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REVIEW

Recent advances in rapid pathogen detection method based on biosensors

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Abstract

As strain variation and drug resistance become more pervasive, the prevention and control of infection have been a serious problem in recent years. The detection of pathogen is one of the most important parts of the process of diagnosis. Having a series of advantages, such as rapid response, high sensitivity, ease of use, and low cost, biosensors have received much attention and been studied deeply. Moreover, relying on its characteristics of small size, real time, and multiple analyses, biosensors have developed rapidly and used widely and are expected to be applied for microbiological detection in order to meet higher accuracy required by clinical diagnosis. The main goal of this contribution is not to simply collect and list all papers related to pathogen detection based on biosensors published recently, but to discuss critically the development and application of many kinds of biosensors such as electrochemical (amperometric, impedimetric, potentiometric, and conductometric), optical (fluorescent, fibre optic and surface plasmon resonance), and piezoelectric (quartz crystal microbalances and atomic force microscopy) biosensors in pathogen detection as well as the comparisons with the existing clinical detection methods (traditional culture, enzyme-linked immunosorbent assay, polymerase chain reaction, and mass spectrometry).

Keywords Biosensor · Pathogen · Medical detection methods · Comparison

Introduction

Nowadays, the incidence of infectious disease is increasing year by year, which has become a significant harmful factor that cannot be underestimated for human health [1]. As a result, the prevention and treatment of infection become more critical. The rapid and early identification of pathogen is the key to set the best anti-infectious therapy. However, even trickier is that the universality of strain variation [2] and the increasing degree of multiple drug resistance [3, 4] are putting forward a great challenge to clinical works.

Until now, the main method of bacteria detection used in clinical studies still relies on the traditional method. This process often includes bacterial culture multiplication, bacteria identification, and antibiotic susceptibility tests, which need 3–5 days or even more time to yield results [5, 6]. This may lead to loss of valuable time of treatment. Enzyme-linked immunosorbent assay (ELISA) is one of the immune methods which is used widely. Applying a simple antigen-antibody reaction, ELISA involves less time to achieve results compared with traditional culture. Various kinds of commercialized kits have been developed for specific antibody or antigen detection. However, cross-reactivity and low sensitivity still limit the further development of ELISA [7]. Due to its high specificity, nucleic acid-based assays have become the most popular pathogen detection technology today, and polymerase chain reaction (PCR) is the most widespread method among these assays [8]. The application of PCR relieves the stress of clinical detection effectively to a great extent, and Table 1 shows the increasing markers used in early diagnosis based

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Table 1 List of common PCR detection types in clinical work

Types of nucleic acid	Pathogens	Abbreviation	Types of sample	References
DNA	Hepatitis B virus	HBV	Serum/anticoagulated blood	[9]
DNA	Human immunodeficiency virus	HIV	Serum/anticoagulated blood	[10]
DNA	Tubercle bacillus	TB	Sputum/hydrothorax and ascites	[11]
DNA	*Mycoplasma pneumonia*	MP	Oropharyngeal swabs/bronchoalveolar lavage	[12]
DNA	EB virus	EBV	Anticoagulated blood	[13]
DNA	Human papillomavirus	HPV	Secretions swabs/serum	[14]
DNA	*Neisseria gonorrhoeae*	NG	Secretions swabs/serum	[15]
DNA	*Chlamydia trachomatis*	CT	Secretions swabs/serum	[16]
DNA	*Ureaplasma urealyticum*	UU	Secretions swabs/serum	[17]
DNA	Herpes simplex virus	HSV	Secretions swabs/serum	[18]
DNA	Human cytomegalovirus	HCMV	Sputum/urine/milk	[19]
DNA	*Treponema pallidum*	TP	Anticoagulated blood	[20]
DNA	*Helicobacter pylori*	HP	Gastric juice	[21]
RNA	Hepatitis C virus	HCV	Serum/anticoagulated blood	[22]
RNA	Influenza A virus	–	Oropharyngeal swabs/bronchoalveolar lavage	[23]
RNA	Influenza B virus	–	Oropharyngeal swabs/bronchoalveolar lavage	[24]
RNA	Enterovirus 71	EV71	Feces	[25]
RNA	Coxsackievirus A16	CA16	Feces	[26]

on PCR. Actually, PCR plays an important part in the detection of virus infections in most hospitals in China, which has become an indispensable technology in microbiological test. But there is still a long way between PCR and ideal detection technology because of its some disadvantages such as complex pretreatment and failure in distinguishing viable from nonviable cells [27]. In recent years, mass spectrometry, a detection tool for proteomics, has becoming more and more developed to provide high-throughput, sensitive, and specific analysis in the microorganism field. An increasing number of big hospitals have used this technology for clinical detection [28, 29], but the expensive equipment of mass spectrometry has made this technique less popular in some small- and medium-sized hospitals. In other words, this method cannot meet most requirements from primary medical institutions. So, finding more perfect techniques remains a huge task.

Biosensors are not a new concept since its introduction. With a series of advantages such as rapid response, high sensitivity, ease of use, low cost, real time, and multiple analyses, biosensors have had a wide range of applications in food safety [30–32], environmental monitoring [33, 34], biomedical and drug sensing [35], and national defense [36]. However, due to complex and diverse clinical samples (blood, urine, feces, sputum, swabs, cephalorachidian liquid, saliva, etc.), there were few papers which refer to clinical diagnosis that have been reported for a long time in the past. In recent years, what is exciting is that more and more clinical applications [37, 38] can be found with the rapid development of biosensors. In this review, we will discuss deeply the principle, development, and application of many kinds of biosensors and also make comparisons with the existing clinical detection methods to discuss their potential in early clinical detection.

Biosensors for pathogen detection

All sensors consist of two main components: recognition element and transducer [39]. The recognition element can recognize and connect the target analyte, and then the transducer converts molecular reaction into some measurable signals. So, biosensors can be grouped into enzyme sensor, antibody sensor, nucleic acid sensor, and whole-cell sensor by the recognition element and into optical, electrochemical, and piezoelectric sensors by the transducer [40], as shown in Fig. 1.

Optical biosensors

Optical biosensors have the advantages of high sensitivity and selectivity. They can offer accurate detection based on many kinds of signal such as absorption, refraction, reflection, dispersion, infrared, polarization, chemiluminescence, fluorescence, phosphorescence, etc. [41]. In fact, optical biosensors can be divided into two parts simply according to whether the labels are needed. One type needs markers and other does not. Here, three kinds of optical biosensors (fluorescence, optical fibers, and surface plasmon resonance) will be discussed emphatically.

