



Nanosensor-Enabled Microfluidic Biosensors for the Detection of Pathogenic Bacteria

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Abstract

The standard techniques to detect pathogenic bacteria include culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). Despite the robustness of the methods, these methods suffer from various drawbacks, such as expensive equipment, tedious steps, and the need for highly trained operators. This has always been a challenge for researchers to achieve a rapid, portable, and inexpensive diagnostic test for the detection of pathogenic bacteria. In recent times, numerous technologies such as nanosensing, microfluidics, aptamers, etc. have been developed to address the aforementioned challenges. In this chapter, we are highlighting the use of nanosensors for detecting pathogenic bacteria. The chapter starts with the introduction section, which covers the pros and cons of the standard techniques to detect pathogenic bacteria. Following this section, the selection of different materials at each development step of a microfluidic chip-based nanosensor is discussed. Then, various microfluidic chip-based nanosensors are reported in the chapter to detect pathogenic bacteria. Along with this, the merits of using nanomaterials in nanosensors (like an increase in sensitivity) are also highlighted in the chapter.

Keywords

Nanosensors · Bacteria · Pathogens · Microfluidic chip · Biosensors · Aptamer

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5.1 Introduction

Disorders due to the existence of a pathogenic microorganism (pathogen), like a virus, bacteria, fungi, protozoa, multicellular parasite, prion, etc., in a host organism are known as infectious diseases (communicable/contagious). These infectious diseases can spread to other individuals by various means, like physical proximity, contaminated food, body fluids, objects, inhalation, water, vectors, etc. (Kumar et al. 2012). If we focus on bacteria, they exist in a variety of environments ranging from soil or water present next to you or harsh environments, like the Antarctica, hot water springs, etc. Interestingly, >99% of bacteria are harmless and help in balancing the environment where we live (Doron and Gorbach 2005). Human-infecting pathogenic bacteria include but are not limited to *Bacillus anthracis*, *Bacillus subtilis*, *Brucella abortus*, *Campylobacter* spp. (e.g., *C. jejuni*), *Clostridium botulinum*, *Escherichia coli* O157:H7, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Yersinia pestis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, etc. (Byrne et al. 2009). These bacterial pathogens cause various water- and foodborne diseases and other illnesses like sepsis, tuberculosis, sexually transmitted infections (STIs), etc. (Doron and Gorbach 2005; Singhal et al. 2021; Zhang et al. 2021). The emergence of resistance to various antibiotics in case of major bacterial pathogens poses a great threat to mankind (Egli et al. 2002; Babaie et al. 2021). The smart and the best possible way to curtail the antibacterial resistance is to treat the illness based on evidence rather than treating empirically. Thus, accurate and timely diagnosis is the key to manage bacterial infections. In search of powerful diagnostic devices for bacterial infections, countless efforts of research communities have been made, resulting in the evolution of diagnostics from centralized laboratory-based diagnostic to point-of-care (POC) diagnostic devices. Historically, classical methods for the detection of bacterial infections were preferably microscopy and bacterial culture (Doron and Gorbach 2005; Srivastava et al. 2018; Bhardwaj et al. 2022). As a consequence of constraints, such as tiresome preparations, longer processing time, low-grade sensitivity (in some cases), etc., the methods were taken over by biochemical (immunoassay/colorimetry-based) and advanced molecular biology and biotechnology (molecular genotyping, polymerase chain reaction (PCR), DNA microarray, etc.) methods (Srivastava et al. 2018; Bhardwaj et al. 2022). These methods depend on the detection of various pathogen-related proteins, toxins, nucleic acids, and antibodies for the diagnosis of bacterial infections (Bhardwaj et al. 2022). Though these methods are leading the market due to rapidity and better sensitivity (in comparison to classical methods), the requirement of complicated equipment and proprietary reagents limit their prominence in low- and middle-income countries.

As per the guidelines of the World Health Organization (WHO), selection criteria called ASSURED criteria was designed for the selection of diagnostics in developing countries. According to this criteria, the diagnostic device/test must be (i) **A**ffordable, (ii) **S**ensitive, (iii) **S**pecific, (iv) **U**ser-friendly, (v) **R**apid and **R**obust,

(vi) *Equipment-free*, and (vii) *Deliverable* to end users (Martinez et al. 2010; Tay et al. 2016). The enlisted features demanded in a diagnostic test can help clinicians conduct a timely and appropriate diagnosis of infections to control/prevent the spread and treat the disease.

The features here mentioned by the WHO to select diagnostic devices are not satisfied by any of the above-listed methods and can be fulfilled by integrating microfluidic chip biosensors and nanotechnology (Mark et al. 2010; Tay et al. 2016). The resultant tool is called a microfluidic chip nanosensor. In the case of microfluidic chip biosensors, microfluidics principles are employed to ease the biosensing of a target analyte. In microfluidics, fluid is processed/manipulated/analyzed within micrometer-sized channels. This technology hand overs several advantages to a tool, like portability, requirement of low sample volume, prospects of implementation in remote areas, low power utilization, reduced possibility of human errors, multiple analyte diagnosis, etc. (Fiorini and Chiu 2005). Biosensors are comprised of biomolecular recognition elements (BRE), like antibodies, antigens, aptamers, whole cells, etc., in close contact with a transducer for the identification of a specific analyte. A transducer converts a physiochemical event into an electrical signal, which is further processed to a readable form. BRE offers selectivity and specificity to a biosensor. Along with, the correct selection of transducer hand overs high analytical sensitivity to a biosensor (Bhardwaj 2015). Microfluidic chip biosensors propose all the qualities in the resulting diagnostic device, such as compactness, faster processing time, specificity, selectivity, sensitivity, easeful use, etc., which are necessitated for satisfying the WHO's ASSURED criteria to select diagnostics (Taylor et al. 2014). Further, to advance sensitivity, specificity, simplicity, and limit of detection (LOD), nanomaterials (NMs) are incorporated in microfluidic chip biosensors to obtain better performance of the sensor (Beltrán-Pineda et al. 2021). The reported nanostructured materials employed in the fabrication of nanobiosensors are nanowires, carbon nanotubes (CNT), thin films, nanoparticles (NPs), nanodots, nanocomposites, and polymer nanomaterials (Hu et al. 2017; Abdel-Karim et al. 2020).

In this chapter, we highlight the role of microfluidic chip nanosensors for the identification of pathogenic bacteria. Moreover, the current scenario in the selection of BRE, NMs, microfluidic chip substrates, design, and transducer for the development of microfluidic chip nanosensor is also illustrated. Along with this, the merit of using NMs in each reported nanosensor (like an increase in sensitivity) is also highlighted in the chapter.

5.2 Development of a Microfluidic Chip Nanosensor

A microfluidic chip biosensor consists of three main components: the design and material of the microfluidic chip, BRE, and transducer (detection). In microfluidic chip-based nanosensors, one additional part is included, i.e., NM. All the parts of the microfluidic chip nanosensor are represented in Fig. 5.1. As per the design and function of the microfluidic chip nanosensor, pretreatment of samples, like

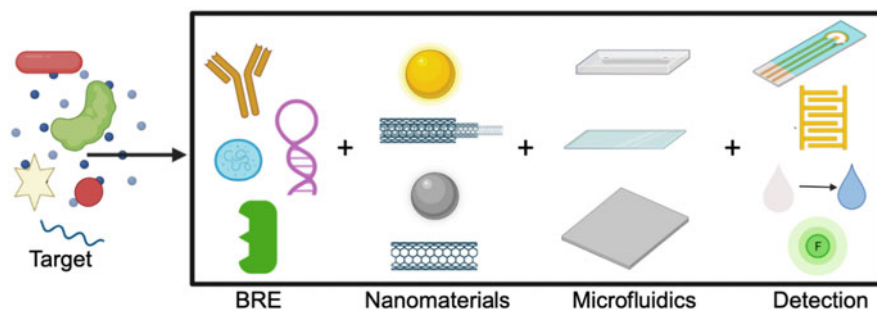


Fig. 5.1 A schematic representation of different parts of a microfluidic chip nanosensor for detection of target analyte, i.e., BRE, NMs, microfluidics (strategy and material), and detection (transducer). Created using [BioRender.com](https://www.biorender.com)

microfluidic separation, NM-based isolation, etc., might be required prior to analysis, which can also be added to the microfluidic chip (Reddy et al. 2022).

