
5 Microfluidic-Based Plasmonic Nanosensors for Biological and Chemical Threats

*Özgecan Erdem, Eylül Gulsen Yilmaz,
Beyza Nur Küçük, Fatih Inci, and Yeşeren Saylan*

5.1 INTRODUCTION

The potential for diseases or harmful chemicals to spread quickly is greater than ever in the modern world due to expanding trade, quick international travel, and the increased rate of communication. If hazardous chemical and biological threats cannot be detected early and accurately, they can cause severe public health problems and large-scale catastrophes. Recently, the world has faced one of the most urgent pandemics due to the severe coronavirus disease 2019 (COVID-19), which caused approximately 7 million deaths worldwide. Since early diagnosis is crucial to initiate treatment and prevent the spread of the virus, during that period many researchers gave priority to developing sensing devices (Ji et al., 2023; Torres et al., 2021). In 1995, as a result of a bioterrorism attack, thousands of people were affected in the Tokyo subway Sarin attack which was caused by a colorless and toxic nerve agent that can cause death in minutes. After such an attack, the detection of toxic chemicals, which can create residues and contaminate water and soil, also became important for protecting public health in mass living areas (Tan et al., 2006). In addition, research into biological pathogen detection, including *Salmonella* and *Escherichia coli* in food products, has been ongoing for decades (Wang et al., 2019). In the developing world and contemporary analytical science—in light of newly emerging infectious diseases and pollution owing to released hazardous substances—the requirement for enhanced, robust, and rapid sensing methods able to detect biological and chemical threats with high sensitivity and specificity has reached extraordinary levels. Since conventional detection methods, characterized by requiring high sample volume and prolonged analysis time, are not sufficient, researchers are trying to develop new sensor platforms and adapt conventional techniques to them (Figure 5.1).

One of the biggest contributions to the development and progress of biotechnological systems has been the adaptation of microfluidic platforms (Sackmann et

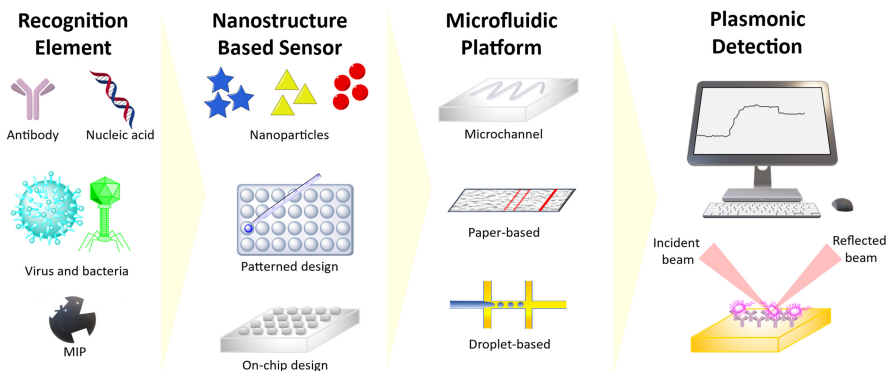


FIGURE 5.1 Scheme of microfluidic integrated nanosensors via various recognition elements. Readapted from C. Jin et al. (2022) with the permission of Elsevier.

al., 2014; Whitesides, 2006). Microfluidics can be defined as a micro total analysis system (μ TAS) that allows the manipulation of micro or less volume of liquid (10^{-9} to 10^{-18} L) in submillimeter-sized channels. By applying a drawing or repulsing force in a controlled manner, sample liquid and analyte molecules in the sample such as cells, micro and nanoparticles, protein, or nucleic acid residues, etc. can be directed and detected with high sensitivity from a small amount of sample (Oh, 2020). Considering the parallelized structure of the microfluidics, more than one transaction can take place, which provides high throughput processing and miniaturized sensor units (Lakhera et al., 2022). Microfluidics can be integrated with different types of nanosensor platforms, such as electrochemical (Schmidt-Speicher and Länge, 2021), mechanical (Thies et al., 2017), or optical (Tokel et al., 2015). These small-scale functional devices can be defined as lab-on-a-chip (LOC) platforms. After their surface is modified with antibody, nucleic acid, or molecularly imprinted polymer (MIP) as target-specific recognition elements, they can handle multi-step processes including sample loading, washing, signal amplification, and conversion. Among the optical nanosensors, plasmonic nanosensors such as surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), and nanostructured photocrystals are well-established systems in terms of monitoring molecular interactions without the need for educated or expert people (Estevez et al., 2012). These methods are thorough-paced compared to regular methods in terms of label-free detection, real-time monitoring, short response time, and reusability (Shrivastav et al., 2021). Plasmonic nanosensors typically consist of a plasmonic material (generally a thin metallic film) and a dielectric material (such as liquid solution). When exposed to light, the free electrons in these continuous metallic structures collectively oscillate at the boundary of the plasmonic surface, creating what is known as SPR (Masson, 2020). These resonances are extremely responsive to alterations in the immediate surroundings, such as fluctuations in the refractive index, brought on by the presence of analytes on or close to the nanosensor's surface, such as cells, pathogens, or proteins. With the development of nanotechnology in recent years, the

