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REVIEW ARTICLE

Advances in airborne microorganisms detection using biosensors: A critical review

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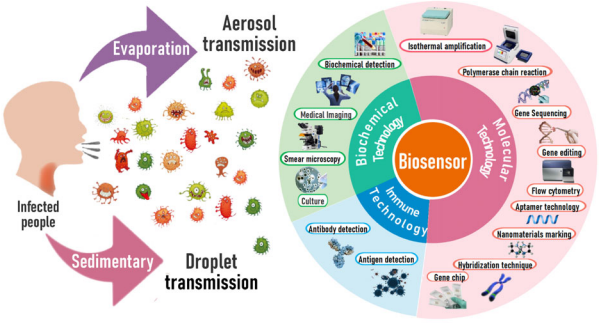
HIGHLIGHTS

- Airborne microorganism detection methods are summarized.
- Biosensors play an important role in detecting airborne microorganisms.
- The principle of biosensor detection of airborne microorganisms is introduced.
- The application and progress of biosensor in recent years is summarized.
- The future perspectives of biosensor are identified.

GRAPHIC ABSTRACT



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ABSTRACT

Humanity has been facing the threat of a variety of infectious diseases. Airborne microorganisms can cause airborne infectious diseases, which spread rapidly and extensively, causing huge losses to human society on a global scale. In recent years, the detection technology for airborne microorganisms has developed rapidly; it can be roughly divided into biochemical, immune, and molecular technologies. However, these technologies still have some shortcomings; they are time-consuming and have low sensitivity and poor stability. Most of them need to be used in the ideal environment of a laboratory, which limits their applications. A biosensor is a device that converts biological signals into detectable signals. As an interdisciplinary field, biosensors have successfully introduced a variety of technologies for bio-detection. Given their fast analysis speed, high sensitivity, good portability, strong specificity, and low cost, biosensors have been widely used in environmental monitoring, medical research, food and agricultural safety, military medicine and other fields. In recent years, the performance of biosensors has greatly improved, becoming a promising technology for airborne microorganism detection. This review introduces the detection principle of biosensors from the three aspects of component identification, energy conversion principle, and signal amplification. It also summarizes its research and application in airborne microorganism detection. The new progress and future development trend of the biosensor detection of airborne microorganisms are analyzed.

1 Introduction

1.1 Hazards of airborne infectious diseases and airborne microorganisms

The corona virus disease 2019 (COVID-19) is considered the largest global pandemic since the 20th century, and it

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has not yet been effectively controlled globally. Indeed, frequent outbreaks of major airborne infectious diseases have been recently reported, which have had a significant impact on human life and health and on the global economy and society. According to the official report of the World Health Organization, the major infectious diseases in recent years are summarized, as shown in Table 1. Every infectious disease is caused by specific microorganisms, including viruses, bacteria, fungi and parasites. Among them, microorganisms that spread through air are called airborne microorganisms (Després et al., 2012). Airborne microorganisms can be transmitted through human exhalation (Doremalen et al., 2020) and show strong survivability in air. Airborne infectious diseases can be spread from person to person through airborne microorganisms (Hoehl et al., 2020; Yu et al., 2020; Jiang et al., 2021). They spread rapidly and extensively (Setti et al., 2020), which can easily cause social panic. (Zheng et al., 2018; Wang et al., 2019a).

1.2 Necessity and challenge of airborne microorganism detection

Timely identification, monitoring, and investigation of airborne microorganism transmission in the human environment is particularly important to prevent the outbreak of airborne diseases in the population. At present, however, most of the test samples of airborne microorganisms come from clinical samples, which mainly include upper respiratory tract (nasopharyngeal swab and deep throat saliva), lower respiratory tract (alveolar lavage fluid and sputum), and body fluids (Cui and Zhou, 2020). The collection of different types of samples can affect microorganism detection. Clinical samples often have high detection efficiency and accuracy, but they require professional operation and bring discomfort to the test subjects. Most technologies require on-site sampling and further tests in the laboratory, with complex operation process and long detection time (Wang et al., 2019a). Correspondingly, the direct detection of air samples has received widespread attention in recent years, and air samples mainly include exhaled breath and aerosols (Razzini et al., 2020). On-site air sample detection features a short detection time, flexibility, and convenience. However, it is easily affected by environmental factors such as wind speed, temperature, light intensity, and air humidity. In addition, the content of airborne microorganisms in the environment is low, with a wide variety of species and large number of impurities, which makes on-site detection difficult.

1.3 Airborne microorganism detection methods

The detection methods for airborne microorganisms can be roughly summarized as biochemical, immune, and molecular technologies. After years of development, some detection methods have become mature and new technologies are emerging constantly. However, most of the existing technologies have outstanding performance in aspects of detection time, specificity, and sensitivity, while some limitations exist in other aspects, which are difficult to meet the requirements of airborne microorganism detection. Several common detection methods are compared in Table 2.

In recent years, in view of the advantages and disadvantages of different detection technologies, diversified technology combinations have emerged, greatly improving the detection capabilities of airborne microorganisms (Zheng et al., 2018). As an interdisciplinary field, biosensors have been extensively studied in recent years, Figure 1 summarizes technologies that have been successfully applied to biosensors or have the potential to be combined with biosensors. They have been widely used because of their short detection time, fast analysis speed, and flexible portability. As a routine laboratory microbial detection technology, biochemical technology is used in combination with biosensors for the preliminary treatment of samples (Peláez et al., 2020). On the basis of the specific

Table 1 Major incidents of airborne infectious diseases in recent years

Airborne diseases	Airborne microorganisms	Parasitifer	Duration	Impact
SARS	SARS-CoV	Bat	2002.11–2003.07	8069 confirmed cases and 774 deaths (as at July 2003)
H1N1 Flu	Influenza virus A	Birds and mammals	2009.04–2010.08	68474274 confirmed cases and 18449 deaths (as at August 2009)
MERS	MERS-CoV	Camel	2012.09–2018.09	2562 confirmed cases and 881 deaths (as at September 2020)
H7N9 avian influenza	AIV	Poultry	2013.03–2017.09	1564 confirmed cases and 609 deaths (as at October 2017)
COVID-19	SARS-CoV-2	Bat*	2019.12–	More than 107 million confirmed cases and 2.3 million deaths (as at February 2021)

Note: *the potential parasitifer.