The selection of the correct strategy is the crux of designing a sensitive microfluidic chip nanosensor. The strategy includes the selection of specific and selective BRE, sensitive transducer, suitable NM for amplifying functionality of the biosensor, materials for chip fabrication, and simple design for microfluidic operations. Following are the crucial steps undertaken for the development of a microfluidic chip nanosensor.

5.2.1 Selection of Biomolecular Recognition Element

It has always been a challenging task to identify a single pathogen from a complex sample, like clinical, food, water, etc. To resolve this issue, BRE is utilized for the specific detection of the pathogen in microfluidic chip nanosensors. BRE is a biomolecule that has selectivity and specificity for a target analyte and can help in the recognition of the target analyte when present in the sample. BRE can be aptamers, antibodies, proteins, enzymes, etc. (Thévenot et al. 2001; Bhardwaj 2014; Khan and Song 2020). Primarily, the selection of a BRE is the first step toward the development of a biosensor. The selection criterion for a BRE is its specificity and selectivity for its target analyte, stability in working condition, and compatibility with the detection technique.

For diagnostic applications, bacterial surface antigenic markers, like lipopolysaccharide (LPS), teichoic acid (TA), membrane proteins, etc., toxins, and nucleic acids are employed for determining pathogen in a sample (Kant et al. 2018). Mostly reported BREs for the detection of the pathogens are antibodies and aptamers (Chen et al. 2017; Kant et al. 2018). Various bacterial pathogens, like *Listeria monocytogenes* (*L. monocytogenes*) (Lu et al. 2016), *Pseudomonas aeruginosa* (*P. aeruginosa*) (Krithiga et al. 2016), *Salmonella pullorum* and *Salmonella gallinarum* (*S. pullorum* and *S. gallinarum*) (Hu et al. 2014), *Salmonella enterica*

(*S. enterica*) (Brandão et al. 2013), and *Yersinia enterocolitica* (*Y. enterocolitica*) (Savas and Altintas 2019), have been detected using antibodies as BREs. Due to various limitations of antibodies, such as low stability, small shelf-life, high cost, complex in vivo generation, batch-to-batch variation, immunogenicity, and inability to target low immunogenic analytes, aptamers are found to have better performance over antibodies and, hence, prioritized these days (Dhiman et al. 2018; Komarova and Kuznetsov 2019; Kumari et al. 2019; Wang et al. 2019b; Mastronardi et al. 2021). Using aptamers as BRE, detection of bacterial pathogens, such as *Salmonella typhimurium* (*S. typhimurium*) (Wu et al. 2014), *L. monocytogenes* (Liu et al. 2018), *Staphylococcus aureus* (*S. aureus*) (Cheng et al. 2016), *P. aeruginosa* (Roushani et al. 2019), and *S. enterica* (Muniandy et al. 2019), has been reported in the literature.

5.2.2 Selection of Microfluidic Chip Material

In microfluidic chips, the process of selection of chip material depends on the application and design of the chip (microfluidic strategy), while the production process, the selection of material decides the fabrication technique. This section is discussed in this chapter because the selection process highly affects the affordability, simplicity, and commercialization of a microfluidic chip nanosensor that is required to fulfill criteria for present and future generation diagnostics (ASSURED criteria).

Various materials are available for the construction of microfluidic chips, such as silicon (Fallahi et al. 2019), glass (Fallahi et al. 2019), polymers (Convery and Gadegaard 2019), etc. Silicon and glass need complex and expensive micromachining fabrication techniques, like wet and dry etching, photolithography, electron beam lithography, etc., requiring extreme hands-on training in a clean room environment (Beebe et al. 2002; Fiorini and Chiu 2005). Due to this fact, other materials, like polymers, are preferred over silicon and glass (Beebe et al. 2002). Polymers present compelling features, such as affordability, accessibility to a different range of shapes and sizes, easy and simple fabrication, no advanced demands, etc. Microstructuring in polymers can be performed using easy and inexpensive techniques, such as injection molding, hot embossing, replica molding, micromilling, laser ablation, casting, etc. (Roberts et al. 1997; Soper et al. 2000; Attia et al. 2009; Greener et al. 2010; Hong et al. 2010; Guckenberger et al. 2015; Ansari et al. 2016; Convery and Gadegaard 2019). Particularly, fluoropolymer, such as polytetrafluoroethylene, due to its soft nature and easy-milling technique, and an additional group of polymers, i.e., thermoplastic polymers, due to easy hot embossing or injection molding, are documented as real-world materials for structuring microfluidic channels (Ansari et al. 2016). Poly(methyl methacrylate) (PMMA), polycarbonate (PC), polystyrene, and cyclic olefin copolymer (COC) are thermoplastic polymers that are most commonly investigated materials in microfluidics (Convery and Gadegaard 2019). These polymers have many options for bonding techniques, such as thermal or glue/solvent-assisted bonding, and

promote mass production. In literature, the use of thermoset polymer material, such as polydimethylsiloxane (PDMS), is also reported. To construct microchannels in PDMS, a master for molding of PDMS is prepared with materials, like silicon or smooth metal (Friend and Yeo 2010; Convery and Gadegaard 2019). The bonding technique for PDMS is known to be highly simple and reliable. PDMS is the most preferred material for research laboratories and has characteristics like flexibility, low thermal conductivity, hydrophobicity, biocompatibility, low toxicity, low-curing temperature, simple-to-cast ability, and tight sealing with many smooth surfaces (Gervais et al. 2011; Giannitsis 2011; Ansari et al. 2016; Faustino et al. 2016; Jigar Panchal et al. 2020). For commercially viable products, paper is preferred due to its disposable nature (Bhattacharya et al. 2019). Patterning and customized origami-based methods are the two fabrication techniques majorly employed in paper microfluidics. The patterning methods are further categorized into cutting, printing, deposition, stamping, lithography, and etching (Li et al. 2008; Haller et al. 2011; Asano and Shiraishi 2015; Kim et al. 2018; Mathaweesansurn et al. 2020; Zargaryan et al. 2020; Nishat et al. 2021; Ramana et al. 2022).