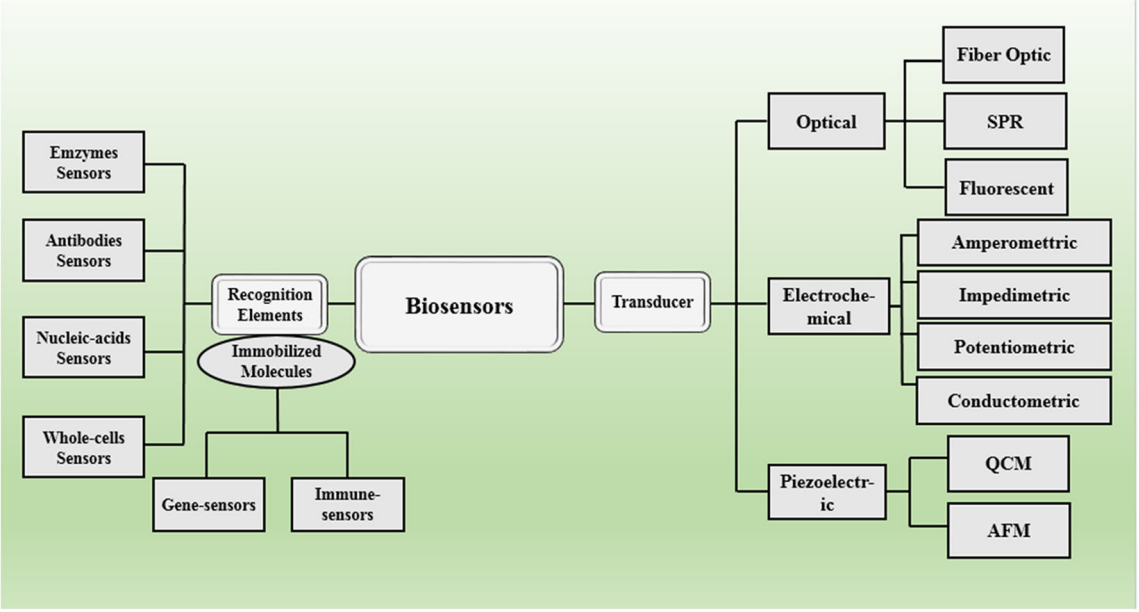

Fig. 1 The different categories of biosensors

Fluorescence biosensor

Fluorescence is the most popular detection method used among optical biosensors. By absorbing sufficient light energy, the valence electron of a fluorescent substance will be excited from ground state to excited state. Fluorescence occurs when the electron returns to its original ground state [42]. Using this feature, the molecular reaction can be tracked well by combining the fluorescent substance to the reactants. Fluorescence technology can provide high detection sensitivity, which fits the detection of trace samples [43]. The more important points are that it does not only have faster response time but also have the advantage of labeling with multicolor dyes synchronously. This makes it possible to detect a variety of pathogens in complex samples simultaneously in a short time. The labeling reagent is the key to fluorescent biosensors. The common biomarkers include organic dyes, nanoparticles, rare-earth elements, and so on. In the past, organic dyes occupy a dominant position in fluorescent technology. However, with the development of new materials, quantum dots, which are composed of elements from groups II–VI to III–V in the periodic table such as CdSe, CdTe, PbS, HgTe, InP, PbSe, PbTe, InAs, and GaAs [44], have become one of the most popular fluorescent labeling materials. Besides the advantages of broad absorption with narrow photoluminescence spectra, low photobleaching, high quantum yield, and resistance to chemical degradation, the most outstanding characteristic of QDs is that it can change the particle size to tune their wavelength of fluorescence emission by altering their synthesis procedures and chemical composition [45]. And this is also an important reason why QDs can be found in many fields as well as in pathogen detection area.

Xue et al. reported a fluorescence measurement method which uses water-soluble QDs as fluorescence markers for rapid detection of *Escherichia coli* and *Staphylococcus aureus* [46]. The CdSe QDs used in the experiment were synthesized by the chemical reaction between Cd^{2+} and NaHSe in the aqueous phase. The advantages of this material are that it has broad excitation spectra and narrow emission spectra. It means that more excitation lights can be chosen compared with traditional organic dye, and the fluorescent detection system can be simplified greatly. Narrow emission spectra also provide higher recognition degree and fluorescence intensity. And all of these provide a good start in the exploration of pathogen detection in aqueous samples, such as urine and seroperitoneum. Then, Xue et al. used highly luminescent and stable CdSe quantum dot-immunoglobulin G as well as propidium iodide fluorescent labeling to develop a method of detecting live/dead *Staphylococcus aureus* cells [47]. It allows a high level of detection accuracy and can help doctors to judge the stage of infection better. Later, the two-color quantum dots strategy improved the development of fluorescence QDs. Wang et al. developed a novel two-color QDs strategy which used fluorescence resonance energy transfer (FRET) and fluoroimmunoassay technology as donors and acceptors simultaneously for the detection of *Salmonella* Enteritidis. The limit of detection of this method was 10 CFU/mL without sample enrichment within 1–2 h [48].

Recently, fluorescent carbon dots (CDs) have attracted increasing concerns with the unique features of having no intrinsic toxicity, elemental scarcity, or complex and rigorous preparation process [49]. What's more, CDs do not have any heavy metals, and therefore, they are much safer than QDs [45]. Duan et al. had an exploration for the availability of this

new material and received a good result. They used two-color quantum dots as donors and novel amorphous carbon nanoparticles (CNPs) as acceptors. This method allows the simultaneous detection of the pathogens *Vibrio parahaemolyticus* and *Salmonella typhimurium* [50]. The detection limits of *V. parahaemolyticus* and *S. typhimurium* are as low as 25 and 35 CFU/mL.

Fiber-optic biosensor

The fiber-optic biosensor (TFOBS) is another common optical biosensor. It is a fiber-derived device which is used in the optical field to measure biological species (cells, proteins, DNA, and so on). Owing to the fact that most identifications of molecular reactions need fluorescent labeling, the fiber-optic biosensor can also be seen as a kind of fluorescence biosensor. However, its difference from other fluorescence biosensors is that the fiber-optic biosensor uses fiber to transmit light. Using tapered fiber, the excitation laser light is introduced at the proximal side. And after a series of total internal reflections, the light transmits along the fiber to the distal side and gets to the detection surface in the end [30, 51], as shown in Fig. 2. Its unique features such as free from electromagnetic interference and corrosion resistance make them have more superiorities than other detecting equipment. Moreover, the small size of the fiber makes the biosensor more portable. TFOBS also can use various optical transduction mechanisms such as changes in refractive index, absorption, fluorescence, and surface plasmon resonance to reflect the molecular reaction occurring in the sensitive film [52]. Because of these advantages, fiber-optic biosensors have been widely used to finish most detection of protein and DNA [53–55].

Conventional immunologic diagnosis technology (such as ELISA) has been a common method of virus antibody detection, but such approaches are often detained by poor sensitivity [56]. Fiber-optic sensors are made up for this shortcoming perfectly. Petrosova et al. developed an optical immunosensor which coupled optical fiber with specific antigen-antibody reaction for the detection of antibodies against the Ebola virus strains Zaire and Sudan. They used a newly photoactivatable electrogenerated polyfilm as the site of antibody immobilization and immune reaction. And then the film was deposited on the surface fiber optic which was modified with indium tin oxide (ITO). Through a coupled chemiluminescent reaction, antibodies in animal and human sera can be detected. The modification of the reaction surface can reduce the nonspecific reaction and improve the sensitivity. So, the titer of subtypes Zaire and Sudan using this method can reach 1:960,000 and 1:1,000,000, which has a clear superiority than conventional ELISAs [57]. A chemiluminescent optical fiber immunosensor, reported by Atias's team, for the detection of anti-dengue virus IgM in human serum also supported the advantages of fiber-optic biosensors. The new diagnostic tool has sensitivity and specificity of 98.1 and 87.0%. By comparing other ELISAs, this assay was found having a lower detection limit which is 10 times lower than the chemiluminescent ELISA and 100 times lower than the colorimetric ELISA [58].