utilization of especially gold (Au) and silver (Ag) nanostructures (particles, rods, pillars, etc.) have gained importance for enhancing the plasmonic properties of nanosensor platforms with their improved electrical and optical features (Anker et al., 2008; Inci et al., 2020a). The utilization of nanostructures in nanosensor technologies was initiated with the use of metallic nanoparticles for LSPR. Unlike surface plasmons (SP), localized surface plasmons (LSP) are collective electron oscillations confined to nanoparticles or nanostructures which are significantly smaller than the wavelength of incident light (Hutter and Fendler, 2004). Both material type (Au, Ag, titanium, etc.) and morphological features of the nanostructures (size, size distribution, shape, etc.) can significantly affect the absorption bands of the plasmonic materials (Brahmkhatri et al., 2021).

In this chapter, microfluidic integrated plasmonic nanosensor applications for biological and chemical threat detection will be covered in detail. The fabrication methods of microfluidics and commonly used microfluidic nanosensor types, the basics of plasmonics, the detection of biological and chemical targets via these systems, advantages and disadvantages, and future expectations are discussed in the next sections of the chapter.

5.2 MICROFLUIDICS

Microfluidic technology, a fast-developing branch of science distinguished by microscale effects, has become more important in a variety of applications, from micro-arrays to cellular biophysics. The term “lab-on-a-chip” was initially used by Tabeling in 2005 to describe these devices, which typically range in size from 10 to 100 micrometers (Tabeling, 2023). With this cutting-edge technology, numerous processes—including sample processing, reactions, separation, and detection—are combined onto a little chip that is only a few square centimeters in size. The main goal is to carry out analyses that are quick, effective, and resource-efficient. The creation of tiny devices that painstakingly control physicochemical processes inside fluid containers is where microfluidic technology thrives. These microstructures support a variety of microfluidic phenomena, including diffusion, laminar flow, surface tension, capillary effects, and rapid heat conduction, providing fine fluid control and prompt responses (Mou et al., 2016). Fluids have particular analytical capabilities because of the peculiar features they exhibit due to their micron-scale structures that are not visible at macroscopic scales. These chips’ diminutive size and larger surface area improve analytical throughput and mass transfer (Zhang et al., 2022). The need for significant sample and reagent volumes is greatly reduced by this breakthrough, which also makes multiplexing and high-throughput screening possible (Cui and Wang, 2019). In addition to its broader applications, microfluidics is increasingly playing a pivotal role in several specialized fields. For instance, microfluidics empowers the creation of highly precise microenvironments for regulating cell growth and differentiation in tissue engineering, thus enabling the generation of functional tissue for applications in regenerative medicine (Gharib et al., 2022). In the realm of drug discovery, microfluidic systems enhance high-throughput screening by facilitating the rapid evaluation of multiple drug candidates

within controlled settings. These systems emulate natural physiological processes and contribute to the assessment of drug effectiveness and safety. Conversely, microfluidic-based sensing devices offer a level of accuracy and sensitivity in the detection of a wide array of biological substances, a capability of paramount importance for tasks such as environmental monitoring, point-of-care diagnostics, and biomedical research.

5.2.1 MATERIALS FOR MICROFLUIDICS

Making a careful choice of the best material for creating devices is a crucial step in microfluidic applications. This decision is crucial because materials' features are greatly enhanced at the microscale, where they may have an impact on the properties of newly created nanomaterials (Song et al., 2008). Shorter retention durations, laminar flows, increased mass, and heat transfer rates, as well as significant surface-to-volume ratios, especially in capillary microfluidics, bring out different phenomena (Ren et al., 2013). Contrary to macroscale vessels, aqueous solution wetting behavior and contact angles on chip materials are crucial. Material selection involves considering several crucial factors beyond simple composition. These include but are not limited to resilience, fabrication simplicity, translucency, suitability for biological applications, compatibility with planned reagents, the ability to withstand required temperature and pressure conditions during reactions, and the potential for surface customization (Shakeri et al., 2019).