Table 2 Comparison of detection methods for airborne microorganisms

Detection method	Advantage	Disadvantage	Reference
Culture	1. Relatively simple operation 2. Low cost, and less equipment investment 3. Used for strain typing and drug resistance detection	1. Large workload, and long detection time 2. Low sensitivity 3. Difficult to cultivate some microorganisms or require high biological safety	Hudu et al., 2016; Gupta and Kakkar, 2018
Medical imaging	1. Short detection time 2. fast analysis speed	1. Need professional equipment 2. Low specificity 3. Invasive 4. Not suitable for early-stage patients	Brenner and Hall, 2007; Seibel et al., 2020
Immune technology	1. Medium sensitivity, capable of determining small or limited amounts of enzymes in samples 2. Medium specificity, not easily affected by impurities 3. Medium detection time, suitable for large number of samples	1. Prone to "false positives" affecting the results 2. Many measurement steps and complicated operation 3. High measurement cost	Phunpae et al., 2014; Fronczek and Yoon, 2015; Mekonnen et al., 2020
Polymerase chain reaction	1. High sensitivity 2. High specificity, low sample purity requirements 3. Used for strain typing and drug resistance detection 4. Medium detection time	1. High measurement cost 2. Complex cyclic process, high technical requirements, and professional equipment 3. Unable to distinguish between living and dead microorganisms	Weile and knabbe, 2009; Paolucci et al., 2010; Eddabra and Ait Benhassou, 2018
Gene Sequencing	1. Good stability, and specificity 2. High detection accuracy	1. Large workload, and long detection time 2. High measurement cost	Schlaberg et al., 2017
Biosensor	1. High sensitivity, and high specificity 2. Short detection time, and fast analysis speed 3. Flexible and portable, suitable for on-site testing 4. Low cost	1. High sample purity requirements, weak anti-interference ability 2. Poor detection stability 3. Poor repeatability	Nidzworski et al., 2014; Cui and Zhou, 2020

Note: High sensitivity means that the lowest detection concentration is roughly less than 10 CFU/mL, medium sensitivity means that the lowest detection concentration is roughly between 10 and 1000 CFU/mL, and low sensitivity means that the lowest detection concentration is roughly higher than 1000 CFU/mL; High specificity means single base mismatches can be detected, medium specificity means that specific identification substances of microorganisms can be detected, low specificity means that different types of microorganisms can not be detected well.

combination of antibody and antigen, immune technology introduces biosensors to construct an immunosensor, which has been extensively used in the detection of airborne microorganisms (Shen et al., 2009; Mavrikou et al., 2020). Good results have been obtained for detecting SARS-CoV (Park et al., 2009), Influenza virus A (Nidzworski et al., 2014), AIV (Huang et al., 2016), SARS-CoV-2 (Seo et al., 2020), and other airborne microorganisms. Molecular technology, as a new technology, can improve sensors, and it is mainly used for the identification of components and signal amplification of sensors (Xu et al., 2016; Freije et al., 2019). Zhang et al. developed a nanosensor combined with RT-PCR amplification and achieved the rapid detection of dengue virus using a PNA probe binding on it (Zhang et al., 2010). As an interdisciplinary field, biosensors integrate the advantages of many technologies and have bright prospects in the detection of airborne microorganisms.

2 Biosensor detection principle

Biosensor is a special device that uses component identification as the biological sensing unit, converting biological signals into detectable signals using an appropriate energy conversion principle. It also uses appropriate methods to achieve signal amplification with high selectivity to the target object. Its basic composition is shown in Fig. 2.

2.1 Components identification

The detection of biosensors is realized by the specificity of component identification. According to the different component identification used, biosensors can be divided into two categories. The first type is the cell-based biosensor (Mavrikou et al., 2020), which has certain requirements on the state and activity of the cell. It monitors and analyzes the changes in metabolites during cell respiration (Xu et al., 2016). The second category is the biosensor based on the detection of microbial metabolites, including sensors based on aptamers (Wu et al., 2019), antibodies (Seo et al., 2020), and nucleic acids (Liu et al., 2018c). This type of biosensor has no special requirements for the survival state of microbial cells and is a commonly used for the component identification of biosensors. Gopinath et al. selected the 16 kDa heat shock protein of *Mycobacterium tuberculosis* (MTB) for component identification and coupled it to gold nanoparticles

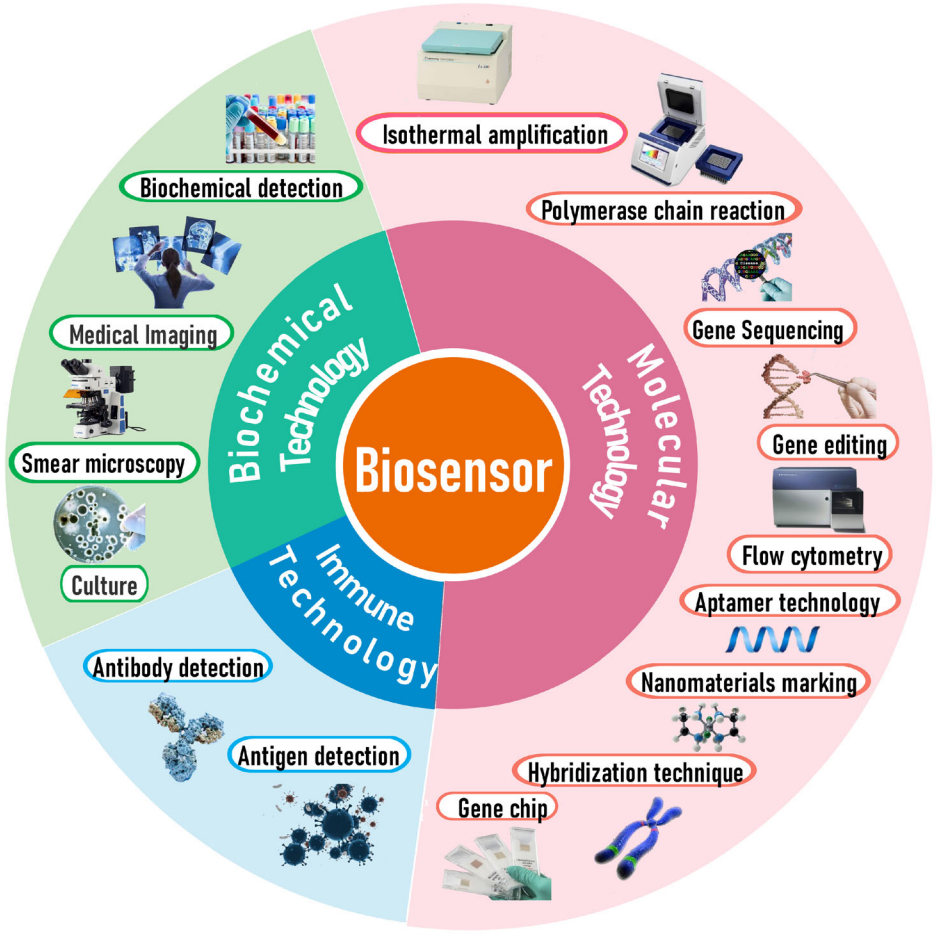

Fig. 1 Airborne microorganism detection methods based on biosensors.