In terms of the detection of bacteria, Janssen et al. may have a more practical exploration in clinical studies. They used co-immobilization of glycol (PEG) diluents or "back filling" of the DNA sensing layer to reduce nonspecific protein adsorption, which can almost completely prevent specific DNA hybridization in a fiber-optic SPR-based melting assay [59]. These approaches used in this paper pave a good way for further researches on how to apply new technologies to

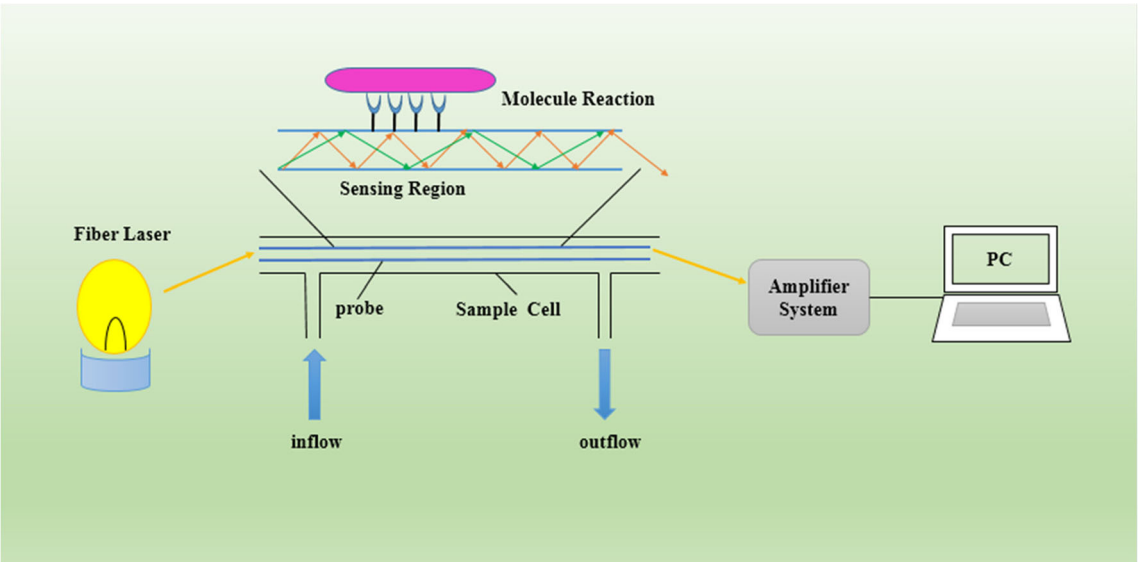

Fig. 2 The schematic representation of fiber-optic biosensors

practical clinical pathogen diagnostics when the samples are so complex.

However, like most fluorescence biosensors, fiber-optic sensors have a fatal shortcoming because the lifetime of fluorescent molecules is limited [60]. So, it needs a detection system that has a faster response time to get an accurate interpretation. In the routine work of clinical microbiology detection, this disadvantage will retard the rechecking of controversial results. In terms of this point, the traditional culture and ELISA are more practical. In addition, the choice of fluorescent labels should receive much concern. Although there are increasing new fluorescence materials with the advantages of broad excitation spectra and narrow emission spectra, which allows higher specificity and stronger signal, the high cost is still a question that should be taken into consideration in the practical work.

Surface plasmon resonance biosensor

Surface plasmon resonance is a physical optics phenomenon. When light occurs as a total internal reflection on a thin metal film which closes to a glass prism, a small portion of the incident light energy without reflection will penetrate into the metal film. This small portion of light is usually called the evanescent wave, which can trigger the free electrons in the metal to generate surface plasmon. With an appropriate angle of incidence or wavelength, the surface plasmon and evanescent wave can have an equal frequency, which is called resonance. Then, the incident light will be absorbed and the energy of the reflected light drops sharply. Later, a resonance peak, which means the lowest value in the reflected spectrum, will appear. The position of the resonance peak can vary with the different conditions of the thin metal film. So, the molecular reaction can be quantified according to this principle [61]. The visual description of the principle is shown in Fig. 3. In 1990, the company of Biacore AB developed the first commercial SPR biosensor by combining SPR technology with biosensors in the world [62]. Then, the detection of *E. coli* O157:H7 based on SPR biosensor by Fratamico et al. was reported in 1998 [63]. Later, the detection limit of *E. coli* had been demonstrated between 10^6 and 10^7 CFU/mL [64]. With the development of SPR, this technology has been widely developed in agriculture [65], food [66, 67], environment monitoring [68–71], and disease prevention and control [72–74].

In order to have early diagnosis and appropriate treatment of hemorrhagic colitis, Tawil et al. developed and described a kind of SPR biosensor for the detection of *E. coli* O157:H7 and methicillin-resistant *Staphylococcus aureus*. They used T4 bacteriophages to detect *E. coli* while a specific phage was used to detect MRSA. The system permits specific and rapid detection of pathogens, for the concentrations of 10^3 colony forming units/mL, in less than 20 min [75]. Chen et al. made some improvements in immobilization. The mixed self-assembled monolayer (SAM) was used in SPR, which can greatly enhance the immobilization ability of the metal surface. And this method was used to simultaneously and qualitatively detect different HPV genotypes successfully [76].

It has been a very tricky problem all the time that some bacteria need strict cultivation conditions, which makes a bar for early detection and diagnosis. *Neisseria meningitidis* is one of these bacteria. Gurpreet et al. pointed toward a promising application based on SPR in the detection of fastidious bacteria. They used the RF sputtering technique to deposit the ZnO thin film on gold-coated glass prisms and the physical adsorption method to immobilize *Neisseria meningitidis* DNA on ZnO film. This configuration named Kretschmann had a sensitive response toward target DNA, and the limit of detection was as low as 502 ng/μL [77].