There are several materials competing to fulfill these strict requirements and be acceptable for producing microfluidic devices. Glass, silicon, polymers, and ceramics are common substrates, while new materials of variable quality are constantly being developed. Based on the intended use, each material has a unique set of benefits and disadvantages. Due to its chemical inertness, thermostability, electrical insulation, rigidity, biocompatibility, and ease of surface functionalization, glass is an extremely suitable material for microreactors (Shakeri et al., 2019).

Glass microreactors are the best choice for carrying out chemical reactions when pressures, temperatures, and solvents are high due to these characteristics (Hwang et al., 2019). In comparison to other materials, glass microcapillary reactors have finer resolution at the micrometer scale, allowing for more exact control over the creation of polymeric nanoparticles and emulsions (Ofner et al., 2017). Excellent optical transparency, cost-effectiveness, and the ability to integrate active components are additional benefits of glass over silicon. Despite difficulties in production and clean room preparation, glass is still one of the most generally utilized materials for microfluidic chips due to its compatibility with biological samples, which makes it ideal for biochemical studies (Nielsen et al., 2019).

Silicon is a popular choice for microfluidic chips owing to its chemical compatibility, availability, and thermostability (Campbell et al., 2020). It offers easy fabrication, flexibility of design, semiconductor features, and surface modification potential, making it the dominant material in microfluidics for years. However, it has limitations, notably its opacity, which is unsuitable for visible and ultraviolet optical detection (Nielsen et al., 2019). In-situ imaging may require non-silicon

components, and silicon's fragility and high modulus pose challenges for integrating active elements like valves and pumps. Silicon is also relatively expensive. Despite this, it finds use in medical diagnostics and drug screening (Jiang et al., 2022).

Polymers have gained traction in microfluidic chip manufacturing, moving away from silicon and glass, due to their cost-effectiveness and ease of production. These versatile platforms find applications in nanoparticle synthesis, fluid manipulation, and more, accommodating temperatures up to 200°C, ideal for large-scale production. These transparent polymeric materials offer optical access for monitoring reactions, crucial for processes like nanocrystallization. Common polymers used include polydimethylsiloxane (PDMS), poly (methyl methacrylate) (PMMA), cyclo-olefin polymers, fluoropolymers, and copolymers (COPs/COCs) and hydrogels. PDMS, a representative polymer, stands out for its affordability, ease of molding, optical transparency, and gas permeability (Syed et al., 2014). Valued for prototyping, it finds use in bio-related research, disease diagnosis, and cellular assays. However, its porosity limits compatibility with certain solvents and leads to water evaporation issues. PMMA, another choice, offers good solvent compatibility, optical transparency, and mechanical properties, suitable for research settings, organ-on-a-chip platforms, and micro-physiological systems (Kotz et al., 2020; Nielsen et al., 2019). Perfluorinated polymers, like Teflon, excel with thermal processability, chemical inertness, and antifouling properties. Polytetrafluoroethylene (PTFE), in particular, tolerates a broad range of chemicals and high temperatures, making it useful for synthesis devices (Liao et al., 2019). COPs/COCs have gained interest for their optical transparency, chemical resistance, and minimal water absorption, ideal for aggressive solvents (Bruijns et al., 2019). Hydrogels, though less common as the primary material, offer biocompatibility, low cytotoxicity, and tunable pore sizes, suitable to encapsulate cells in tissue engineering and substance delivery (Yilmaz et al., 2023). These materials can enhance microfluidic component functionality, including semi-permeable barriers and smart valves, with chips primarily composed of rigid materials (Goy et al., 2019).