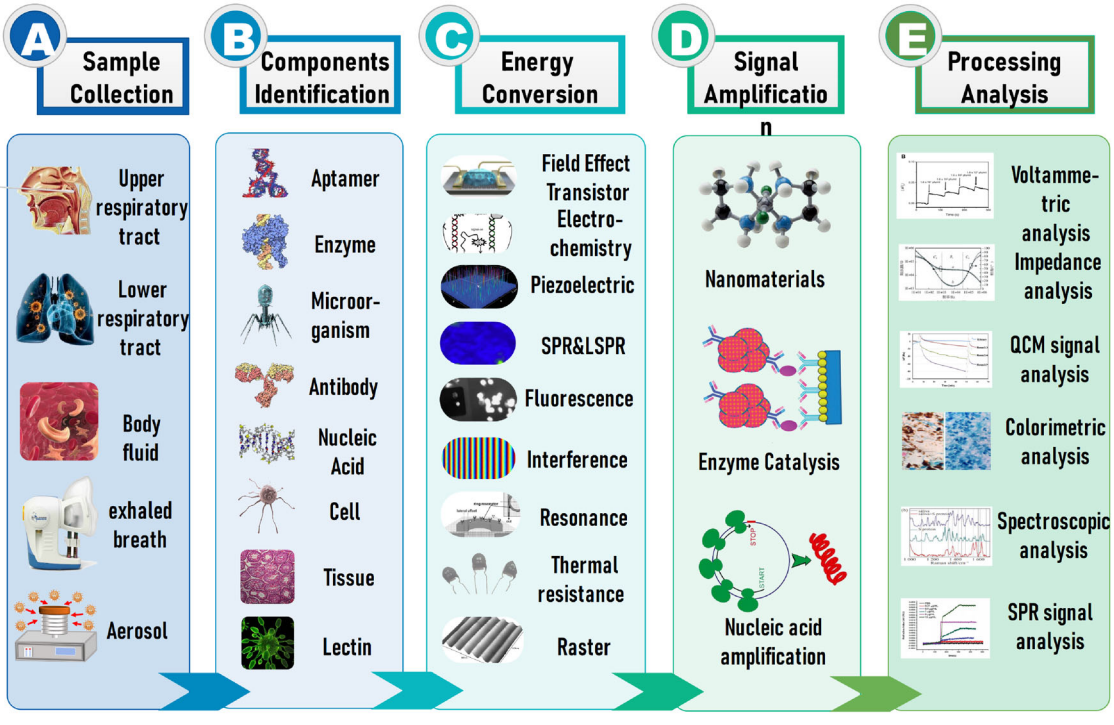

Fig. 2 Principle flow chart of airborne microorganisms detection using biosensors.

with a detection limit as low as 100 fM (Gopinath et al., 2016).

Aptamers are artificially synthesized short single-stranded DNA or RNA, which can develop high-affinity molecules to specifically recognize the desired target. Aptamers have many advantages compared with antibodies, such as short generation time, low manufacturing cost, high variability, good thermal stability and broad application (Zhang et al., 2019c). Kwon et al. used aptamer biosensors to directly detect avian influenza virus in clinical samples of chicken serum, with a detection limit of 5.9 pM (Kwon et al., 2020).

2.2 Principle of energy conversion

The biosensor uses the principle of appropriate energy conversion to convert identifiable biological signals into detectable electrical, optical, acoustic, or thermal signals. In recent years, electrical and optical biosensors have developed rapidly.

2.2.1 Electrical biosensor

Electrical biosensors are the most widely used and the earliest developed biosensors in the field (Cesewski and Johnson, 2020). This type of biosensor mainly uses electrical signals for detection, such as electrochemistry, field-effect transistor (FET), and piezoelectric sensors. The electrochemical biosensor uses the electrochemical signal generated by the biorecognition process on the electrode surface for detection. Depending on the signal type, electrochemical biosensors can be divided into three types of sensors: volt-ampere (Seo et al., 2020), impedance (Xu et al., 2016), and ampere (Bhattacharyya et al., 2016). The FET biosensor uses the biological recognition process to cause changes in the electronic characteristics of semiconductor channels for detection. The Piezoelectric sensor uses the biometric process to detect surface charges when piezoelectric materials are pressed. Mavrikou et al. used a new type of electrochemical sensor to detect the S1 spike protein expressed on the surface of the virus SARS-CoV-2. The results are provided within 3 min, and the detection limit is 1 fg/mL (Mavrikou et al., 2020). Seo et al. constructed a FET biosensor, detected the spike protein of SARS-CoV-2 at a concentration of 1 fg/mL, and successfully detected the culture medium (detection limit 16 PFU/mL) and clinical specimens (detection limit 2.42×10^2 copies/mL) of SARS-CoV-2 (Seo et al., 2020). Zhang developed a new type of piezoelectric sensor combined with aptamer technology to detect MTB with a limit of 100 CFU/mL (Zhang et al., 2019b).

The electrical biosensor is an important branch of biosensor, which fixes the bio-recognition element to the electrode surface, and converts the chemical or pressure signal generated by the combination of the target microorganism and the recognition element into a measurable electrical signal. It has been widely studied for its high sensitivity, fast response, high specificity, portability, and low cost (Cesewski and Johnson, 2020).

2.2.2 Optical biosensor

The optical biosensor is a biosensor that converts the signal of the detected object into a detectable optical signal. Optical biosensors mainly utilize the properties of light, such as fluorescence (Wu et al., 2019), surface plasma resonance (SPR) (Peláez et al., 2020) and colorimetric (Briceno et al., 2019) sensors. The fluorescence sensor uses the unique photophysical properties of fluorescent nanomaterials for labeling and detection of microorganisms (Zheng et al., 2019). The SPR biosensor uses the interaction of biomolecules to cause the instantaneous light signal change on the surface of the nano-layer metal film and then convert it into an electrical signal for detection. The colorimetric biosensor is based on the change in the number of target microorganisms in the sample, which can cause the color change of the detection solution to detect the target microorganisms (Wang et al., 2020a). Wei et al. developed a fluorescent immunological biosensor that uses fluorescent dyes to modify DNA probes, which can be used to detect H5N1 antibodies in serum samples (Wei et al., 2013). Peláez et al. used the SPR biosensor for the direct and label-free detection of the HspX recombinant antigen of MTB. Moreover, their process involved simple pretreatment of sputum specimens without any additional amplification steps, with a detection limit of 0.63 ng/mL (Peláez et al., 2020). Briceno et al. used a colorimetric biosensor to complete detection within 20 min and can reach the sensitivity level of the culture method (Briceno et al., 2019).

The optical biosensor is an emerging research direction of sensing in recent years. Through biological or chemical luminescence sensing, real-time detection of the object can be realized without modifying the label of the target. The optical biosensor belongs to the category of traditional physical sensing, with sensitive response and strong anti-interference ability.