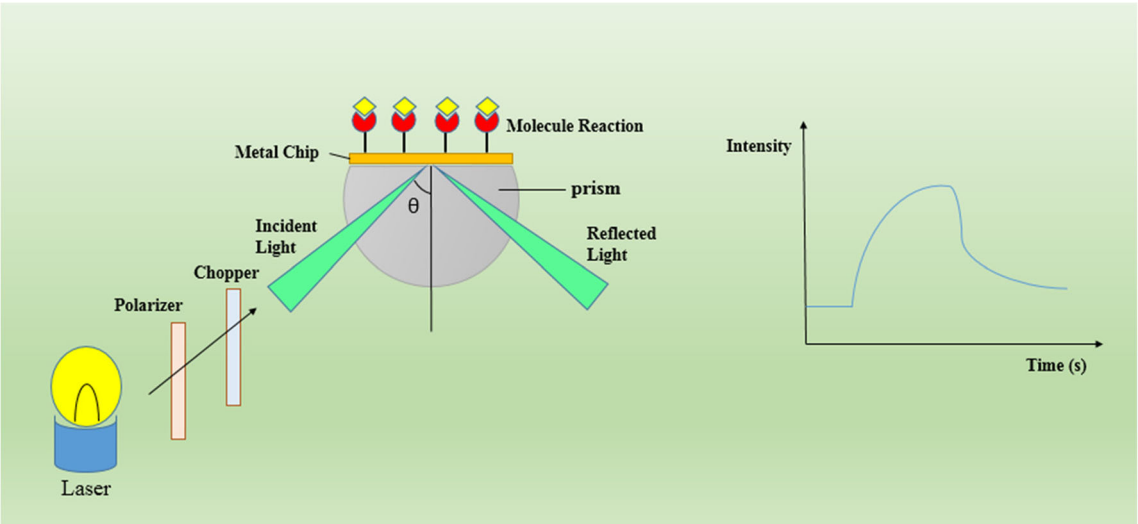

Fig. 3 The schematic representation of SPR biosensors

As a label-free assay, the results of SPR biosensors rely on the decline of reflected light caused by the molecular reaction on the metal film. With simple pretreatment, SPR biosensors have a shorter time to yield results compared with traditional immunological labeling ways. The high resolution of optical detection can distinguish the slight differences among different molecules and get different resonance peaks. So, SPR biosensors possess higher specificity than routine fluorescence-marked methods on the condition that a nonspecific reaction cannot be avoided. All the time, the big size of the SPR biosensor is still a problem which needs to be solved. But what is exciting is that there are already some explorations on the combination of SPR and fiber [78–80]. This new design compromises the merits of the small size of the fiber and being label-free of SPR. Even though there are few examples of fiber-optic surface plasmon resonance-based biosensor in microbiology test, the innovation integration between the two technologies is a good start in the development of a detection method.

Electrochemical biosensors

With the advantages of simple structure, high sensitivity, low cost, and rapid response, electrochemical biosensors express characters of biosensors perfectly and are considered as the most promising technology which is appropriate for microorganisms being tested in real time. Electrochemical sensors usually consist of a working electrode, a counter electrode, and a reference electrode. The reaction on the electrode surface is collected and converted to electrochemical signals which are proportional to analyte concentration present in the sample [81], as shown in Fig. 4. And based on the observed parameters such as current, impedance, conductance, and potential, electrochemical biosensors can be classified into amperometric, impedimetric, conductometric, and potentiometric [51]. There are some rough statistics shown in Figs. 5 and 6 according to the papers we collected. They reveal the situations of using electrochemical subclasses and the application in various types of bacteria.

Amperometric biosensor

Compared to other electrochemical sensors, amperometric biosensors have higher sensitivity and earlier application. The principle of the amperometric biosensor is to convert molecular reactions on the surface of electrodes into a detectable current signal and perform further analysis [82]. In the 1960s, Clark and Lyons developed a glucose enzyme electrode for the detection of glucose. This is the simplest amperometric biosensor which created a precedent in biosensor research [83]. After that, the enzyme electrode, a kind of biosensor element that combines enzyme-sensitive membrane with electrodes, has been developed rapidly and has been used widely with its high sensitivity in amperometric sensors. A few years ago, many scholars had explored amperometric biosensors based on enzyme electrode deeply and applied it to the detection of the microbial field [84, 85].

Utilizing the characteristic of bismuth nanofilm having a sensitive response for 4-nitrophenol which is converted by β-d-glucuronidase that is released by *Escherichia coli*, Zhang et al. developed a new amperometric sensor for the rapid detection of *E. coli* with the detection limit of 100 CFU/mL [86]. Like the current clinical identification methods, the principle of this testing is an enzyme-substrate reaction. The specific β-