Though various materials are available for the fabrication of microfluidic chips, choice of the correct material as per the required application and design strategy of the chip plays a major role. For instance, the material selected for one application may not be ideal for another, like materials used for laboratory research applications may not always be used in the commercialized products. For research applications, materials such as PDMS, glass, and silicon are used (Ren et al. 2013). Glass and silicon demand highly complex techniques, which are unsuitable for scale-up and manufacturing perspectives (Buckner et al. 2016). PDMS has become a trademark in proof-of-concept studies because of its easy obtainability and well-known, rapid, and simple protocol (rapid prototyping), but its application is hampered for commercialization due to cost concerns and reusability (Buckner et al. 2016). Thermoplastics can be the perfect material from the perspective of commercialization but cannot be a great option for economic research applications due to the requirement of an expensive and high-heat sustainable metal or hard material for molding. Along with, paper can be the adjoining material for economical research applications due to its higher chances of mass production, ease in use, and disposability. The second example of the selection of material is the use of a stiff and smooth material (glass) for patterning electrodes instead of flexible material (PDMS) (Li 2006; Toepke and Beebe 2006; Giannitsis 2011). On the other side, for various elastic parts of the chip, like membranes and valves, PDMS or similar material is employed (Convery and Gadegaard 2019). In such cases, glass or any other non-flexible material/polymer cannot find application. Similarly, for applications, such as biological cell culture, which necessitates the requirement of gas permeability, PDMS is used over other materials, such as silicon, glass, PMMA, and PC (Ren et al. 2013). Depending on detection techniques, the materials can also be chosen, like a combination of PDMS/glass is selected for electrochemical research studies (Sia and Whitesides 2003; Vullev et al. 2006; Bhardwaj et al. 2020). For optical-based detection methods, the use of transparent material is highly promoted, like

PMMA (Bhardwaj and Kumar Jha 2018). Hence, systematic navigation of materials is essential before the fabrication of a microfluidic chip nanosensor.

5.2.3 Selection of Nanomaterial (NM)

The selection of NM for sensing depends on the properties of NM, microfluidic strategy, and the choice of the detection technique. Depending on the properties of NMs and detection technique, they are selected for a specific application, which improves sensitivity, LOD, and signal-to-noise ratio of the sensor, for example, (1) NMs with a large surface to volume ratio are used for immobilization of BRES; (2) highly conductive NMs as signal amplifiers, mediators, nanoprobe, and nanozymes are majorly employed in electrochemical sensing; and (3) NMs with fluorescent features are used for fluorescent-based detection methods, etc. (Dhar Malhotra and AzaharAli 2018). Along with, many other NMs can also be additionally selected as per the requirement of the microfluidic chip design. For example, few chips involve preconcentration steps, in which magnetic nanoparticles (MNPs) are employed majorly due to easy magnetic field (MF)-based separation (Reddy et al. 2022).

In literature, NMs, like quantum dots (QD), metal-based NPs, graphene oxide, and up-conversion NMs, are used for fluorescence-based detection. Noble metal-based NPs, such as gold (Au), platinum (Pt), palladium (Pd), and silver (Ag), are reported for surface-enhanced Raman spectroscopy (SERS) and colorimetric detection (Reddy et al. 2022). For instance, Pd-Pt core-shell NPs were used in nanozyme-based colorimetric immunosensing to detect *S. enteritidis* and *E.coli* O157:H7 (Cheng et al. 2017). Pd-Pt NPs are popular for peroxidase-like catalytic activity, increased surface area, and robustness. Here, the NPs replaced hydrogen peroxidase (HRP) in detection antibodies and increased sensitivity and stability. On adding 3,5,3',5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2), a blue color appeared proportional to the concentration of bacteria due to the peroxidase-like catalytic activity of NPs. Similarly, for colorimetric analysis of *E.coli* in the milk sample, nanozyme-based lateral flow assay was designed in which Pd-Pt NPs were used as nanozyme attached to monoclonal detection antibodies (Han et al. 2018). To determine *S. typhimurium* and *E.coli*, colorimetric aptasensor was designed in which unmodified AuNPs were immobilized with aptamers specific for *S. typhimurium* and *E.coli* (Wu et al. 2012). The absence/presence of bacteria in the sample induced conformational changes in the aptamer's structure, which controlled the aggregation of AuNPs in the solution resulting in a change in the color of the solution. For detecting *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *S. typhimurium*, dual fluorescence resonance energy transfer (FRET)-based biosensor was reported using green-emitting quantum dots (gQDs) and red-emitting quantum dots (rQDs) as donors and amorphous carbon nanoparticles (CNPs) that acted as acceptor (Duan et al. 2015). The fluorescence of aptamer immobilized QDs was quenched by CNPs in the absence of pathogen, which increased in the presence of pathogen due to the

protective layer provided by capturing the pathogen on the surface of aptamer immobilized QDs.

For electrochemical detection methods, high conductivity and stable NMs are employed, like silicon, noble metal-based NMs (Pt, Ag, and Au), carbon-containing NMs (graphene and CNTs), and inorganic two-dimensional nanosheets (Reddy et al. 2022). Incorporation of magnetic separation-based step in nanosensing includes utilization of MNPs, such as metallic iron, iron oxides, cobalt, nickel, etc. (Reddy et al. 2022). For instance, a multiwalled CNT (MWCNT) fiber electrode was used for sensitive electrochemical detection of *L. monocytogenes* in food samples because of the excellent electrical properties of MWCNTs (Lu et al. 2016). For detection of *S. pullorum* and *S. gallinarum* in the poultry industry, screen-printed carbon electrodes were deposited with AuNPs, which worked as an electron transfer agent. Additionally, AuNPs provided a large surface area for the immobilization of biomolecules (antibodies) for detection (Hu et al. 2014). For the determination of *S. enterica*, an electrochemical magneto-immunosensing assay was reported based on MNPs (Brandão et al. 2013). Anti-*Salmonella* antibodies were immobilized on MNPs and used for capturing the bacteria. Here, MNPs fulfilled two purposes, i.e., provided a large surface area for immobilization of antibodies and used for magnetic separation prior to analysis. To detect *Salmonella* in milk, MWCNTs layered with tyrosinase were used for detection instead of enzyme-linked secondary antibodies as nanoprobe (Chumyim et al. 2014). MWCNTs provided a high surface area for enzyme loading, which enhanced the performance of the method. Furthermore, MWCNTs improved electron transfer reactions and electrocatalytic activity. In advanced nanosensors, enzymes have been totally replaced with nanostructures, which have electrocatalytic properties too. Along with, they improve the sensitivity as well. For instance, graphene QDs (GQDs) possess peroxidases-like catalytic activity, leading to decomposition of H_2O_2 (replacement of HRP enzyme). Moreover, these nanozymes display better stability in comparison to enzymes and, hence, provide a stable and reproducible signal. Additionally, GQDs have faster performance in electrochemical reactions, a wider linear range, and low LOD. Such a nanozyme-based amperometric immune nanosensor was developed to detect *Y. enterocolitica* in milk and human serum (food and clinical applications) (Savas and Altintas 2019).

5.2.4 Selection of Detection Technique

Various detection methods are available for analysis in nanosensors, like electrochemical (amperometric, potentiometric, impedimetric), optical (absorption, fluorescence, chemiluminescence), and piezoelectric (Bhardwaj 2015; Sharma et al. 2021). The piezoelectric detection method comprises a quartz crystal, which measures variation in mass due to interaction between the target analyte and BRE. This method is used for bioaffinity reactions majorly. Due to high sensitivity, the method necessitates the use of isolation equipment to minimize the noise effect from the environment (Saylan and Denizli 2020). The scope of this chapter is restricted to

electrochemical and optical detection methods due to their prevalence in research and practical environment over piezoelectric sensing.