5.2.2 FABRICATION METHODS FOR MICROFLUIDICS

The fabrication of microfluidic chips demands a meticulous approach rooted in biological principles. Various methodologies exist for crafting these chips, each offering distinct advantages and limitations (Akceoglu et al., 2021). As shown in Figure 5.2, soft lithography, for instance, stands as one of the most prevalent techniques, employing flexible materials like polymers to generate microfluidic channels and structures (Scott and Ali, 2021). This process involves molding or patterning the polymer, followed by curing it through heat or ultraviolet radiation. It excels in crafting intricate three-dimensional structures, proving cost-effective and user-friendly. Photolithography represents another avenue for microfluidic chip fabrication. Here, light is harnessed to pattern a photosensitive material, like SU-8 photoresist, on a substrate (Lee et al., 2015). The developed photoresist yields a patterned mask, guiding the etching of desired channels and structures in the substrate (Ma et al., 2010). While photolithography boasts high resolution and the ability to craft complex

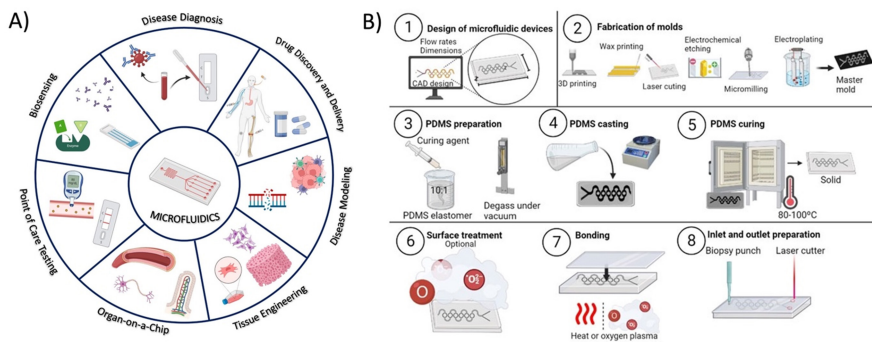


FIGURE 5.2 Common applications (A) and fabrication methods of microfluidics (B). Reproduced from Gharib et al. (2022) with the permission of MDPI and reproduced from Rodríguez et al. (2023) with the permission of Frontiers.

structures, it can be costly, time-consuming, and less suited for high-volume production (Nguyen et al., 2022).

Micromachining emerges as a third fabrication technology, involving cutting, drilling, or etching microfluidic channels and structures into a substrate through various tools and techniques (Juang and Chiu, 2022). For instance, concentrated ion beams may be employed to shape channels in silicon wafers or drill holes in glass substrates. While this method enables the creation of exceedingly small and intricate structures, it can be costly and challenging to control. Micro-molding, another prevalent approach, utilizes a mold to craft desired structures in a polymer material, such as PDMS (Scott and Ali, 2021). Typically, molds are fashioned using photolithography or other methods and are employed to create channels and structures in polymeric materials. Micro-molding offers advantages like affordability and high throughput and can be readily scaled up for extensive production. However, it may lack the precision of photolithography and can pose challenges in crafting complex structures. Lastly, microfluidic systems can also be manufactured using 3D printing techniques, necessitating specialized printers capable of fine-resolution material deposition, including polymers and metals. Typically guided by computer control, these printers allow for precise construction of intricate 3D structures and enable the customization of designs (Jin et al., 2022).

5.2.3 TYPES OF MICROFLUIDICS

Droplet microfluidic technology harnesses tiny liquid droplets enclosed within an immiscible fluid, serving as minuscule independent reaction chambers, whether in continuous or segmented flow (Cui and Wang, 2019). These methods bring forth notable benefits, including minimized sample usage, accelerated reaction rates, and heightened reliability and consistency, all while enabling precise control over composition within these extremely minute volumes. Also, these methods have a variety of uses, including chemical synthesis, drug screening, and biochemical analysis.

In contrast, “Digital Microfluidics” elevates droplet manipulation to an art form by using electrodes and automation to precisely direct discrete droplets over open surfaces (Zhai et al., 2020). This digital precision streamlines laboratory processes, making it essential for uses like single-cell analysis and digital polymerase chain reaction (PCR). Contrarily, “Paper-Based Microfluidics” values affordability and simplicity, relying on capillary action to support point-of-care diagnostics even in environments with low resources. This method offers quick and affordable testing solutions that are essential for both environmental monitoring and medical diagnosis (Nishat et al., 2021). “Lab-on-a-chip Systems” are microchip-sized wonders that can house full laboratories. These systems combine tasks ranging from sample preparation to analysis, revolutionizing analytical chemistry, DNA sequencing, and medical diagnostics (Francesco et al., 2019). “Organ-on-a-chip Technology,” however, transports us to a world where physiological organ conditions are precisely duplicated in tiny settings (Bhatia and Ingber, 2014). By bridging the gap between controlled lab trials and real-world circumstances, this breakthrough redefines drug testing, disease modeling, and organ function studies. In summary, the field of microfluidics is a multidimensional setting where accuracy and miniaturization intersect. These various microfluidic technologies, each equipped with special capacities, demonstrate the enormous potential of microscale activities. They enable scientists to rethink possibilities and shape the future of science and technology, from high-throughput chemical synthesis to mimicking human organ functioning.