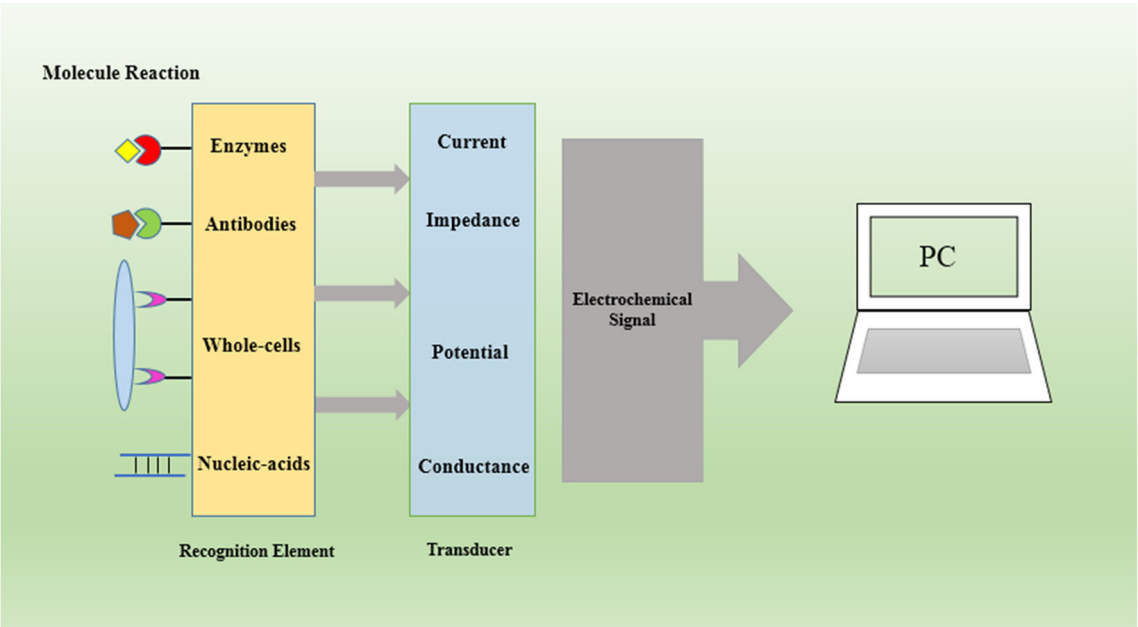

Fig. 4 The schematic representation of electrochemical biosensors

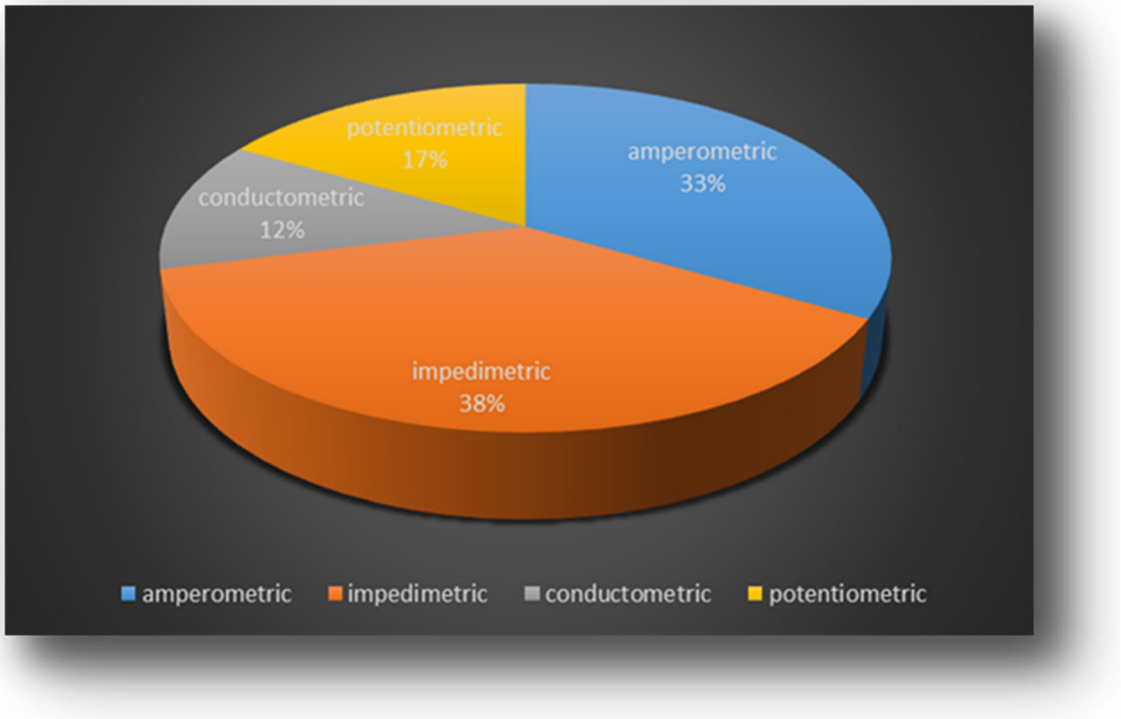

Fig. 5 The proportion of electrochemical subclasses used in pathogen detection

d-glucuronidase released by *E. coli* can hydrolyze the substrate named 4-nitrophenyl β-d-glucuronide (PNPG) in the culture medium. The new product, 4-nitrophenol, is an electroactive substance which can be used for electrochemical instruments to quantify *E. coli*. But the difference is that an amperometric biosensor has faster reaction time (3 h) than traditional bacteria identification (24 h). In addition, this biosensor allows enrichment and identification in the same

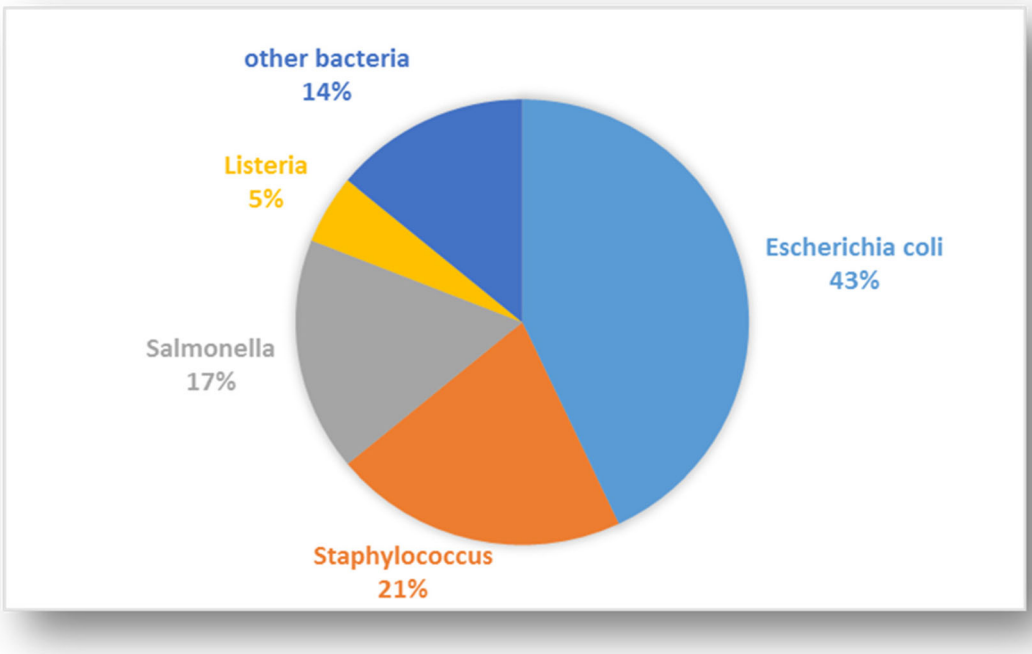

Fig. 6 The distribution of bacteria detected by electrochemical biosensors

culture medium, which omitted the procedure of isolation. From the point of view of cost saving, amperometric biosensors have an obvious advantage than traditional culture.

Certainly, as a detection equipment, the purchasing cost of amperometric biosensors is far beyond the cost of a simple culture medium. Many scholars have been working on how to reduce the costs to make amperometric biosensors more applicable. Screen printing is a great favorite technique used in amperometric sensors for many years with its low cost. Pure screen-printed electrodes discovered have been applied to detect pathogenic bacteria [87, 88]. Besides, compared with DNA hybridization, the low specificity of the enzyme-substrate reaction is the biggest shortcoming of amperometric biosensors. Now, the simple enzyme-electrode amperometric sensors have become rarer, while many new technologies are combining with each other in order to improve specificity. The introduction of DNA hybridization makes the situation better than before [88–90]. And the application of magnetic bead also gives more possibilities to clinical application. Campuzano et al. reported disposable amperometric magneto immunosensors, which are based on functionalized magnetic beads and gold screen-printed electrodes, to detect *Streptococcus pneumoniae* quantitatively [91].

Impedance biosensor

Impedance biosensors can detect and/or quantify analyte by recording the change of impedance value caused by the biomolecule reaction on the electrode surface. Electrochemical impedance spectrum (EIS), which was first discovered in an experiment by the famous Holland physical chemist Sluyters in the early 1960s, plays an important role in impedance biosensor development [92, 93]. The basic principle of EIS is to add small amplitude sine wave perturbations to an electrochemical system in a wide frequency range. And then, the detector can measure the responding signals as a function of frequencies [94]. When bacteria attached to the reaction surface of the electrodes, the current will be inhibited and the impedance will increase. So, in terms of the detection of bacteria, the EIS is a reliable method [95]. By immobilizing specific antibody to a screen-printed electrode via a cysteamine monolayer activated with glutaraldehyde, Farka et al. developed a label-free immunosensor for the rapid detection of *Salmonella typhimurium*. The immunosensor allowed the detection of 1×10^3 CFU/mL in 20 min with negligible interference from other bacteria [96].