2.3 Methods of signal amplification

In the detection of airborne microorganisms, the actual sample content is particularly low, so analysis and detection using conventional biological analysis methods are difficult to achieve. Certain methods are required to achieve signal amplification and improve the sensitivity of the sensor. Common signal amplification strategies include nanomaterial amplification technology (Xiao et al., 2020), enzyme catalysis amplification technology (Xie et al., 2019a), and nucleic acid-based amplification technology (Wu et al., 2019).

2.3.1 Nanomaterial amplification technology

The physical and chemical properties of nanomaterials are different from those of macroscopic substances, showing unique properties in optics, electricity, magnetism, biology, and other aspects. Nanomaterials have been extensively applied in the research of biosensors, greatly promoting the development of biosensors. Nano-functionalized materials are used as electroactive markers (Xiao et al., 2020), enrichment materials (Briceno et al., 2019), signal carriers (Gao et al., 2018), and catalysts (Xie et al., 2019a) for signal amplification.

In recent years, nanomaterials have been introduced into sensors to manufacture a large number of high-sensitivity sensing systems, which have excellent performance and long-term stability (Gao et al., 2018). Shen et al. combined sensors with silicon nanowires to develop a real-time bioaerosol sensing system, which can observe the conductance changes of H3N2 viruses in a few seconds (Shen et al., 2011). The hybrid structure of nanomaterials has attracted much attention due to its synergistic amplification effects. A platinum nanoparticle hybrid ZIF-8 composite biosensor can detect *Salmonella* at 11 CFU/mL (Wang et al., 2020a). The gold nanoparticle hybrid fullerene nanoparticle/nitrogen-doped graphene nanosheet biosensor can detect MTB at 3 fM (Bai et al., 2019).

2.3.2 Enzyme catalysis amplification technology

Enzymes are organic macromolecules with high selectivity and catalytic ability produced by living organisms. In biological analysis, enzymes are one of the most common signal markers. The catalytic effect of enzymes on substrates can transform the biochemical signals that are difficult to be detected into optical or electrical signals; meanwhile, the biological signals can be amplified to improve the detection sensitivity. Biological enzymes are subject to certain restrictions in application due to their high price and easy inactivation. In recent years, researchers have discovered that immobilizing enzymes on the surface of nanomaterials can not only increase the amount of enzyme immobilized, but also immobilize multiple enzymes at the same time, realizing the further amplification of the detection signal, and constructing nanocatalysts with mimic enzyme properties. Enzyme catalysis amplification technology has been used in the development of biosensors for its low cost, stable performance, and adjustable catalytic activity (Xie et al., 2019a).

2.3.3 Nucleic acid-based amplification technology

Nucleic acid-based amplification technology is an amplification method that can transform a small number of nucleic acid molecules into a large number of nucleic acid amplification products. It is mostly used to detect specific accounting fragments of airborne microorganisms. Nucleic acid amplification technology can be divided into non-isothermal amplification technology (polymerase chain reaction [PCR] technology) and isothermal amplification technology (such as chain replacement amplification technology and rolling circle amplification technology) depending on reaction conditions. Zhang developed a biosensor based on Exonuclease III (Exo III)-assisted target recovery, which can recognize hybrid double strands and selectively digest DNA capture probes. This process improved the sensor's sensitivity, and it can detect 20 CFU/mL MTB (Zhang et al. 2019b). Liu et al. improved the silicon photonic microcircle sensor using recombinase polymerase amplification technology, which increased the detection sensitivity of the sensor by three times (Liu et al., 2018c).

3 Application of sensors to detect airborne microorganisms

Biosensors have made considerable progress in the detection of airborne microorganisms. Tables 3, 4, and 5 summarize the applications of biosensors in the detection of airborne viruses, bacteria, and other microorganisms, respectively, and present the sensor types, sample types, detection concentration range, detection limit, response time and detection target for the detection of different microorganisms. Figure 3 summarizes the response time and detection limit of several common sensors for the detection of specific substances of airborne microorganisms. Thus, the response time of the sensor is mostly concentrated in the minute level, and the detection limit for specific substances can be as low as "zM" (10^{-21} mol/L). Compared with other airborne microorganisms, the virus has a lower detection limit and a shorter response time. Electrochemical sensors have been extensively used, with detection limits spanning multiple orders of magnitude of dynamic range, and can quickly detect low-concentration microorganisms.

4 Optimization of biosensor performance

In recent years, some new technologies have been used to optimize biosensors to detect airborne microorganisms, and the performance and efficiency of the biosensors have improved. For example, air sampling technology is used to solve of low content of microorganisms to be tested in environmental aerosol samples and improve the sensitivity of the biosensor. Purification and separation technology can improve the anti-infection ability of biosensors and solve the problems of excessive impurities in environmental aerosol samples. Microfluidic technology can

Table 3 Application of biosensor in airborne virus detection

Virus	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
SARS-CoV-2	FET biosensor	Culture medium; Nasopharyngeal swabs	Protein: 1 fg/mL – 100 pg/mL; SARS-CoV-2 in culture medium: 1.6×10^1 – 1.6×10^4 PFU/mL; Clinical samples: 1×10^1– 1×10^5 copies/mL	Protein: 1 fg/mL; SARS-CoV-2 in culture medium: 1.6×10^1 PFU/mL; Clinical samples: 2.42×10^2 copies/mL	50 s	SARS-CoV-2 antigen protein, cultured SARS-CoV-2, or SARS-CoV-2 from clinical samples	Seo et al., 2020
	Electrochemical biosensor	Cell-containing medium	10 fg/mL – 1 μg/mL	1 fg/mL	3 min	SARS-CoV-2 S1 spike protein	Mavrikou et al., 2020
	SPR biosensors	Oligonucleotide	0.1 pM – 1 μM	0.22±0.08 pM	–	RNA-dependent RNA polymerase-COVID sequences	Qiu et al., 2020
SARS-CoV	SPR biosensor	Rabbit anti- SARS coronaviral surface antigen (SCVme)	200 ng/mL – 100 μg/mL	200 ng/mL	10 min	Anti-SCVme	Park et al., 2009
	Nanowire FET biosensor	Nucleocapsid (N) protein	0.6 – 10 nM	0.6 nM	10 min	N protein	Ishikawa et al., 2009
H1N1 Influenza virus	Paired surface plasma waves biosensor	Throat swab	18 – 1.8×10^6 PFU/mL	30 PFU/mL	20 min	Swine-origin influenza virus	Su et al., 2012
	FET biosensor	H1N1 HA	50 aM – 5 nM	50 aM	–	HA	Hideshima et al., 2013
	Electrochemical immunesensor	Throat swab	10 – 100 pg/mL	20 pg/mL (80 – 100 virions/μL)	30 min	Structural protein in the virion	Nidzworski et al., 2014
	SPR biosensor	Recombinant influenza virus A	10 pg/mL – 10 μg/mL	50.5 pg/mL	–	Anti-hemagglutinin (HA)	Ahmed et al., 2017
	Electrochemical biosensor	Nasal swab	10^3 – 10^8 PFU/sample	10^2 PFU/sample	15 min	H1N1 influenza virus	Cui et al., 2017
	Electrochemical impedance aptasensor	Inactivated H1N1 viruses	9 – 900 ng/L	0.9 pg/uL	30 min	Inactivated influenza A virus subtype H1N1	Bai et al., 2018
	Electrochemical impedance sensor	Influenza virus DNA	1 pM – 10 nM	8.4 pM	–	Influenza virus DNA	Lee et al., 2018
	Electrochemical biosensor	Mini-HA protein and H1N1 viruses	0 – 10^6 PFU/mL	3.7 PFU/mL	30 min	Mini-HA protein and H1N1 viruses	Bhardwaj et al., 2019
H7N9 Influenza virus	Upconversion luminescence resonance energy Biosensor	H7 oligonucleotide	1 pM – 10 nM	7 pM	2 h	H7 oligonucleotide	Ye et al., 2014
	Electrochemical DNA biosensor	Throat swab	1 pM – 100 nM	100 fM	100 s	HA gene sequence	Dong et al., 2015
	Electrochemical immunesensor	Inactivated H7N9 avian influenza virus (AIV)	0.01 – 20 ng/mL	6.8 pg/mL	–	H7N9 AIV	Wu et al., 2015
	Electrochemical biosensor	H7N9 virus DNA	50 fM – 100 pM	9.4 fM	150 min	H7N9 virus DNA	Yu et al., 2015
	Electrochemical immunesensor	AIV H7	1.6 pg/mL – 16 ng/mL	1.6 pg/mL	30 min	AIV H7	Huang et al., 2016