5.3 MICROFLUIDIC-BASED PLASMONIC NANOSENSORS

5.3.1 PRINCIPLES OF PLASMONIC NANOSENSORS

A surface plasmon is created as photons interact when the surface of a metal dielectric with SPPs is struck, and the wavelengths in the SP are sensitive to the refractive index of the medium (Akgönüllü et al., 2023) (Figure 5.3A). Because of these properties, there are several different types of plasmons that can be generated from SPP and used in optical-based measurements, including SPR and LSPR (Duan et al., 2021). Both these nanosensors mentioned are sensitive to local refractive index changes that happen when the target is bound to the surface. Therefore, these nanosensors can generate a response without labeling (via chromophore and fluorophore, etc.), unlike conventional optical nanosensors (Choi and Choi, 2012). In SPR, the interacting electromagnetic field at the metal and dielectric interface initially excites collective coherent oscillations of free electrons in the metal’s conduction band, and SPPs are generated with subsequent charge density oscillations as shown in Figure 5.3B. After penetrating a few nanometers into the surrounding matrix, SPPs generate an electric field that decays exponentially (Srivastava et al., 2018). The capacity of nanoparticles to resonantly couple incoming photons to collective oscillations of free electrons on their surface is defined as LSPR, as indicated in Figure 5.3C (Yildirim et al., 2021). Optical nanosensors based on metallic nanoparticles’ LSPR have gained growing popularity for detecting various chemical and biological species (Taghavi et al., 2020; Tian et al., 2021; Versiani et al., 2020).

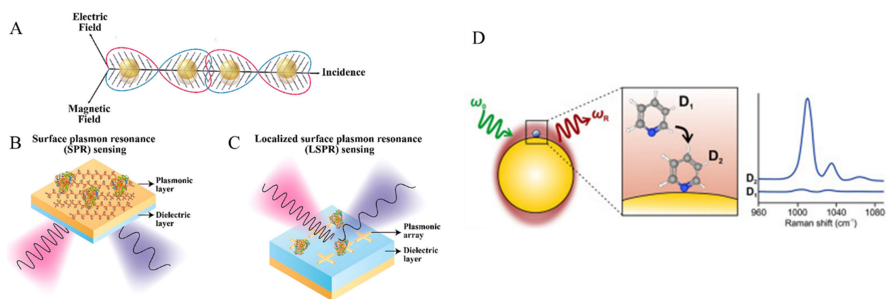


FIGURE 5.3 Illustration of plasmonic effects which are created by collective oscillation of free electrons in response to a component of the incident light (A), geometry-dependent properties of plasmonic materials of SPR (B) and LSPR (C), and conduction band electron resonance oscillation and the generation of a localized strong electromagnetic field zone on the surface of materials related to SERS enhancement (D). Reproduced from Amrollahi et al. (2021) with the permission of ACS Publications and reproduced from Leong et al. (2022) with the permission of Elsevier.

Because the stimulation of surface plasmons is deeply dependent on the dielectric medium's refractive index, every contact between the analyte and the plasmonic nanoparticle modifies the resonance state (Amirjani et al., 2022). Gold nanoparticles (AuNPs), in particular, when paired with a thin metal surface, improve the SPR response in nanosensors, whereas silver nanoparticles create a robust and acute plasmon resonance (Saylan, 2023). When exposed to incoming light, these resonances are caused by the collective oscillation of free electrons on the nanoparticle surface. This interaction with light produces stronger electromagnetic fields surrounding nanoparticles, which can be exploited for sensing purposes (Liu et al., 2018).