Interdigital array microelectrode (IDAM) is a reformative electrode which plays an important role in impedance biosensor, which is the difference between the impedance biosensor and other biosensors. IDAM consists of a pair of microstrip electrode arrays. Each array is composed of a plurality of finger electrodes with a width and spacing of micrometers in parallel. The electrodes are meshed with each other to form an interdigitated electrode array, as shown in Fig. 7. Because of the micrometer size, on the one hand, the IDAM has higher sensitivity and shorter detection time than the common electrode. On the other hand, the volume of samples will be reduced greatly, which means that there are few interference backgrounds in the results. Dastider et al. had tried to detect *Escherichia coli* O157:H7 using IDAM-based impedance biosensor. They applied dielectrophoresis to impedance spectroscopy to develop an interdigitated electrode array (IDEA) impedance biosensor, which improves the detection capability for *Escherichia coli* O157:H7. The dose response was

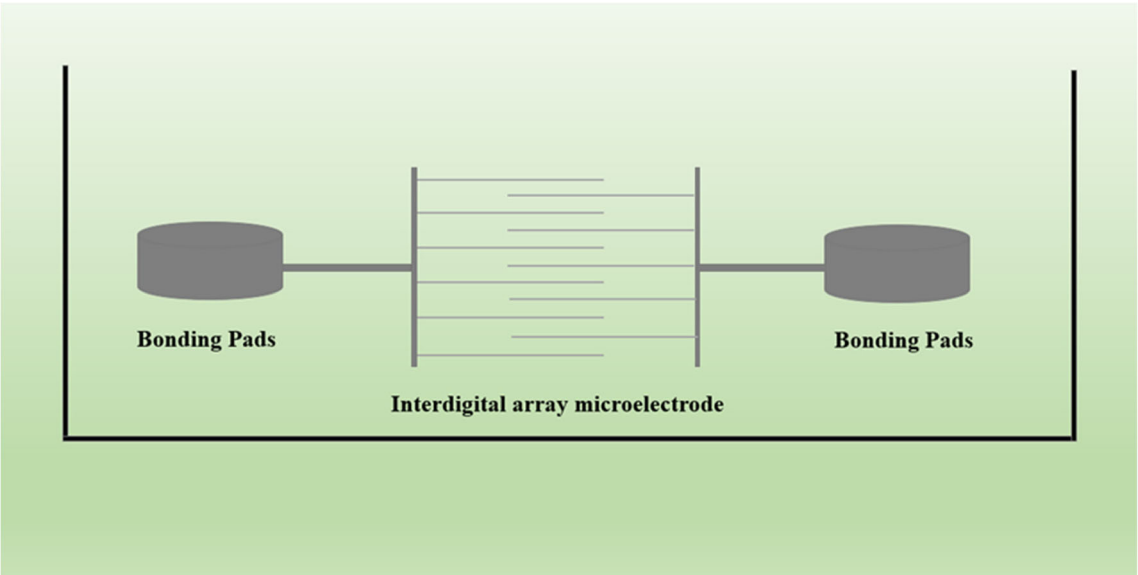

Fig. 7 The schematic representation of IDAM

between 3×10^2 and 3×10^6 CFU/mL. This is 10-fold better than the detection limit previously reported [97].

However, there are still some questions of IDAM that have not been solved. Firstly, the detection of IDAM usually uses the principle of immunology, which needs to immobilize the antigen or antibody on the electrode. The trouble is that the immobilization technology of IDAM is still not very mature, which leads to a low rate of capture [98]. Secondly, the antigen or antibody immobilized on the electrode is not easy to clean. This can cause few frequencies of repeated tests and higher costs.

Potentiometric biosensor

Conventional potentiometric biosensors are composed of an ion-selective electrode (pH, ammonium, chloride, and so on) or a gas-sensing electrode (pCO_2 and pNH_3) coated with an immobilized microbe layer [99]. Using a high impedance voltmeter, potentiometric biosensors usually measure electrical potential difference or electromotive force (EMF) between two electrodes when near zero current [41]. The changes of pH, ionic, or redox at the surface can be converted to corresponding electrical signals by a transformer proportional. Thus, potentiometric biosensors are often used to detect metal ions [100], toxin [101, 102], carbohydrates [103], and so on.

However, though there are some examples of potentiometric biosensors applied to the medical field, few reports can be found in the literature about the detection of pathogenic bacteria based on simple constructed potentiometric biosensors. So, many strategies have emerged for further development of potentiometric biosensors. The modification of electrode is one of the most common methods [104, 105], and the application of aptamer is a breakthrough among these ways.

By comparison, Zelada-Guillén et al. demonstrate that easy-to-build aptamer-based SWCNT potentiometric sensors are highly selective than the conventional and can be used to detect living microorganisms in an assay close to real time [106]. After that, they detected *Escherichia coli* and *Staphylococcus* successfully [107]. Similarly, Hernández et al. used two different strategies (covalent and noncovalent) to attach the aptamer to the graphene oxide (GO) and reduced graphene oxide (RGO) layer for the detection of living *Staphylococcus aureus* [108].

It is known that different phases of pathogen growth are very important for infection as the bacteria in clinical specimens of different phases (latency, active stage, quiescent stage, incipient stage or advanced stage, etc.) may determine different treatments. But many methods used in clinics only have the capacity of identifying the pathogen. For this problem, Zeladaguillén et al. seemed to open a new view for us. Eleven ssDNA sequences from different families of *Staphylococcus aureus* were selected by the SELEX procedure. By further assay, five high affinity and specific aptamers that can recognize different sites were screened and composed. This potentiometric biosensor has not only extraordinary selectivity for *S. aureus* but also can identify the different phases of the pathogen through different positive results composed of five aptamers [107].

Conductometric biosensor

The conductometric biosensor is an analytical device that can interpret specific biological recognition reaction as electrical conductance. Compared with the other types of biosensor transducers, conductometric biosensors were produced through inexpensive thin film standard technology and there is no reference electrode needed [109]. So, it is not difficult to find conductometric biosensors, especially gas sensors [110–113], being applied in many fields [114–117]. However, conductometric biosensors have a notable shortcoming in that there is a significant background conductivity when analyzing liquid samples, which caused low selectivity [109]. It may be one of the reasons why conductometric biosensors rarely have applications in the field of clinical microbiological detection. However, there are still some examples which can offer some references for us.

Okafor et al. fabricated a polyaniline-based conductometric biosensor for the detection of serum antibody (IgG) against the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) which causes Johne's disease (an important gastrointestinal disease) [118]. Then, this team used a capture membrane with limited variability in the immunomigration channel and an optimal concentration of the secondary anti-bovine antibody to further optimize conductometric biosensor based on MAP-specific antibodies. The result showed that it has a moderate agreement with the result of the commercially available antibody detection kit of ELISA [119]. Ichi et al. used addressable magnetic nanoparticles coupled with anti-LPS antibodies to develop conductometric immunosensors for the generic, rapid, and sensitive detection of Gram-negative bacteria. This approach can not only detect *Escherichia coli* or *Serratia marcescens* successfully but also allow the direct detection of 10–10^3 CFU/mL of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains that were undetectable using standard immunoblot methods [120].