(*Continued*)

Virus	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
	Electrochemical immunesensor	AIV H7	1 – 25 ng/mL	0.43 ng/mL	20 min	AIV H7	Tian et al., 2017
	SPR biosensor	H7N9 virus mixed with nasal mucosa	2.3×10^2 – 2.3×10^5 copies/mL	402 copies/mL	10 min	H7N9 virus	Chang et al., 2018
H5N1 Influenza virus	SPR biosensor	Poultry swab	0.128 – 12.8 HAU/50 μL	0.128 HAU/50 μL	1.5 h	H5N1 AIV	Bai et al., 2012
	Electrochemical immunesensor	Chicken red blood cells	10^1 – 10^3 EID_{50}/mL	10^3 EID_{50}/mL	2 h	H5N1 AIV	Lum et al., 2012
	Electrochemical DNA biosensor	H5N1 AIV HA and neuraminidase (NA)	8 – 100 nM	18 nM	–	HA and NA	Grabowska et al., 2013
	Fluorescence biosensor	H5N1 antibody	5.0 nM – 1.0 μM	1.6 nM	–	H5N1 antibody	Wei et al., 2013
	Electrochemical impedance aptasensor	H5N1 virus and chicken swab	0.125 – 16 HAU/50 μL	H5N1 virus: 0.125 HAU/50 μL chicken swab: 1 HAU/50 μL	–	H5N1 virus	Karash et al., 2016
	SPR biosensor	H5N1-infected feces	1×10^4 – 1×10^6 EID_{50}/mL	1000 EID_{50}/mL	–	H5N1 AIV	Nguyen et al., 2016
	FET biosensor	Chicken serum	10 pM – 10 nM	5.9 pM	–	HA protein	Kwon et al., 2020
Rotavirus	Photonic crystal biosensors	Culture/ Feces	0.02×10^4 – 5.77×10^4 FFU/mL	0.18×10^4 FFU/mL	30 min	Rotavirus	Pineda et al., 2009
	Fluorescence biosensor	Rotavirus	10^3 – 10^5 PFU/mL	10^5 PFU/mL	–	Rotavirus cell	Jung et al., 2010
	FET biosensor	Pure rotavirus stock and fecal sample	Pure rotavirus stock: 10 – 10^5 PFU/mL fecal sample: 10 – 10^4 PFU/mL	Pure rotavirus stock: 10^2 PFU/mL fecal sample: 10^3 PFU/mL	50 min	Rotavirus	Liu et al., 2013
	3D photonic crystal biosensor	Rotavirus antigen	2.54 – 127 μg/mL	6.35 μg/mL	–	Rotavirus	Maeng et al., 2016
Dengue virus	Innovative silicon nanowire FET sensor	Viral RNA mini kit	1 – 100 fM	10 fM	30 min	RNA	Zhang et al., 2010
	Electrochemical impedance biosensor	Vero cells	5.5×10^3 – 8.4×10^5 $TCID_{50}$/mL	8.4×10^2 $TCID_{50}$/mL	–	Dengue virus	Wasik et al., 2017
	Optical DNA-based biosensor	Saliva and urine	0.1 fM – 0.1 nM	0.2 aM	90 min	DNA	Ariffin et al., 2018
	Solid-state optical DNA biosensor	Serum, Urine, and Saliva	1 fM – 1 × mM	0.121 fM	15 min	Dengue virus serotype 2 genome	Mazlan et al., 2019
Vaccinia virus	Evanescent wave biosensor Optic biosensor	Throat culture swab specimens	1.3×10^1 – 1.3×10^8 PFU/mL	2.5×10^5 pfu/ml	–	Variola virus	Donaldson et al., 2004
	Electrochemical impedance biosensor	Human blood cells	0 – 3500 PFU/50 μL	330 PFU/50 μL	–	Variola virus	Labib et al., 2012