Surface-enhanced Raman scattering (SERS), in addition to the previously described SPR and LSPR nanosensors, is employed for the quantitative measurement and accurate determination of (bio)chemical compounds. Raman spectroscopy is fundamentally based on the inelastic scattering of a photon by molecules. The vibrational or rotational modes of the molecules are relaxed or excited during this scattering process, creating a shift in the wavelength of the photons as depicted in Figure 5.3D. Because each chemical species has unique vibrational energy levels, a fingerprint Raman spectrum for each molecule is theoretically possible (Demirel et al., 2018). SERS has evolved as a strong, label-free sensing approach, capable of capturing “fingerprint” data from biological cells, organelles, and molecules down to the single-molecule scale (Bai et al., 2019; Lv et al., 2016; Nikelshparg et al., 2022). Due to local field enhancement, surface plasmons are essential in increasing Raman signals for target molecules near plasmonic nanostructures (Peng et al., 2021).

Overall, in the context of nanosensors, the interaction between plasmonic nanomaterials and target analytes can be understood in several key steps: (i) Surface functionalization of plasmonic nanomaterials with antibodies (Panchal et al., 2022; Pereira et al., 2021), aptamers (Shi et al., 2013; Xiao et al., 2016), or MIPs

(Ahmad et al., 2015; Erdem et al., 2023; Yılmaz et al., 2022) which enable the selective binding of the target analyte; (ii) Local environment changes after binding, i.e., changes in the refractive index (Omrani et al., 2021), electric field distribution (Ndukaife et al., 2014), and dielectric constant (Farmani et al., 2020) of the environment of the nanomaterials; (iii) Shifts in plasmon resonance, which is related to the presence and concentration of the target analyte (Li et al., 2019; Ye et al., 2022).

5.3.2 INTEGRATION OF MICROFLUIDICS AND PLASMONIC NANOSENSORS

Microfluidic lab-on-a-chip systems, which are miniaturized and require only small volumes of liquid, have recently been created and are suited for a variety of applications such as infectious illness (Kanithamniyom et al., 2021; Saylan and Denizli, 2019) and cancer diagnostics (De Oliveira et al., 2018; Zilberman and Sonkusale, 2015), as well as environmental monitoring (Li et al., 2022; Tavakoli et al., 2022). Integrating microfluidic platforms and optical imaging systems joins the benefits of LOC systems and optical technology. Photonics and plasmonics (i.e., SPR, LSPR, nanostructured photonic crystals) are at crossroads of optics and nanotechnology, assisting in developing reliable, accurate, and simple-to-use sensor platforms. Plasmonic LOC systems may be created as low-cost platforms for point-of-care (POC) testing by utilizing disposable chips (Aslan et al., 2023).

Integrating microfluidics with plasmonic nanosensors offers many advantages, creating a powerful platform for varied sensing and analytical applications. Microfluidic devices have the capability to handle minimal sample volumes while providing a controlled flow of liquids at the micro and nanoscale (Gimondi et al., 2023). Reduced diffusion times enhance interactions between plasmonic nanomaterials and analytes, resulting in faster and more reliable detection (Srivastava et al., 2018). Multiple parallel channels make the simultaneous analysis of numerous analytes possible (Qu et al., 2020). By combining multiple channels and different channel plans, the platform can handle more than one sample/chip, and it can be changeable for detecting multiple targets on the same chip (Inci et al., 2020b). The combination of microfluidics with plasmonic nanosensors offers a lot of opportunities for POC applications. Microfluidic systems' real-time monitoring and continuous measuring capabilities make it simple to keep track of the quick reactions brought on by the analyte's binding in plasmonic nanosensors (Zhou et al., 2016). Additionally, the mobility and compactness of microfluidic tools, and the receptiveness of plasmonic nanosensors (Duan et al., 2021), can provide rapid and precise diagnosis for bedside applications (Zhou et al., 2019).

In summary, the combination of microfluidics with plasmonic nanosensors affords an adaptable platform for a variety of applications, including healthcare (Ameen et al., 2015; Escobedo et al., 2013; Tian et al., 2022) and environmental monitoring (Mishra et al., 2022; Pilát et al., 2018; Rasheed et al., 2023). This integration improves sensitivity, selectivity, speed, and automation, making it a significant tool in enhancing sensing technology (Luka et al., 2015).