Piezoelectric biosensors

Quartz crystal microbalances biosensor

Quartz crystal microbalances (QCM), which are highly sensitive to the change of mass on the surface of the quartz crystal, have been considered as a representative device among the piezoelectric biosensors [121]. When the mass increases due

to the interactions between molecules, the frequency of oscillation of the crystal will decrease. If the voltage applied to the quartz crystal causes it to oscillate at a specific frequency, the change in mass will directly relate to the change in frequency of the oscillating crystal [122]. We can have a clear recognition of the principle of QCM through Fig. 8. In 1959, Sauerbrey discovered the relation between quartz oscillation frequency and change in surface mass [123]. And then, with constant explorations by researchers around the world, the QCM technique has obtained great progress and had many applications in microbiological detection.

Like the SPR biosensor, the quartz crystal microbalance is also a label-free technology. So, the simple pretreatment and subsequent automatic detection are two advantages compared with some labeling methods. And based on the change of resonant frequency, the QCM can have an extraordinary sensitivity which can touch the mass change of subnanogram. This is also the most notable feature of this biosensor.

Poitras and Tufenkji had an early try for the detection of *E. coli* O157:H7 using the QCM biosensor and received a satisfactory selectivity [124]. Later, Guo et al. made further improvement of the QCM detection system to achieve enrichment and detection at the same time. They immobilized the specific captured antibodies onto the QCM chip. And then, the broth containing *E. coli* O157:H7 was circulating in the circulating flow QCM system for 18 h. The negative controls of *Listeria monocytogenes* and *Salmonella typhimurium* were used to prove the specificity of this biosensor. Finally, they got the detection limit of 0–1 log CFU/mL or g [125]. Actually, this real-time monitoring method is similar to blood culture being used in clinical studies at the present. But the traditional blood culture does not have a fast response time.

The descriptions above were applied for detection in food. However, we know that there are so many components in clinical samples than food samples. This can cause high background and nonspecific reactions in the detection. And this is also a question that cannot be ignored in label-free methods. How to reduce the nonspecific reactions under a label-free circumstance is still an obstacle to the development of the method's clinical application. Ozalp et al. seem to give a good inspiration for this question. They introduced the magnetic bead separation system to the QCM sensor for the specific detection of *Salmonella*. This new technology improved not only the speed of enrichment but also the effect of purification. So, the system they used received a detection limit of 100 CFU/mL less than 10 min [126].

Atomic force microscopy biosensor

Atomic force microscopy (AFM) is an excellent scanning probe microscopy technique which can analyze samples of a few nanometers to a few micrometers by moved raster scanning with a sharp tip (about 10 nm) [127], shown as Fig. 9. Due to escaping the limitation of diffraction, AFM has a high-resolution imaging [128]. With its advantages such as not needing pretreatment, fixation, or labeling, this technology is now the focus of interest and has been used as an effective tool for structural determination, identification, and characterization of the cell wall structure of many pathogens [129].

There were many successful applications of *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Bacillus subtilis*, *Staphylococcus aureus*, *Lactococcus lactis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and so on [130–134]. However, in view of the remarkable feature of ultra-low spatial resolution which allows the study of a single agent of infection viral particles, AFM has more applications in the detection of the virus. Yuri et al. developed a method for

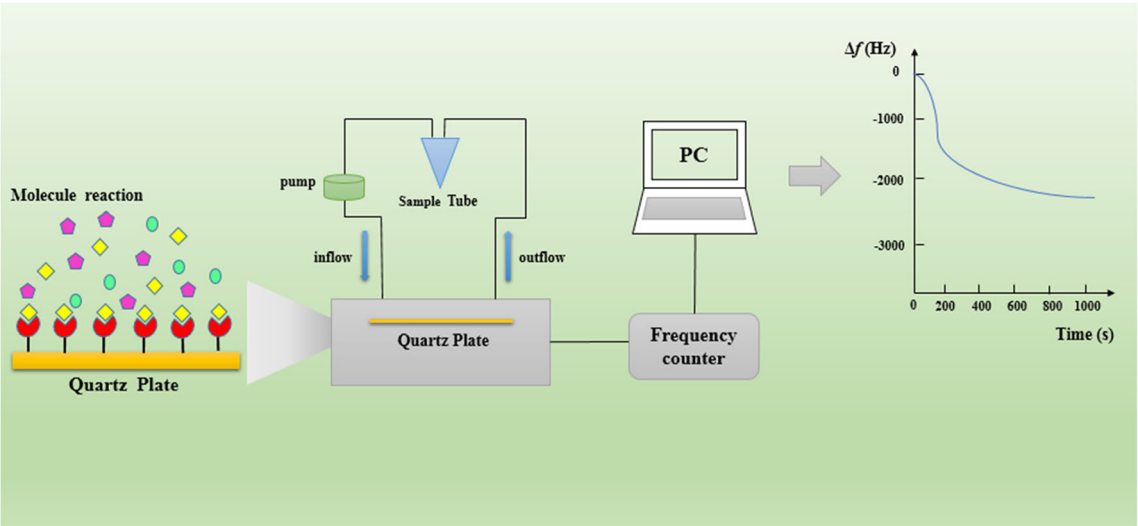

Fig. 8 The schematic representation of QCM

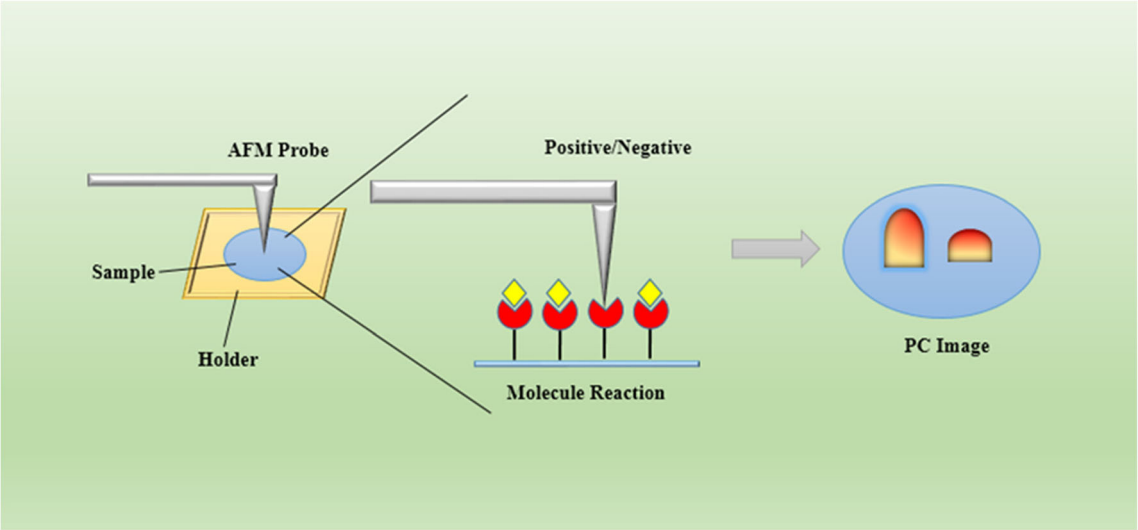

Fig. 9 The schematic representation of AFM

the detection and identification of core antigen of hepatitis C virus (HCV CoreAg) in the serum based on the combination of reversible biospecific AFM fishing and mass spectrometry (MS) [135]. Ivanov et al. fabricated stable bioactive arrays of human rhinovirus particles serotype 2 based on native-protein nanolithography (NPNL). This system allows the detection of the virus without prelabeling and preamplification under physiologic conditions, which were suggested to detect single viral particles in a variety of clinically relevant samples [128]. Bocklitz et al. detected five different virus species, namely Varicella-zoster virus, Porcine tescho virus, Tobacco mosaic virus, Coliphage M13, and Enterobacteria phage PsP3, using AFM. Automatic discrimination quantified the morphology of the virions and the accuracy of this classification model was 96.8% [136].