Table 4 Application of biosensor in airborne bacteria detection

Bacteria	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
Pneumococcus	Electrochemical biosensor	Serotype	10^0 – 10^4 CFU/sample	10^2 CFU/sample	15 min	*S. pneumoniae*	Cui et al., 2017
	Electrochemical impedance biosensor	Bacteria in Mueller-Hinton medium	10^1 – 10^7 CFU/mL	10^1 CFU/mL	–	*K. pneumoniae*	Silva Junior et al., 2018
Yersinia pestis	Phosphor biosensor	Lung tissue homogenates infected Balb/c mice	10^4 – 10^8 CFU/mL	10^4 CFU/mL	30 min	The whole cells of *Y. pestis*	Yan et al., 2006
	Fiber optic biosensor	Serum	0 – 10^3 ng/mL	10 ng/mL	40 min	Anti-F1 antibodies	Wei et al., 2007
	Magnetic biosensor	Buffer and human blood serum	25 – 300 ng/mL	2.5 ng/mL	–	*Y. pestis antigen F1*	Meyer et al., 2007
Staphylococcus aureus	Electrochemical biosensor	Apple juice samples and water	2.0 – 2.0 × 10^6 CFU/mL	2 CFU/mL	2 min	*S. aureus*	Bhardwaj et al., 2016
	Fluorescent MOF biosensor	Culture medium and cream pastry samples	40 – 4 × 10^8 CFU/mL	31 CFU/mL	20 min	*S. aureus*	Bhardwaj et al., 2017
	Autoinducer peptide-based electrochemical biosensor	AIP-I isolated from *S. aureus* cultured	10 – 1000 nM	0.5 nM	4 h	Autoinducer peptide	Lubkowicz et al., 2018
	Love wave biosensor	Synthesis	0 – 10 nM	1.86 pM (12.4 pg/mL)	30 min	S. aureusgene sequences	Ji et al., 2020
Bacillus	Electrochemical immune biosensor	*B. cereus*, *Bacillus megaterium*, and *Bacillus thuringiensis*	10^0 – 10^7 CFU/mL	10^1 CFU/mL	–	*Bacillus*	Pal et al., 2007
	Electrochemical biosensor	Synthesis	0.1 fM – 20 fM	0.08 fM	120 min	DNA	Hu et al., 2014
	Single-walled carbon nanotubes-based electrochemical biosensor	*B. subtilis* KCCM 11316	10^2 – 10^{10} CFU/mL	10^2 CFU/mL	10 min	B. subtilis	Yoo et al., 2017
Corynebacterium diphtheriae	Array fluorescent biosensor	Human serum	5 – 20 μg/mL	100 fg	–	Human antibodies	Moreno-Bondi et al., 2006
	SPR biosensor	Monoclonal anti–diphtheria IgG sample	0 – 1000 ng/mL	10 ng/mL	1 h	Anti-diphtheria IgG	Zeinoddini et al., 2018
	Electrochemical immune biosensor	Human saliva	10^{-4} – 10^{-1} Lf/mL	10^{-4} Lf/mL	–	Diphtheria toxoid	Ziółkowski et al., 2019
Streptococcus	Electrochemical biosensor	Group B *Streptococus* nucleic acid detection kit	1 fM – 1 nM	0.4 fM	2 h	DNA	Yuan et al., 2016

(*Continued*)

Bacteria	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
	Electrochemical immune biosensor	*S. agalactiae* reference strain	$10^1 - 10^7$ CFU/mL	10^1 CFU/mL	90 min	*S. agalactiae*	Vásquez et al., 2017
	Electrochemical biosensor	Human serum	$50 - 5 \times 10^4$ CFU/mL	50 CFU/mL	–	*S. pneumoniae*	Chang et al., 2020
Acinetobacter baumannii	Electrochemical DNA biosensor	Blood or sputum	$27.5 - 8.25 \times 10^7$ mg/mL	0.825 ng/mL (1.2 fM)	15 min	*DNA*	Yeh et al., 2010
	Lateral flow biosensor	Sputum	10 ng/uL – 1 fg/uL	100 fg/uL	1 h	DNA	Hu et al., 2019
	Optical DNA biosensor	Synthesis	1 μM – 1 zM	1 fM	–	Oligonucleotide sequences	Bahavarnia et al., 2020
Escherichia coli	Electrochemical biosensor	*E. coli* O157:H7	$10 - 10^5$ CFU/mL	79 CFU/mL	10 min	*E. coli* O157:H7	Muhammad-Tahir and Alocilja, 2003
	Electrochemical impedance biosensor	*E. coli* strains ORN 178 and ORN 208	$1.2 \times 10^2 - 2.5 \times 10^3$ CFU/mL	120 CFU/mL	–	*E. coli*	Guo et al., 2012
	FET biosensor	*E. coli* O157:H7	$10 - 10^4$ CFU/mL	10 CFU/mL	100 s	*E. coli* cells	Chang et al., 2013
	Electrochemical immunesensor	E. coli O157:H7	$30 - 3 \times 10^8$ CFU/mL	30 CFU/mL	–	E. coli O157:H7	Güner et al., 2017
	Quartz crystal microbalance (QCM) sensor	Stock cultures of *E. coli* O157:H7	$10^2 - 10^7$ CFU/ml	1.46×10^3 CFU/mL	50 min	*E. coli* O157:H7	Yu et al., 2018
	Electrochemical biosensor	Urine	$15 - 1.5 \times 10^8$ CFU/mL,	1 CFU/mL	140 min	*E. coli*	Li et al., 2018
	Electrochemical biosensor	*E. coli* strain ATCC 11303 culture collection	313, 10, and 1 CFU/mL	1 CFU/mL	6 – 8 h	*E. coli*	Zuser et al., 2019
	Microfluidic colorimetric biosensor	Chicken *E. coli* O157:H7	$50 - 5 \times 10^8$ CFU/mL	50 CFU/mL	–	*E. coli* O157:H7	Zheng et al., 2019
Hemophilus influenzae	Electrochemical biosensor	Culture/urine	0.1 – 2500 nM	0.02 nM	–	Chloramphenicol	Yadav et al., 2014
	Electrochemical DNA biosensor	Synthesis	1 zM – 1 μM	1 zM	50 min	DNA	Mobed et al., 2019b
	Optical DNA biosensor	Synthesized *H. influenza* sequences	1 μM – 1 zM	1 zM	2 h	DNA	Hassanpour et al., 2020
Legionella pneumophila	Electrochemically biosensor	Synthesis	$1 \times 10^{-14} - 1 \times 10^{-6}$ M	2.3×10^{-14} M	30 min	DNA	Rai et al., 2012

(*Continued*)