5.3.3 DETECTION OF BIOLOGICAL THREATS

Pathogenic bacteria and viruses pose a significant threat to security through food contamination, bioterrorism, or biological threat (Walper et al., 2018). Most food-borne diseases are caused by a pathogenic bacteria/virus or toxins released from microorganisms. For bioterrorism/biological threats, examining the relationship between aerosol contagion and toxicity and the amount of the substance reduces the number of active substances that can cause the highest number of casualties (Kirsch et al., 2013). Any contagious organism is likely to be misused for criminal or threat approaches. Some biological agents pose a more significant threat than others due to factors such as their virulence or toxicity, as well as the agent's stability in the environment, capacity to infiltrate the host organism, and so on (Pohanka, 2019).

The threat from biological agents is scaled via the Centers for Disease Control and Prevention (CDC) using the letters A, B, and C, from highest to lowest, to identify them according to their hazard level (Centers for Disease Control and Prevention, 2018). Table 5.1 provides an overview of significant biological threat agents.

Determining infectious disease threats is a fundamental function of public health. The early detection of a bioterrorism event or the danger of a naturally occurring disease depends on clinicians diagnosing the first few cases or the

TABLE 5.1

Bioterrorism Agents and Diseases (Centers for Disease Control and Prevention, 2018)

Category	Biological Agent	Disease/Toxins
A	<i>Bacillus anthracis</i>	Anthrax
	<i>Clostridium botulinum</i>	Botulism
	<i>Yersinia pestis</i>	Plague
	<i>Variola major</i>	Smallpox
	<i>Francisella tularensis</i>	Tularemia
	Filoviruses (Ebola, Marburg)	Viral hemorrhagic fevers
	Arenaviruses (Lassa, Machupo)	
B	<i>Brucella</i> sp.	Brucellosis
	<i>Clostridium perfringens</i>	Epsilon toxin
	<i>Salmonella</i> sp., <i>E. coli</i> O157:H7, <i>Shigella</i> sp.	Food safety threats
	<i>Burkholderia mallei</i>	Glanders
	<i>Chlamydia psittaci</i>	Psittacosis
	<i>Coxiella burnetii</i>	Q fever
	<i>Ricinus communis</i>	Ricin toxin
	<i>Staphylococcus aureus</i>	Staphylococcal enterotoxin B
	<i>Rickettsia prowazekii</i>	Typhus fever
	Alphaviruses	Viral encephalitis
	<i>Vibrio cholerae</i> , <i>Cryptosporidium parvum</i>	Water safety threats
C	Nipah virus, Hantavirus	Emerging infectious diseases

identification of suspicious clinical appearances by infectious disease specialists. However, recognizing a rare illness that may be seen for the first time is a challenging prospect (National Research Council, 2011). Traditional detection methods, such as biochemical and immunological recognition-based assays and biomolecular techniques such as PCR, or cell culture, are used to detect pathogenic microorganisms or toxins, but detection times are lengthy and necessitate the use of specialized personnel. These approaches must be faster and more selective for environmental monitoring and initiating treatment of exposed persons (Rowland et al., 2016).

The detection platform for POC applications must be simple to apply and understand, inexpensive, durable, stable across a broad variety of operating conditions, and preferably disposable and portable (Wang et al., 2021). One of the other significant requirements for pathogen detection equipment is the capacity to run multiplex testing. To address the present constraints of traditional approaches, LOC and microfluidic devices are gaining traction (Mondal et al., 2020). Owing to the rising security concerns and ongoing war on terrorism, the requirement for fast detection nanosensors against threat agents for civil and military defense applications has risen. However, the availability of rapid, low-cost, accurate diagnostic tests is the primary requirement for the monitoring and controlling of illnesses. Microfluidic systems are a significant option with the attributes discussed in this context (Srinivasan and Tung, 2015). This section briefly summarizes microfluidic integrated nanosensors developed for the fast and accurate detection of biological threat agents.

Bacillus anthracis (*B. anthracis*), which causes anthrax, is a zoonotic disease primarily transmitted from animals and contaminated soil and has also been determined by the CDC as a Category A potential threat agent. The bacterium was utilized as a weapon in the First and Second World Wars and was the cause of deaths in the Soviet Union in 1979, Japan in 1995, and the United States in 2001. *B. anthracis* has aerobic/facultative anaerobic, Gram-positive properties and dormant spores that can survive for decades in soil and are very resistant to extreme environmental conditions. These spores can be transmitted easily from one host to another (Savransky et al., 2020). In a study, a fully automated, SERS-based microfluidic chip was developed for poly- γ -D-glutamic acid (PGA) detection, which is an anthrax biomarker (Gao et al., 2015) (Figure 5.4A–C). In the system developed utilizing anti-PGA-immobilized magnetic beads and AuNPs with PGA in a microfluidic environment, magnetic immune complexes were analyzed by directly measuring their SERS signals after being captured by solenoids placed inside the chip. To increase mixing effectiveness, a mixer was integrated to the channel as shown in Figure 5.4D–E. The limit of detection (LOD) value of PGA in serum in the microfluidic nanosensor was determined as 100 pg/mL (Figure 5.4F).