In electrochemical nanosensing, an electrochemical reaction occurs on the surface of the transducer (electrode) between BRE and target analyte integrated with NMs (for improving the signal), leading to variation in current, voltage, or impedance (Bhardwaj 2015; Dhar Malhotra and AzaharAli 2018). Majorly, the BREs are immobilized on NMs (Chumyim et al. 2014), or NMs modified electrodes (Lu et al. 2016) for signal amplification. NMs can also be utilized as nanozymes for catalyzing substrate-based reactions (Savas and Altintas 2019). The electrochemical nanosensors can be further categorized depending on the type of input and output signal, such as amperometric, voltammetric, potentiometric, and impedimetric. In amperometric nanosensing, a constant voltage is applied as input, and variation in output current is measured to study the interaction between the target analyte and BRE. Amperometry has high sensitivity with low detection limits (Bhardwaj 2015). In voltammetric nanosensing, a varying voltage is applied as input, and output current is measured for studying the binding interaction of the target and BRE. Different types of voltammetric techniques are available, such as potential step, cyclic, linear sweep, differential pulse, and square wave, which can be selected as per the requirement of sensitivity in the application (Bhardwaj 2015). On the other hand, in potentiometric nanosensing, a constant current is applied to measure variation in voltage generated due to the reaction between the target analyte and BRE. This method is less sensitive than amperometry and presents high detection limits (Bhardwaj 2015). In impedimetric nanosensing, a constant voltage is applied, and the impedance of the sensor is measured, which is generated in response to the reaction between the target analyte and BRE. In this type of sensing, changes in resistance or dielectric constant are measured for studying the interaction between the analyte and BRE. This method is majorly employed for bioaffinity reactions, not for biocatalytic reactions (Bhardwaj et al. 2020). The impedimetric detection method has the advantages of compact design, rapidity, inexpensiveness, and easy integration with other technologies (Mi et al. 2022). The most used electrochemical method for nanosensing of pathogenic bacteria is amperometry, voltammetry, and impedimetry. These methods have been applied for the detection of various bacterial pathogens, like *E.coli* (Wang et al. 2018; Yao et al. 2018), *L. monocytogenes* (Lu et al. 2016), *S. aureus* (Tang et al. 2010), *P. aeruginosa* (Krithiga et al. 2016), *S. pullorum* (Hu et al. 2014), *S. gallinarum* (Hu et al. 2014), *S. enterica* (Brandão et al. 2013), *Y. enterocolitica* (Savas and Altintas 2019), and Gram-positive bacteria (Han et al. 2020).

In optical-based nanosensing, an optical transducer, NMs, and BRE are integrated together to measure physical/chemical changes that occurred due to the reaction between BRE and target analyte. In response, variation in fluorescence, absorption, color, chemiluminescence, surface plasmon resonance (SPR), etc. is generated (Bhardwaj 2015; Dhar Malhotra and AzaharAli 2018). The optical sensing method has anti-interference ability, easy miniaturization, high sensitivity, and rapid response (Mi et al. 2022). In addition, they are known to be highly sensitive over electrochemical detection methods. On further integration of optical sensing with

microfluidic chips and NMs, the resulting device provides an ultrasensitive, high-throughput, compact, portable, inexpensive, and automated device, requiring a low quantity of samples (Mi et al. 2022). Majorly, NMs are used as tags/labels, carriers for biomolecule loading, target-induced aggregation/morphological reaction in NPs, and signal amplification in optical detection nanosensing.

Basically, the optical method can use label-free or label-based detection. In the label-free optical method, optical signals are generated directly on the reaction between the target and BRE. On the other hand, the label-based optical method utilizes a label or tag for the generation of the optical signal, like in the case of colorimetric, fluorescence, chemiluminescence, etc. (Saylan and Denizli 2020; Li et al. 2021). Although label-based optical methods are better in sensitivity, they involve the utilization of expensive labels and labeling steps (Saylan and Denizli 2020). The mostly employed optical method in nanosensing is colorimetry and fluorimetry. Chemiluminescence-based nanosensing is not commonly used but can be an attractive detection method in the future due to its fast response, low background noise, high sensitivity, and wide dynamic range (Deng et al. 2021; Mi et al. 2022). Colorimetric sensing is found attractive from the perspective of visual color change in the detection of the pathogen in the sample (Mi et al. 2022). The main drawbacks of this detection method are its low sensitivity and long detection time (Mi et al. 2022), which can be improved by integration with microfluidics and nanotechnology. Few colorimetric nanosensors are reported in the literature for the detection of various bacterial pathogens, like *S. enteritidis* (Cheng et al. 2017), *E. coli* (Cheng et al. 2017; Han et al. 2018; Filby et al. 2020), *E. sakazakii* (Zhang et al. 2017), *L. monocytogenes* (Liu et al. 2018), *R. rhodochrous* (Filby et al. 2020), *S. aurantiaca* (Filby et al. 2020), and *S. aureus* (Yin et al. 2022). Fluorescence-based detection has the benefits of high sensitivity and easy readability and is found to be better in sensitivity than colorimetric detection (Mi et al. 2022). Fluorescence-based nanosensing has been demonstrated for the detection of bacterial pathogens, such as *V. parahaemolyticus* (Duan et al. 2015, 2016), *S. typhimurium* (Duan et al. 2015, 2016; Li et al. 2018), *S. aureus* (Duan et al. 2016; Yu et al. 2017; Qiao et al. 2018), and *E. coli* (Li et al. 2018).

The above-discussed parts (BRE, NM, microfluidic chip material/strategy, and detection technique) are integrated together for the development of a microfluidic chip-based nanosensor. A few examples of such microfluidic chip-based nanosensors to detect pathogenic bacteria are discussed in the next section.

5.3 Microfluidic Chip Nanosensors for Detecting Pathogenic Bacteria

5.3.1 Electrochemical Microfluidic Chip Nanosensors

Following are the few electrochemical microfluidic chip nanosensors for the detection of pathogenic bacteria. All the nanosensors are summarized in Table 5.1, and their working principles are pictorially represented in Fig. 5.2.

Table 5.1 Microfluidic chip nanosensors for the detection of pathogenic bacteria

Target	Nanomaterial used	Microfluidic chip material	Principle	Application	LOD	Ref.
Electrochemical ELISA-based nanosensors						
<i>E. coli</i>	AuNPs modified with HRP-labeled secondary antibodies	Silicon dioxide wafer	Sandwich ELISA: antibody-antibody	Water	50 cfu/mL	Altintas et al. (2018)
<i>Salmonella</i>	1. MNPs coated with monoclonal antibodies 2. Polyclonal antibodies and urease coated AuNPs	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	10 cfu/mL	Hou et al. (2020)
<i>Salmonella</i>	MNPs immobilized with monoclonal antibodies	Glass capillary	Sandwich ELISA: antibody-antibody	Food	33 cfu/mL	Hou et al. (2019)
<i>E. coli</i>	1. MNPs modified with polyclonal antibodies 2. AuNPs conjugated with urease and aptamers	PDMS/glass	Sandwich ELISA: antibody-aptamer	Food	12 cfu/mL	Yao et al. (2018)
<i>L. monocytogenes</i>	1. MNPs modified with monoclonal antibodies 2. AuNPs modified with polyclonal antibodies and urease	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	1.6×10^2 cfu/mL	Chen et al. (2016)
<i>E. coli</i>	AuNPs as catalyst for silver enhancement	PDMS/glass	Positive dielectrophoresis	Water and food	500 cfu/mL	Wang et al. (2018)
Optical ELISA-based nanosensors						
<i>E. coli</i>	1. cMNPs used for immobilization of antibodies 2. Aggregation of AuNPs for sensing	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	50 cfu/mL	Zheng et al. (2019)
<i>Salmonella</i>	1. Polyclonal antibodies modified MNBs 2. Monoclonal antibodies modified Au@PtNCs	PDMS	Sandwich ELISA: antibody-antibody	Food	350 cfu/mL	Qi et al. (2022)

(continued)

Table 5.1 (continued)