Bacteria	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
	Electrochemical DNA biosensor	Synthesis	1 zM – 1 μM	1 zM	20 min	DNA	Mobed et al., 2019a
	Antimicrobial peptide biosensor	*L. pneumophila*	10^3 – 10^6 CFU/mL	10^3 CFU/mL	2 h	*L. pneumophila*	Islam et al., 2020
Campylobacter jejuni	QCM sensor	Against C. jejuni	10^4 – 10^9 CFU/mL	150 CFU/mL		*C. jejuni*	Masdor et al., 2016
	Fluorescence immunosensor	*C. jejuni* in poultry liver	10 – 10^6 CFU/mL	10 CFU/mL	1.5 h	*C. jejuni*	Dehghani et al., 2020
Salmonella	Electrochemical impedance biosensor	Culture	3×10^3 – 3×10^6 CFU/mL	3×10^3 CFU/mL	3 h	*Salmonella*	Dastider et al., 2015
	Microfluidic nano-biosensoR	Culture/chicken	0 – 10^6 CFU/mL	10^3 CFU/mL	30 min	*Salmonella*	Kim et al., 2015
	Electrochemical biosensor	Culture/apple juice	10^2 – 10^8 CFU/mL	3 CFU/mL	45 min	*Salmonella*	Sheikhzadeh et al., 2016
	Colorimetric biosensor	Culture/spiked Chicken carcass	10^1 – 10^4 CFU/mL	11CFU/mL	2.5 h	*Salmonella*	Wang et al., 2020a
	Electrochemical aptasensor	Culture/spiked Chicken carcass	10^2 – 10^6 CFU/mL	80CFU/mL	2 h	*Salmonella*	Wang et al., 2020b
	Electrochemical biosensor	Milk	1.5×10^1 – 1.5×10^4 CFU/mL	150 CFU/mL	30 min	*Salmonella*	Malvano et al., 2020
Mycobacterium tuberculosis	Electrochemical DNA biosensor	Synthesis	1 pM – 10 nM	0.26 pM	100 s	DNA	Hong et al., 2012
	Electrochemical impedimetric immunosensor	Synthesis	100 fM – 1 nM	100 fM	–	16 kDa HSP	Gopinath et al., 2016
	Multichannel series piezoelectric quartz crystal (MSPQC) sensor	Culture /sputum	1×10^3 – 1×10^7 CFU/mL	10^2 CFU/mL	70 min	H37Rv	Zhang et al., 2017
	Silicon photonic microring sensor	Sputum	5 fg/uL– 500 pg/uL	3.2 copies	1 h	DNA	Liu et al., 2018c
	MSPQC sensor	Culture /sputum	1×10^2 – 1×10^8CFU/mL	20 CFU/mL	3 h	H37Ra	Zhang et al., 2019a
	Electrochemical Sensor	Culture	10^2 – 10^7 CFU/mL	10^2 CFU/mL	2 h	H37Rv	Zhang et al., 2019b
	Electrochemical sensor	Synthesis	1 fg/mL – 1 ng/mL	0.33 fg/mL	–	Protein	Chen et al., 2019
	SPR biosensor	Sputum	2 – 125 ng/mL	0.63 ng/mL	35 – 40 min	Protein	Peláez et al., 2020

Table 5 Application of biosensor in airborne bio-substances detection

Bio-substance	Sensor type	Sample	Range	Detection limit	Response time	Detection target	Reference
Aspergillus flavus	Electrochemical DNA biosensor	Aflatoxin B1 in pistachio nuts	1 nM – 10 μM	0.55 nM	4 h	DNA	Sedighi-Khavidak et al., 2017
Aspergillus niger	Cantilever sensor	Fungal strain *A. niger*	–	10^3 CFU/mL	4 h	Fungal spores	Nugaeva et al., 2007
Mycoplasma	Cantilever Sensors	Cell culture	10^3 – 10^7 CFU/mL	10^3 CFU/mL	Less than 1h	*Mycoplasma*	Xu et al., 2010
	Electrochemical gene sensor	Synthesis	0.1 pM – 20 nM	0.03 pM	2 h	DNA	Liu et al., 2016
	Fluorescence biosensor	Sheep serum	10^2 – 10^6 copies/μL.	1.042 copies/μL	Less than 15 min	*Mycoplasma* ovipneumoniae	Chen et al., 2017
	Lateral flow biosensor	Oropharyngeal swab specimens	60 fg/uL – 60 ng/uL	600 fg/uL	1 h	DNA	Wang et al., 2019b
	Lateral flow biosensor	Oropharyngeal Swab specimens	5 fg/uL – 5 ng/uL	50 fg/uL	1 h	DNA	Wang et al., 2019c
	Fluorescence biosensor	Human saliva	5 – 300 nM	3.96 nm	10 min	DNA	Li et al., 2019
Rickettsia	Optical biosensor	Blood plasma/Liver biopsy samples	5×10^1 – 5×10^4 copies/reaction	5×10^1 copies/ reaction	20 min	DNA	Koo et al., 2018
Chlamydia	Optical DNA biosensor	Human urine	0.25 – 20 nM	0.25 nM	–	DNA	Parab et al., 2010
	Nanoplasmonic biosensor	Culture/ Urine	10^1 – 10^7 CFU/mL	300 CFU/mL	–	*Chlamydia* trachomatis	Soler et al., 2017
Leishmania spp	Electrochemical DNA biosensor	Genomic sequence of *Leishmania* major	0.5 – 20 ng/μL	0.07 ng/μL	–	DNA	Moradi et al., 2016

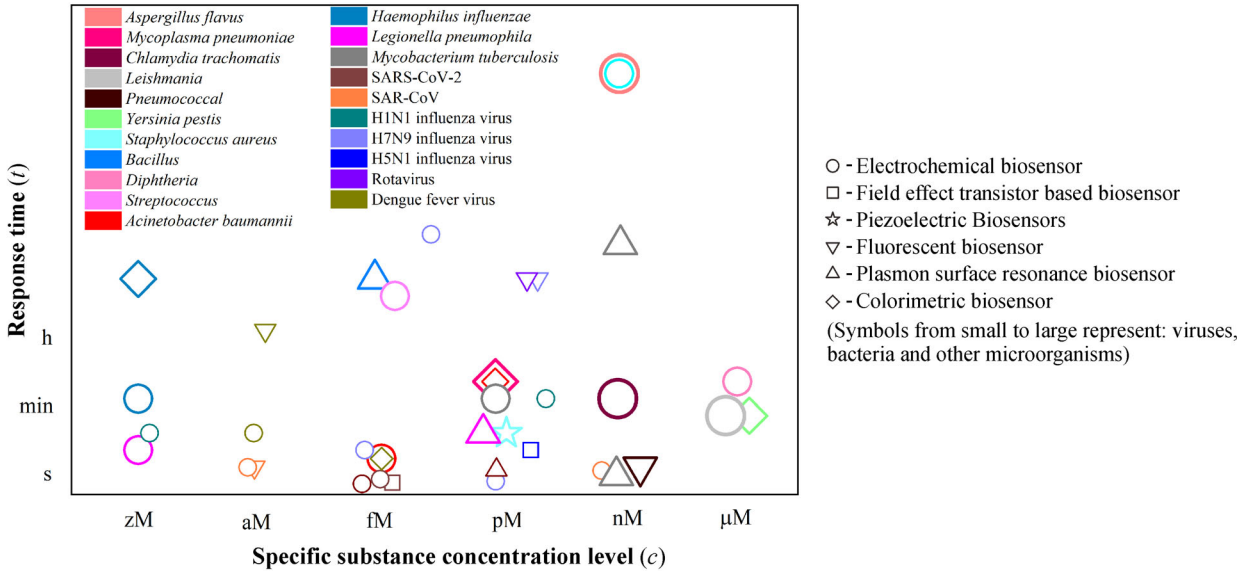

Fig. 3 Performance chart of airborne microbial-specific substances of common biosensors.

reduce sample consumption, reduce the size of detection equipment, and improve detection anti-interference ability; it is flexible and portable and convenient for field operations. Multiple detection technology can perform multiple detections at the same time, thereby improving the detection efficiency of biosensors. Smart devices can improve the visual operation and remote operability of the biosensors.

