogens. Jorge et al. selected Hechtia argentea lectin as a material to capture target bacteria for application in a biosensor. It was extracted from the Hechtia argentea, which belongs to the Bromeliaceae family. This lectin had the selectivity towards D-mannose, a substance existing on the cell wall of Salmonella. In the study, this extracted lectin was fixed on a screen-printed gold electrode to serve as identification. When the recognition occurred on the electrode, the impedance of electron transferring happened on the surface of the electrode changed because the presence of self-assembled monolayers altered the rate of electron transfer and the interface capacitance, ultimately leading to an increase in impedance[60]. The overall detection scheme is shown in Figure 7a. From the experimental results, the measured impedance obtained increased with the concentration of Salmonella bound to the biosensor surface and it was linear from 15 to 2.57×107 CFU/mL, with a low limit of 5 CFU/mL. The biosensor was used for the detection of Salmonella in egg samples and the results obtained were consistent with the official test results[60]. Besides, the biosensor had the advantages of short detection time and simple operation and had a broad application prospect.

For electrochemical biosensors, the weak current signal cannot meet the detection requirements, so signal amplification has been a key issue for this type of biosensor. In recent years, the application of polymer materials, metal nanoparticles, and carbon materials has greatly improved the performance of electrochemical biosensors. Among them, ferroferric oxide (Fe3O4) has been widely used because of its large specific surface area, low cost, and excellent biocompatibility, but it also has the defects of poor mechanical stability, weak electrical conductivity, and electrocatalytic ability. However, in 2-dimensional materials, graphene has exactly some advantages that Fe3O4 does not have. Combining Fe3O4 with graphene can overcome the inherent limitations of metal oxides. In the study, an electrochemical biosensor equipped with electrodes modified with Fe3O4@graphene nanocomposite was designed by Feng et al[61]. The synthesis of the Fe3O4@graphene and the construction process of the electrochemical immunosensor are shown in Figure 7b. Combining the large specific surface area of Fe3O4 with the mechanical stability and electrical conductivity of graphene, the complex Fe3O4@graphene could generate ideal electrical signals and improve the sensitivity of biosensor detection[61]. Scientists used electrochemical techniques to immobilize gold nanoparticles on Fe3O4@graphene composites. Salmonella was captured by the antigen-antibody reaction when it passed through the biosensor. In the experiment, the complex exhibited a faster electron transfer rate and stronger current response. Under the optimal conditions, the concentration of Salmonella showed a good linear relationship from 2.4×102 to 2.4×107 CFU/mL, with a low limit of 2.4×102 CFU/mL. This type of biosensor had good stability, accuracy, and selectivity for the detection of the target bacteria, and was suitable for the detection of Salmonella in milk.

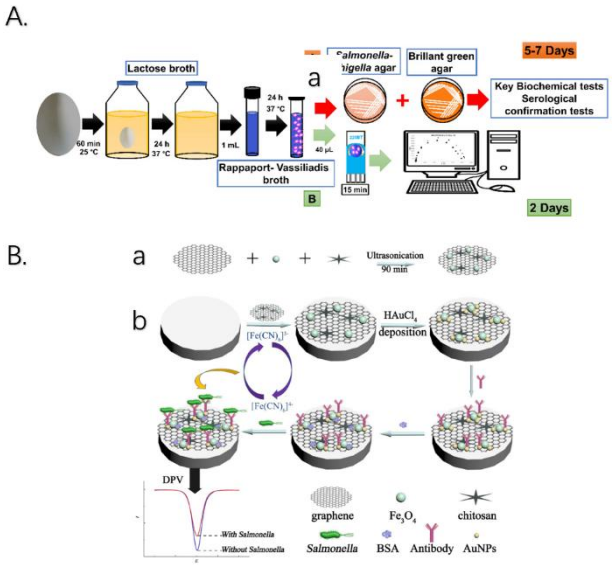

Fig 7 A. Analysis scheme for Salmonella spp. detection. The sample is placed for pre-enrichment in lactose broth (LB) and enrichment in Rapaport-Vassiliadis broth (RVB). (a) Analysis of presumptive colonies, employing the microbiology method, involving inoculation in solid media (Salmonella-Shigella and brilliant green agars); (b) Analysis employing the biosensor, using 40 µL of RVB[60]; B. Schematic illustration for (a) synthesis of the Fe3O4@graphene nanocomposite and (b) the construction process of the electro-chemical immunosensor. DPV = differential pulse voltammetry. I = current (µ A); E = potential (V)[61].

3. Conclusions and Outlook

In recent years, food contamination caused by Salmonella has become a growing concern. Testing methods for foodborne Salmonella are constantly being updated. Traditional detection methods, such as immunological assays and nucleic acid analysis, have limitations in some aspects[62]. The biosensor assays based on nucleic acid analysis and immunological analysis have been developed due to their simplicity, high sensitivity, and short detection time.

In the development of biosensors, the use of new nanomaterials and signal amplification techniques have further broadened the areas in which they can be applied. Optical and electrochemical biosensors, which are often used, are also undergoing continuous innovation. Different isolation strategies and identification methods from the original ones have greatly improved the performance of biosensors in detecting target strains from samples, and they have reduced detection time and greatly improved sensitivity, specificity, and interference resistance during detection. At the same time, the portability and ease of operation of biosensors have made them less demanding to use in food production processes, enabling rapid and accurate detection of Salmonella levels and gaining more popularity[62].

However, there are still some problems with the biosensors currently in use. Firstly, most of the biosensors studied now can only achieve the detection of one specific type of Salmonella and cannot make the simultaneous detection of two or more Salmonella species. Secondly, the detection results of biosensors often need to be converted and then read using instruments, which may affect the cost and portability of their manufacture. In addition, since most of the recognition elements for biosensors to capture target bacteria use antibodies, the long preparation cycle of antibodies makes the production cycle of biosensors longer. In order to overcome the above problems, we can choose a new type of antibody with high affinity and specificity, such as nanobody, which has high stability and heat resistance and can replace common antibodies and be expressed in large quantities in engineered bacteria, thus improving various properties of the biosensor. Moreover, we combine optical visualization and electrochemical stability to visualize test results, reduce costs, miniaturize

equipment, and improve portability. At this stage, biosensors play an important role in the detection of foodborne Salmonella, and with the continuous improvement of its technology, biosensors will likely be popularized in the future for a variety of target bacterial detection applications.

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