Target	Nanomaterial used	Microfluidic chip material	Principle	Application	LOD	Ref.
<i>S. typhimurium</i>	CdSe/ZnS quantum dots for fluorescence	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	37 cfu/mL	Wang et al. (2015)
<i>S. typhimurium</i>	MNPs modified with monoclonal antibodies	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	58 cfu/mL	Wang et al. (2019a)
<i>E. coli</i>	MNPs modified with antibodies	Plastic	Single antibody-based detection	Clinical	10 cfu/mL	Park et al. (2017)
<i>S. pneumoniae</i>	Ni/NiO NPs immobilized with antibodies	PMMA/PDMS/glass	Sandwich ELISA: antibody-antibody	Clinical	10 ⁵ –10 ⁷ cfu/mL	Weng et al. (2021)
<i>S. typhimurium</i>	Monoclonal antibodies modified MNPs	PDMS/PMMA	Sandwich ELISA: antibody-antibody	Food	150 cfu/mL	Cai et al. (2019)
<i>S. typhimurium</i>	1. MNPs modified with monoclonal antibodies 2. MnO ₂ -QDs NFs functionalized with polyclonal antibodies	PDMS/glass	Sandwich ELISA: antibody-antibody	Food	43 cfu/mL	Hao et al. (2020)

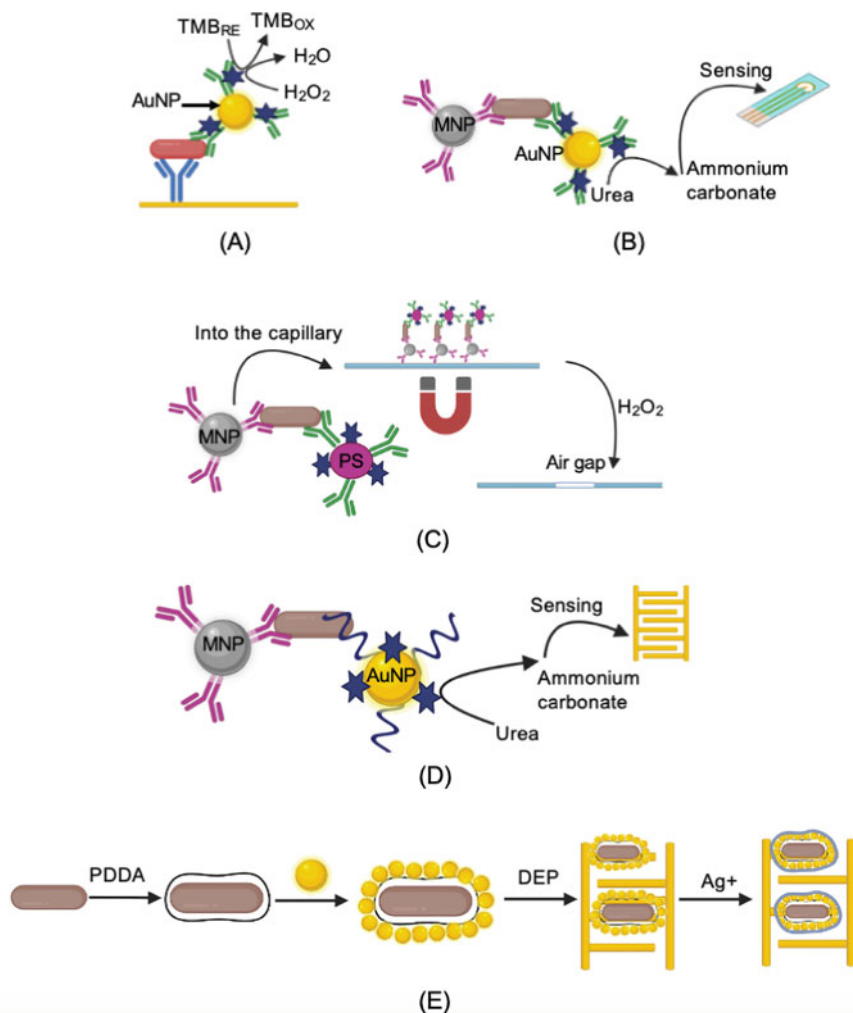


Fig. 5.2 A schematic representation of working principle of electrochemical microfluidic chip nanosensors. (a) Amperometry-based microfluidic chip nanosensor (Altintas et al. 2018). (b). Voltammetry-based microfluidic chip nanosensor (Hou et al. 2020). (c) Microfluidic electrical signal-off nanosensor (Hou et al. 2019). (d) Impedimetric microfluidic chip nanosensor (Yao et al. 2018). (e) Impedimetric microfluidic chip nanosensing using silver enhancement solution (Wang et al. 2018). The representation of working principle in this figure is to explain the mechanism and adapted from the mentioned references. The original research article representation of these chips may vary from the one shown in the figure. Created using [BioRender.com](https://www.biorender.com)

A fully automated microfluidic chip-based electrochemical sensor was developed for the detection of *E. coli* (Altintas et al. 2018). Electrodes present in the chip were immobilized with primary antibodies specific for *E. coli* followed by incubation with the sample containing *E. coli*. Then, quantification of bacteria was performed with

two strategies, one with standard HRP-labeled secondary antibodies for *E. coli* and the other with AuNPs modified with HRP-labeled secondary antibodies. When TMB reagent was added, then the amperometric signal was generated due to oxidation of TMB and reduction of H_2O_2 (Fig. 5.2a). This signal increased proportionally with the increase in the concentration of HRP or bacteria. The current was measured using an automated system, which contained a potentiostat, a LCD display, a digital control circuit, and a communication interface. In the analysis, it was clearly found that the use of AuNPs increased the sensitivity by providing a higher surface area for immobilization of HRP-labeled secondary antibodies, and the LOD was improved to 50 cfu/mL from 1.99×10^4 cfu/mL (improvement by 398-fold).

A voltammetry-based ultrasensitive microfluidic chip biosensor was reported using magnetic grid pre-separation and urease catalysis for the detection of *Salmonella* in food samples (Hou et al. 2020). For this purpose, MNPs coated with anti-*Salmonella* monoclonal antibodies (mAb) were passed through the magnetic separator grid in which MNP chains were obtained. Then, bacterial sample followed by anti-*Salmonella* polyclonal antibodies (pAb) and urease-coated AuNPs was passed through the grid to obtain enzymatic bacteria. Further, in the presence of urea in the grid, ammonium carbonate was formed proportional to the concentration of bacteria present in the sample (Fig. 5.2b). This solution was passed into the microfluidic chip used for the voltammetric detection of bacteria. This chip was made up of PDMS/glass and consisted of screen-printed electrodes for sensing. Linear scan voltammetry (LSV) was performed, and the resistance or current of the system was analyzed. The current increased or resistance decreased proportionally with the increase in the concentration of bacteria in the sample. LOD of the sensor was 10 cfu/mL. The MNPs served the purpose of increasing the sensitivity of the method by immobilizing a higher number of antibodies and preconcentration of the sample. Along with, AuNPs provided a higher surface area to urease and detection antibodies, which also increased the sensitivity by signal amplification.