4.1 Air sampling technology

Although clinical samples such as nasopharyngeal swabs can be used for detection, traditional sampling methods can make patients feel uncomfortable and cause sneezing to produce aerosols, which can cause potential health risks (Cui and Zhou, 2020). At present, biosensors use air sampling systems to directly detect air samples. For infectious disease hotspots, the rapid detection of airborne microorganisms in air samples is necessary, and air sampling is often the first critical step (Shen et al., 2012). Wen et al. developed an air sampling method for Gram-negative bacterial marker endotoxin, optimized the analysis method based on the limulus reagent test (Wen et al., 2017), and detected 37.9–97.6 EU/m^3 endotoxin in the air of a university campus (Liu et al., 2018a). Zheng et al. used an exhaled air condensing device to obtain 300 μL of air sample within 3 min, and combined it with isothermal amplification technology to successfully detect seven airborne microorganisms from exhaled breath (Zheng et al., 2018). Rufino de Sousa et al. developed a large-scale electrostatic air sampler with good air filtration and sample treatment capabilities, and successfully detected *Bacillus* Calmette-Guerin vaccine of about 11 CFU/L-air and MTB of 46 CFU/L-air within 15 min (Rufino de Sousa et al., 2020). Meanwhile, there are other applications for the direct detection of air samples. Bhattacharyya et al. built a titanium dioxide nanotube array sensing platform for the electrochemical detection of tuberculosis volatile organic compound biomarkers, which can detect 0.12 mg/m^3 of methyl anisate (Bhattacharyya et al., 2016).

4.2 Purification and separation technology

The actual sample has impurity interference and the content of microorganisms in environmental aerosols is low. Directly collecting airborne microorganisms can be very challenging. Therefore, the samples for sensor detection must be preprocessed. Immunomagnetic separation has been extensively used in sample pretreatment. However, this method has shortcomings such as high requirements and low efficiency, that limit its application. Wang et al. used a magnetic grid separation column without any pre-enrichment of bacteria to complete the separation of 70% of target *Salmonella* cells in a 50 mL bacterial sample in 2.5 h, greatly improving the sensitivity of the sensor (Wang et al., 2020a). Song et al. proposed an optimized collection and detection scheme for complex air samples, which can break the wall of airborne microorganisms without destroying the internal structure, thereby improving the detection efficiency (Song et al., 2020). Briceno et al. added a magnetic field to the nanoparticles combined with MTB to achieve separation and enrichment, and the concentration rate of MTB could reach 47%, without using any expensive consumables and equipment (Briceno et al., 2019).

4.3 Microfluidic technology

Existing sensors mostly use the drip method to measure samples, making the loading and processing of samples difficult to control. This method is susceptible to interference from external physical factors such as light, humidity, and temperature, resulting in inaccurate measurement and poor sensing stability. Microfluidic technology integrates sample preparation, reagent manipulation, biological reactions, and detection steps on a unique platform, which can simplify complex analysis schemes and reduce sample volume, detection time, and reagent costs (Nasseri et al., 2018). Khan et al. integrated graphene and microfluidic devices to enhance the sensing performance, such as detection limit and sensitivity and continuous monitoring; the detection limit for thrombin reached 2.6 pM (Khan et al., 2020). Xie et al. used a high-throughput microfluidic chip to construct an electrical impedance sensor, and successfully distinguished different forms of yeast, which can be used as a rapid analysis technique to airborne microorganisms (Xie et al., 2019b).

4.4 Multiple detection technology

To improve the detection efficiency of biosensors and the portability of outdoor operations, multiple samples or multiple target microorganisms need to be detected at the same time to increase practicability and flexibility (Liu et al., 2018b). Liu et al. combined four micro-ring sensors to realize real-time measurement and multiplexing of four samples, greatly improving the detection speed (Liu et al., 2018c). Kumar et al. used peptide nucleic acids to induce color changes caused by aggregation of gold nanoparticles, which can be used to simultaneously detect multiple influenza viruses (Kumar et al., 2020).

4.5 Smart device linkage

The combination of biosensors and smart devices can make them flexible and portable; capable of real-time, continuous, and rapid detection; and has unique advantages such as miniaturization, high sensitivity, and absence of tags (Yang and Gao, 2019; Xing et al., 2020). The introduction of smart devices has greatly improved microorganism detection and provided convenient data processing and transmission for demonstration purposes (Nasseri et al., 2018). Mavrikou et al. combined a biosensor with a customized portable readout device operated by a smart phone/tablet computer for the portable detection of the new coronavirus spike protein within 3 min, with a detection limit of 1 fg/mL (Mavrikou et al., 2020). Zheng et al. developed a new type of biosensor and

used a smartphone imaging APP to monitor the color changes of AuNPs to determine the number of bacteria. The detection limit for *Escherichia coli* in chicken samples was 50 CFU/mL (Zheng et al., 2019).

5 Future perspectives

The current recurrence of airborne infectious diseases is not optimistic, and the COVID-19 pandemic threatens to interfere with public health services. Reversing the recent progress in reducing the burden of airborne infectious diseases will lead to a reduction in the detection of infectious diseases and an increase in deaths. Therefore, rapid detection and point-of-care (POC) analysis of airborne microorganisms that cause these diseases are important. Among the various methods used to detect airborne microorganisms, biosensor technology is at the forefront of POC device development. In recent years, scientists have conducted extensive research on biosensor technology. Some biosensors have been gradually used to detect microorganisms in air, and good results have been achieved. However, some challenges in sensors need to be further resolved in the future:

1) The detection of air samples requires further research. Most of the biosensor samples used for the detection of microorganisms in air are tested under laboratory conditions, and the test samples are usually limited to ideal samples, such as recombinant proteins or cell culture fluids, which are often different from actual samples.

2) An intelligent integrated system of sensor air collection and detection should be developed. Such a system integrates air collection, sample pretreatment, specific detection, and other steps. It also minimizes errors caused by manual operation, improves detection efficiency, and realizes fast and portable detection. An integrated system is essential to determine whether the sensor can leave of the laboratory to be tested.

3) Reduce costs, improve stability, and realize commercial production. Given the current outbreak of global infectious diseases, to expand the detection range, costs should be reduced, standardized sensor preparation and functionalization should be carried out, and more sensor characterization methods, such as expressing sensor detection performance in advance through the working characteristic curve, should be developed. Thus, large-scale commercial production can be realized.

4) Optimize the repeatability of the sensor. Given that the recognition and binding of biomolecules is often irreversible, most existing sensors are disposable products, and rebirth is difficult to achieve. The current rebirth effect is also uneven, and there is no unified standard that defines it. This is an important reason for limiting the large-scale application of sensors.

5) Further improve the specificity and sensitivity of the sensor. As a result of the low concentration of air microorganisms, detection is difficult, which affects the detection sensitivity of the sensor, and impurities are likely to cross-react and affect the detection specificity. New and more sensitive specific biological recognition elements must be developed.

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