In fact, apart from the intuitionistic parameters such as length, height, and diameter, AFM can also distinguish viruses through other characteristics like local frictional and adhesion forces, elasticity, and viscosity [137]. It makes AFM to have higher accuracy in virus detection. However, the expensive cost of equipment hinders this technology from clinical promotion. In terms of this point, virus detection by PCR still has an unshakable position in clinical practice. In addition, the interpretation of an image needs professionals. It also has a shortcoming compared with PCR.

Comparison between existing clinical detection methods and biosensors

The approaches of pathogen detection at the present time include traditional culture, spectrophotometry, chromatography, immunology-based assays (latex agglutination tests, immunodiffusion, immunochromatography, and ELISA) [138], nucleic acid-based assays (PCR and gene chip), and proteomic assays such as mass spectrometry. Culture and colony counting, considered as the most reliable and accurate techniques by official agencies [30], is one of the oldest detection methods [139]. As a result of its low cost and simple operation, the traditional culture, often combined with drug sensitivity analysis, is still in use for bacterial detection up to now. But its long inspection cycle [5] and high risk of contamination [31] are two of its outstanding disadvantages. In addition, the discrimination of colony morphology needs experienced laboratory personnel. It means that there are some subjective factors when judged, which may cause a faulty diagnosis.

For the aforementioned problems, mass spectrometry can fill the gap to an extent. Relying on a rich mass spectra database which may contain thousands of species of bacteria and fungi, mass spectrometry allows identifying specific intrinsic marker proteins of pathogen directly from colonies grown on culture plates in a few minutes. So, it can avoid subjective judgment from laboratory personnel effectively [140–142]. But the equipment of this technology is very expensive that many small hospitals cannot afford, which limits its development in the detection of the pathogen.

ELISA is the most popular immunology-based assay based on the combination of the specific antigen-antibody reaction and the efficient label technique of enzyme. In recent years, more and more commercialized kits have been applied for rapid clinical diagnosis. So, ELISA is the best qualitative and semiquantitative choice in in situ real-time monitoring. However, immunology-based assays have some inevitable shortcomings such as high production cost of monoclonal antibodies, low sensitivity, and cross-reactivity. Besides,

Table 2 The comparison of several detection methods

Methods	Advantages	Disadvantages	References
Traditional culture	Authoritative, reliable, low cost, simple	Time-consuming, the requirement of skilled workers, subjective judgment	[5, 6, 31, 141]
ELISA	Rapid, real-time, simple, commercialization	Low sensitivity, cross-reactivity, expensive monoclonal Abs, false positive, false negative	[7]
PCR	Rapid, sensitive, specific	Complex pretreatment steps, failing to distinguish between viable and nonviable cells, sophisticated instruments	[27, 45]
Electrochemical biosensors	Rapid, sensitive, label free, low cost	Low stability and repeatability, low coping ability for complex clinical samples	[78, 109, 110]
Optical biosensors	Rapid, sensitive, specific	Sophisticated instruments, complex pretreatment steps	[41]
Piezoelectric biosensors	Rapid, highly sensitive, specific, label free	Rely on sample preparation, complex pretreatment steps	[122, 131]

ELISA is vulnerable to pollution, which causes a high incidence of false positive and false negative [7].

PCR is a detection technology established on amplification of the target sequence. As a widely used nucleic acid-based assay, PCR has many types including real-time PCR, multiplex PCR, nested PCR, reverse transcriptase PCR as well as digital PCR [143]. And now, the most common one used in clinical works is real-time fluorescent quantitative PCR [144]. There have been so many clinical detection markers (especially in virus detection) which are based on this method that are being developed due to the advantages of rapidity, high specificity, sensitivity, accuracy as well as capacity to detect small amounts of target. Nevertheless, sophisticated instruments and expensive commercial reagents make PCR unsuitable for point-of-care detection [45]. Moreover, the detection of nucleic acid requires the lysis of target cells; in other words, PCR cannot distinguish viable and nonviable cells and there is a series of pretreatment steps before detection. It requires skilled workers to be very careful to prevent not only contamination that can cause false positive during extraction but also nucleic acid degradation that can lead to false negative [27].

For the above problems of existing pathogen detection methods, biosensors seem to have shown clear superiorities to overcome these limitations. Firstly, faster detection time is the most prominent advantage of most biosensors compared with existing detection methods. This is also the problem that needs to be solved predominantly in clinical work. So, from the view of fast response, the biosensor is a technology which is worth developing in depth. Secondly, some biosensors, such as optical biosensors and piezoelectric biosensors, also have high sensitivity. It means that the sample capacity needed tends to be small. When it comes to the detection of samples consisting of small children and critical patients, this advantage will be particularly important. Last but not least, biosensors can provide higher specificity than current immunological methods (such as ELISA). Besides, the accurate, real-time, label-free, low-cost, and reproducible detection platforms are also some reasons why biosensors set off a new wave of substitution. The specific comparison is shown in Table 2. However, there still are some problems that have not been overcome among biosensors. Although real-time PCR and SPR are able to monitor samples in real time, the size of the SPR is bigger and the cost is higher. Although the costs of electrochemical biosensors are very low, its sensitivity and specificity need to be improved. From this point, the existing pathogen detection methods used in practical work have higher credibility. In addition, there are some other problems that need to be given more attention, including biomolecule immobilization on the electrode surface and low stability in electrochemical biosensors, low coping ability for complex clinical samples (blood, urine, feces, sputum, swabs, cerebrospinal liquid, saliva, etc.), and expensive devices in optical biosensors.

Conclusion

From the views of the literature we collected and the comparison shown above, biosensors have a great potential to be used for clinical microbiological detection though there are some deficiencies that need to be addressed. Here, we have neither negated existing methods nor admired biosensors overly. Our original intention is to allow people to reexamine the shortcomings of the existing methods and to learn more about the new detection methods which are possible to be popularized in clinical work. Now, it is clear that the ideal detection method must have higher sensitivity, specificity, and accuracy; lower cost; and smaller size. So, there is still a long way between the ideal detection method and existing methods. But we believe that microbial detection methods will have a bright future with the development of science and the progress of technology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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