A microfluidic signal-off biosensor using magnetic separation and enzymatic catalysis was constructed for the determination of *Salmonella* in food samples (Hou et al. 2019). The microfluidic chip consisted of two inlets and two outlets. Enzymatic bacteria and washing solutions were handled using one pair of inlet and outlet. The other pair of inlet and outlet was used for measuring the electrical voltage of the capillary using gold electrodes, which were connected to a constant power supply and light emitting diode (LED). The voltage of the capillary was monitored online to check for any variation in resistance of the capillary. Glass capillary with acrylonitrile butadiene styrene (ABS) plastic tubing at both ends played the role of incubation channel accommodated in a PDMS mold. Both ends of ABS tubing were connected to the inlet and outlet. In the study, MNPs were immobilized with anti-*Salmonella* mAbs, and polystyrene microspheres (PSs) were modified with anti-*Salmonella* detection pAbs and catalase. Firstly, modified MNPs and PSs were reacted with a sample solution containing *Salmonella* leading to the formation of MNPs/*Salmonella*/PSs complex. Then, the enzymatic bacteria solution was passed into the capillary and captured using external MF. After injecting H_2O_2 solution into the capillary, catalase catalyzed H_2O_2 into oxygen. This phenomenon generated an

air gap in the capillary leading to an increase in the resistance of the capillary and, hence, electrical signal-off (Fig. 5.2c). The voltage across the capillary increased proportionally to the concentration of bacteria due to an increase in the resistance of the capillary. The sensor was able to detect *Salmonella* as low as 33 cfu/mL within 2 h. In this study, MNPs served the purpose of increasing the sensitivity of the method by immobilizing a higher number of antibodies and preconcentration of the sample.

A microfluidic impedimetric microfluidic chip biosensor using MNPs and AuNPs was fabricated to identify *E.coli* O157:H7 in food samples (Yao et al. 2018). Firstly, streptavidin-modified MNPs were modified with biotinylated pAb against *E.coli* O157:H7 bacteria. This unit made a complex with bacteria cells when present in the sample, and the bacteria-pAb-MNP complex was separated from the background sample using a magnetic separator. Then, AuNPs conjugated with urease and aptamers specific for *E.coli* O157:H7 were incubated with bacteria-pAb-MNP complex leading to the generation of urease enzyme-linked bacteria MNP complex, which produced ammonium carbonate on the addition of urea. The production of ammonium carbonate was directly proportional to the concentration of the urease enzyme, which showed the concentration of bacteria present in the sample. After this reaction, MNPs complex was again separated, and the supernatant was passed into a PDMS/glass microfluidic chip containing a gold interdigitated (IDT) electrode for impedimetric analysis (Fig. 5.2d). The impedance of the chip decreased with an increase in the concentration of ion strength (ammonium carbonate) directly proportional to the concentration of bacteria in the sample. LOD of the chip was 12 cfu/mL. The use of NMs, like MNPs and AuNPs, provided a higher surface area for the immobilization of biomolecules, which led to better sensitivity. Along with, the use of microfluidic chip enabled the continuous flow measurement, which reduced detection time and improved sensitivity as well. Similarly, using MNPs and AuNPs, a microfluidic chip was fabricated for the detection of *L. monocytogenes* (Chen et al. 2016). The microfluidic chip was developed from PDMS and glass substrates, which consisted of two parts: a separation chip and a detection chip. MNPs modified anti-*Listeria* mAbs, AuNPs modified anti-*Listeria* pAbs and urease, and a sample containing *L. monocytogenes* was incubated in the separation chip to form MNPs-*Listeria*-AuNPs-urease complex. The complexes were magnetically separated using high gradient MF and then resuspended in the urea solution. This reaction led to the generation of ammonium carbonate, whose concentration was directly proportional to urease or bacteria. This solution was passed into the detection chip, which contained gold IDT electrodes for impedimetric analysis. The impedance of the chip was found to decrease with the increase in the concentration of bacteria. The LOD of the chip was 1.6×10^2 cfu/mL. Along with, the analysis time was 1 h.

In another study, the sensitivity of the impedimetric detection method for the detection of the bacterial pathogen (*E. coli*) in water and food samples was increased using a silver enhancement solution (Wang et al. 2018). A microfluidic chip was fabricated with PDMS and glass, which consisted of two zones, tesla mixing and detection zone. In the detection zone, IDT electrodes were present for performing

positive dielectrophoresis for the enrichment of bacteria on the electrode. Firstly, bacterial cells were coated with cationic polymer diallyldimethylammonium chloride (PDDA) to give rise to a positively charged *E. coli*/PDDA complex. Then, this solution and AuNPs solution was passed into the chip for mixing. After mixing zone, *E. coli*/PDDA/AuNPs complex was generated, which was collected at IDT electrode using positive dielectrophoresis. Lastly, for silver enhancement, silver salt and initiator solution were passed into the chip, and silver salt was reduced to silver ions forming a silver adduct by AuNPs collected on the electrode (Fig. 5.2e). When bacteria concentration increased on the electrode, more silver adducts accumulated at the edge of the electrode, resulting in an increase in the contact area between bacterial suspension and electrodes. This phenomenon decreased the impedance of the sensor and increased the conductivity and double layer capacitance around the electrodes with the increase in the concentration of bacteria in the solution. In this method, the silver enhancement method improved the sensitivity of dielectrophoresis and the impedimetric method. Along with, AuNPs worked as a catalyst for the silver enhancement process. The LOD obtained with the sensor was 500 cfu/mL.

5.3.2 Optical Microfluidic Chip Nanosensors

Following are the few optical microfluidic chip nanosensors for the detection of pathogenic bacteria. All the nanosensors mentioned in this section are summarized in Table 5.1, and their working principles are pictorially represented in Fig. 5.3.

A colorimetric biosensor for detection of *E. coli* in food samples using the principle of aggregation of AuNPs and smartphone imaging HSL (hue-saturation-lightness) app to analyze color changes was developed (Zheng et al. 2019). A microfluidic chip with two serpentine mixing microchannels, one separation chamber, and one detection chamber was fabricated using PDMS/glass. In the study, MNPs were immobilized with capture pAbs, and PSs were modified with detection mAbs and catalase for detection of *E. coli*. Firstly, modified MNPs and PSs were passed into the chip with the sample solution containing *E. coli*. In the first mixing channel, MNPs, PSs, and *E. coli* interacted with each other, leading to the formation of MNPs/*E. coli*/PSs complex. Then, after mixing, the complex was separated in the separation chamber using external MF. Further, H₂O₂ solution was passed into the chamber, which was catalyzed by the catalase enzyme attached to PSs according to the concentration of bacteria. The catalysate from the separation chamber was mixed with cross-linking agents containing AuNPs, HRP, and TYR in the second mixing channel, and the color of the solution was checked in the detection chamber. In the absence of bacteria, catalase enzyme was not found in the separation chamber; hence, H₂O₂ was not converted into H₂O. This led to the aggregation of AuNPs (blue-colored solution) by hydroxyl radical through the crosslinking of phenolic hydroxyl moieties in TYR by the C–C and C–O couplings between aromatic rings. On the other side, in the presence of bacteria, a red-colored solution was obtained due to the conversion of H₂O₂ into H₂O in the separation chamber, which further did

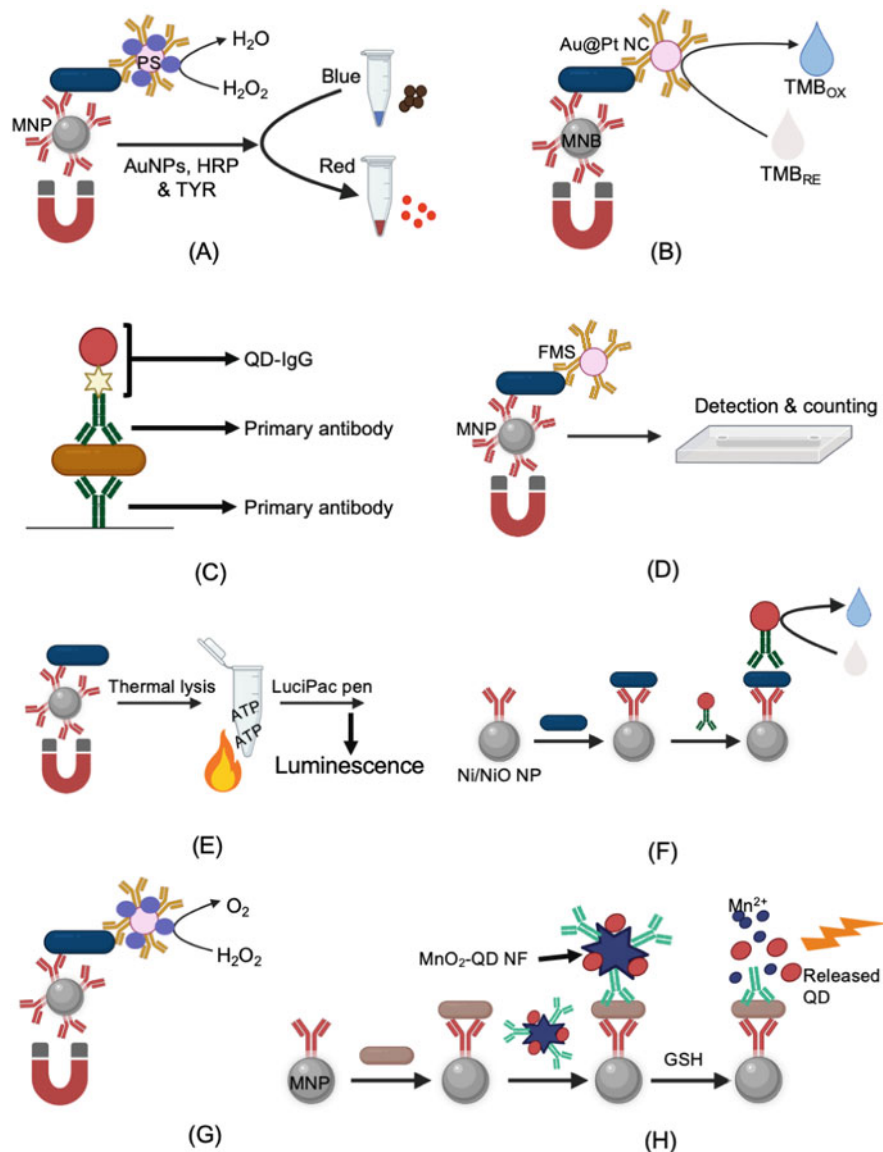


Fig. 5.3 A schematic representation of working principle of optical microfluidic chip nanosensors. (a) Colorimetric microfluidic chip nanosensor (Zheng et al. 2019). (b) Finger-actuated microfluidic chip nanosensor (Qi et al. 2022). (c) Fluorescence-based microfluidic chip nanosensor (Wang et al. 2015). (d) Fluorescence-based microfluidic chip nanosensor (Wang et al. 2019a). (e) Luminescence microfluidic chip nanosensor (Park et al. 2017). (f) Colorimetric microfluidic chip nanosensor (Weng et al. 2021). (g) Colorimetric microfluidic chip nanosensor (Cai et al. 2019). (h) Fluorescence microfluidic chip nanosensor (Hao et al. 2020). The representation of working principle in this figure is to explain the mechanism and adapted from the mentioned references. The original research article representation of these chips may vary from the one shown in the figure. Created using [BioRender.com](https://www.biorender.com)

not initiate the aggregation of AuNPs. The intensity of the red color was in accordance with the concentration of bacteria in the sample. The LOD of the sensor was 50 cfu/mL. In the biosensor, MNPs were used for immobilization of antibodies due to their high surface area, and AuNPs were utilized for detection (Fig. 5.3a).

A finger-actuated microfluidic biosensor was fabricated using immune magnetic nanobeads (MNBs), gold@platinum nanocatalysts (Au@PtNCs), and a smartphone app for the detection of *Salmonella* (Qi et al. 2022). The chip was fabricated with three PDMS layers: the top layer consisted of microfluidic channels, and the middle and bottom layers comprised of pneumatic channels (valves, button, and pumping chamber). The chip had three regions: a finger-actuated one-way micropump, a finger-actuated micromixer, and a colorimetric card. Anti-*S. typhimurium* pAbs-modified MNBs, bacterial sample, and anti-*S. typhimurium* mAbs-modified Au@PtNCs were injected into the micromixer using the finger-actuated micropump. After mixing and incubation in the micromixer, a MNB-*Salmonella*-Au@PtNCs complex was formed. Then, the complex was magnetically separated in the chip, and colorless substrate (H_2O_2 -TMB) was passed into the chip, which was catalyzed by the Au@PtNCs present in the complex to produce blue-colored catalysate (TMB_{ox}) (Fig. 5.3b). Lastly, the color of the catalysate was observed with the naked eyes by comparing it with a colorimetric card or smartphone app for quantitative value. The LOD of the sensor was as low as 350 cfu/mL. In this study, Au@PtNCs worked as nanozymes for the catalysis of H_2O_2 -TMB into a colored product.

To detect *S. typhimurium*, a microfluidic chip was developed using CdSe/ZnS QDs labeling (Wang et al. 2015). The chip was fabricated using PDMS and glass, which consisted of 12 channels, 3 mixing zones, and 6 immune reaction/detection zones. Firstly, primary antibodies against *S. typhimurium* were immobilized in the detection zone, followed by injection of *S. typhimurium* sample in the chip. Then, primary antibodies and QDs-IgG were flowed into the chip through mixing zones to form QDs-IgG-primary antibody complex, which further reacted with captured bacteria in the detection zone (Fig. 5.3c). Lastly, the fluorescence intensity of detection zones was analyzed for quantification using self-assembled light-emitting diode-induced fluorescence detection (LIF) microsystem consisting of a LED, photomultiplier tube, and data acquisition circuit. The LOD of the system was 37 cfu/mL. The QDs here were utilized for supersensitive fluorescent labels because of their bright fluorescence, which helped in improving the sensitivity of the method.

A microfluidic biosensor was reported for online detection of *S. typhimurium* in food samples using fluorescence labeling and smartphone video processing (Wang et al. 2019a). A microfluidic chip was fabricated using PDMS/glass, which consisted of two parts, a fan-shaped cylinder filter and a line-shaped microchannel. The filter avoided the blockage of the chip with large-sized particles, and the microchannel was used to detect and count the fluorescent spots. Firstly, MNPs were modified with mAbs against *S. typhimurium*, which were used to separate the bacteria from the sample. After magnetic separation, fluorescent microspheres (FMSs) functionalized with pAbs were incubated with the MNP-bacteria complex, resulting in the formation of MNP-bacteria-FMS complex (Fig. 5.3d). The resultant complex was reconstituted in buffer after magnetic separation and passed into the chip to detect

and count the bacteria. For detection, an automated smartphone-based fluorescent microscopic system was developed for the analysis. MNPs were used for immobilization of antibodies due to their high surface area, which also assisted in better separation of bacteria using external MF. LOD of 58 cfu/mL was obtained with the system.

For diagnosis of bacterial pathogens in cases of sepsis, a 3D-printed microfluidic magnetic preconcentrator (3D μ FMP) was designed using luminescence-based detection and MNP-conjugated antibodies (Park et al. 2017). The chip was fabricated with plastic using 3D printing. MNPs modified with anti-*E. coli* antibodies were first incubated with the sample containing the bacteria, which resulted in the production of bacteria-Ab-MNP complex. These complexes were concentrated in the chip using a permanent magnet (Fig. 5.3e). Then, the concentrated sample was thermally lysed to extract ATP, and this ATP luminesced on dipping LuciPac Pen (luciferase and powder) in the solution. The ATP concentration was directly proportional to the bacterial concentration in the sample, and hence, the luminescence also increased. In this chip as well, MNPs were used for immobilization of antibodies due to their high surface area and preconcentration. Using the mechanism, a low concentration of *E. coli*, 10 cfu/mL, was detected. Similarly, a magnetic microfluidic ELISA system was constructed for determining *Streptococcus pneumoniae* (*S. pneumoniae*) (Weng et al. 2021). Firstly, paramagnetic surface-oxidized nickel NPs (Ni/NiO NPs) were synthesized to carry histidine-tagged G β 1 protein, which were then used to immobilize specific antibodies (SMU290) against *S. pneumoniae* surface protein. Here, three-dimensional magnetic beads enhanced the area of contact between the antigen and bead, resulting in an increase in the sensitivity of the assay. This complex was mixed with the sample and passed into the microfluidic chip for the detection of *S. pneumoniae*. The chip consisted of three layers: PMMA top layer, PDMS middle layer, and bottom layer of glass. The system was controlled with Arduino and a motor for automation. A layer of trichloro-(1H,1H,2H,2H- perfluorooctyl)-fluorane fluoroalkyl silane (FAS) was coated on the glass layer of the chip in the form of rectangular regions. Further, mineral oil was mixed in the hydrophobic region of the FAS coating to separate the hydrophilic detection zone. PDMS layer consisted of five circles and five ellipses. Holes were drilled in the topmost PMMA layer to operate the PDMS layer. While assembling the chip, the hydrophobic solutions present on the glass layer were entrapped within the ellipse-shaped structures of the PDMS layer. In the hydrophilic region of the glass (circular regions in the PDMS layer), various detection solutions, like HRP labeled secondary antibody solution and TMB reagent, were added, which were manipulated under a MF to cross the hydrophilic/hydrophobic interface. The platform reduced the analysis time to 15 min and increased sensitivity (LOD 10^5 – 10^7 cfu/mL) in comparison to conventional ELISA (10^6 – 10^9 cfu/mL). In comparison to a conventional 96-well plate, the setup can analyze only a single sample; hence, it can be improved further. The working principle of the chip is shown in Fig. 5.3f.

For the detection of food pathogens, like *S. typhimurium*, a microfluidic immunosensor was reported using the principle of sandwich ELISA assay (Cai et al. 2019). The microfluidic chip was fabricated with a top layer of PDMS and a

bottom layer of PMMA (for fixing PDMS). PDMS consisted of different zones: (1) a press chamber prefilled with oil to drive samples and H_2O_2 on pressing, (2) two chambers for sample and H_2O_2 , (3) multiple asymmetric rings for efficient mixing of sample and H_2O_2 , and (4) one chamber for red dye for visual detection. Firstly, anti-*Salmonella* mAbs-modified MNPs were incubated with the sample to separate the bacteria from the sample to form MNPs-*Salmonella* complex. Then, PSs conjugated with anti-*Salmonella* pAbs and catalases were added to further give rise to MNPs-*Salmonella*-PS-catalase complex (Fig. 5.3g). This complex, after magnetic separation and resuspension into the buffer, was passed into the chip. The catalase enzyme converted H_2O_2 into O_2 , which increased the pressure in the prefilled red dye chamber. This led to the movement of the dye out of the chamber into the serpentine microchannel provided with a scale to quantify the concentration of bacteria in the sample. The LOD of the chip was 150 cfu/mL for *S. typhimurium*. This system can lead to the development of an inexpensive and easy diagnostic product in the market.

A microfluidic chip biosensor using NMs was fabricated to determine *S. typhimurium* in food samples (Hao et al. 2020). A microfluidic chip with a mixing channel, incubation channel, and separation chamber was developed using PDMS/glass. MNPs were modified with mAbs specific for *Salmonella*. Amino-modified MnO_2 nanoflowers (NFs) were conjugated with carboxyl-modified QDs to form MnO_2 -QDs NFs, which were further functionalized with pAbs to give rise to MnO_2 -QDs-pAb NFs. A bacterial sample was passed into the chip along with MNPs and MnO_2 -QD-pAb NFs for mixing in the mixing channel. After efficient mixing, a complex of MNP-bacteria-QD- MnO_2 was formed within the incubation channel. Then, the complexes were captured in the separation chamber using external MF, and glutathione (GSH) was injected into the chamber, which dissolved MnO_2 into Mn^{2+} , resulting in the release of QDs (Fig. 5.3h). This phenomenon increased the fluorescence of QDs proportionally to the concentration of bacteria present in the sample. LOD of 43 cfu/mL was achieved with the sensor.

5.4 Conclusions

Pathogen detection is closely associated with human health; therefore, entry of potent tools is always welcome to ensure good health. The major challenge toward pathogen detection is the scarcity of the following listed features altogether, like sensitivity, stability, accuracy, affordability, rapid, simplicity, automated, compact, and no requirement of expensive reagents/equipment, in classical, biochemical, and advanced biotechnology methods (Doron and Gorbach 2005; Srivastava et al. 2018; Bhardwaj et al. 2022). The characteristics here noted are expected to be fulfilled by uniting microfluidics biosensing with nanotechnology (Mark et al. 2010; Tay et al. 2016). The integration of these two different techniques offers all the qualities, such as compactness, faster processing time, specificity, selectivity, sensitivity, easeful use, etc., which are necessitated for satisfying the WHO's ASSURED criteria to select diagnostics (Taylor et al. 2014; Beltrán-Pineda et al. 2021).

Research communities are integrating these two technologies to bring all the qualities in a single product. Unfortunately, out of them, only a handful of products are commercialized and are in the market. Following are a few of the challenges that need to be resolved for further translation of microfluidic chip nanosensors into diagnostic products. For instance, the reproducibility and stability of NMs resist the deployment of nanosensors for clinical diagnosis. This issue can be easily tackled by the standardized techniques for mass production and strict quality check assurance of NMs by industries for the construction of NMs (Liu et al. 2019; Bobrinetskiy et al. 2021). The other issue is the non-specific adsorption of interfering agents on the surface of NMs due to their large surface area. The use of appropriate blocking agents, like bovine serum albumin, skim milk, or ethanolamine, etc., can be a solution to this problem (Liu et al. 2019). The third problem can be due to the presence of high-density surfactants on the surface of NMs used during their synthesis. This leads to difficulty in the immobilization of antibodies on the surface of NMs (Wu et al. 2019). The main challenge remains lie in their practical application due to the lack of achievement of required sensitivity, low cost, and rapidity (Bobrinetskiy et al. 2021). The other challenge is related to customer acceptance, complex and long regulatory approval protocols, and acceptance of microfluidics in the market/industry, which hinders commercialization of microfluidic chip nanosensors in the market. In addition to these issues, improvisation in accuracy, detection unit setup, sensitivity, and performance is required in microfluidic chip nanosensor to match current gold standard methods. Hence, these issues need to be resolved for inviting efficient products into the market.

In the future, we expect the generation of NMs with excellent properties gifting new capabilities to microfluidic chip nanosensors. Up till now, NMs have demonstrated tremendous growth in microfluidic chip nanosensors for the detection of pathogenic bacteria in terms of reaching high sensitivity. Active collaboration of nanotechnology and microfluidic chip biosensing can lead to a POC diagnostic device for detecting pathogenic bacteria, which can bridge the existing gap between research investigations and commercialization. Microfluidic chip nanosensors have great scope for future-generation diagnostic tests for the detection of pathogenic bacteria. It will be interesting to witness the translation of microfluidic chip nanosensors into the laboratory and clinical application for the diagnosis of pathogenic bacteria.

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