Humans and other warm-blooded animals' intestines contain the naturally occurring bacteria *E. coli*, which aids in the body's synthesis of vital vitamins. Some strains, nevertheless, are pathogenic. For instance, the strain *E. coli* O157:H7 can create toxins that can damage the intestinal lining and result in illness (Dhull et al., 2019). With a Gram-negative rod shape and all associated characteristics, *E. coli* O157:H7 belongs to the Enterobacteriaceae family. *E. coli* O157:H7's primary

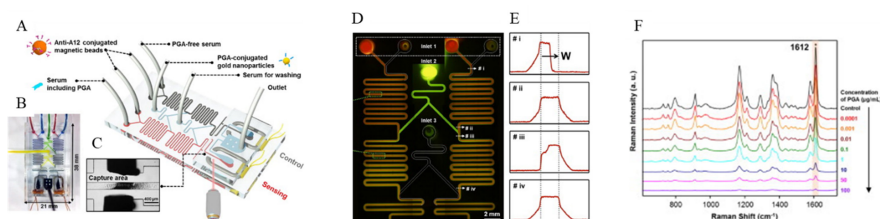


FIGURE 5.4 Scheme of dual-channel of SERS-based microfluidic nanosensor for PGA and control detection (A), photo of chip filled with ink and capture area for magnetic immune complexes (B), process of mixing efficiency analysis using Rhodamine B and Rhodamine 6G solutions (C), photo of fluorescent microfluidic channels (D), fluorescence intensity patterns corresponding to the places indicated via dashed lines (E), and concentration-dependent SERS spectra of PGA detection (F). Reproduced from Gao et al. (2015) with the permission of Elsevier.

reservoir is in the intestines of ruminant animals, which include cattle, sheep, deer, and goats. Animal feces are the pathogen's principal route of environmental release (Sharma et al., 2011). The World Health Organization (WHO) has recently stated that significant *E. coli* O157:H7 outbreaks in the world's highest-income nations, including the UK, Germany, and Canada, have been caused by contaminated food or water (Caprioli et al., 2005). *Salmonella* sp. is another noteworthy pathogen causing gastrointestinal problems in Enterobacteriaceae with Gram-negative, non-spore forming and rod-shaped characteristics. Ingesting *Salmonella*, which is one of the most common causes of diarrhea, by drinking infected water or food, may also result in additional symptoms (Ehuwa et al., 2021). *Salmonella* sp. and *E. coli* O157:H7 have been determined by CDC as a Category B potential agent for biothreat. In a study for the rapid and reliable screening of food-borne bacterial pathogens, an SPR nanosensor depended on very low contamination and functionalizable poly (carboxybetaine acrylamide) (pCBAA) brushes were developed for the detection of bacterial pathogens in raw food samples (Vaisocherová-Lísalová et al., 2016). In this study, raw food samples (cucumber and hamburger) were used. Firstly, the bacteria in the raw food samples were detected by antibodies immobilized to pCBAA. In the second step, the secondary biotinylated antibody (Ab2) was attached to the pre-captured bacteria. Finally, the streptavidin-modified AuNPs were bound to the biotinylated Ab2, increasing the nanosensor response. Also, an SPR nanosensor depended on a pCBAA brush with a thickness as low as 20 nm. In detection studies performed on complex hamburger and cucumber samples, LOD values were 57 cfu/mL and 17 cfu/mL for *E. coli* O157:H7, while these values were 7.4×10^3 cfu/mL and 11.7×10^3 cfu/mL for *Salmonella* sp. A hybrid microfluidic chip was developed in different research to determine *E. coli* O157:H7 using an array of SPR and fluorescence imaging (Zordan et al., 2009). A PDMS microfluidic flow chamber surrounds a sequence of gold dots; each modified with a capture molecule targeting a particular pathogen and supplying a magnetically concentrated sample for testing. To functionalize the gold spots, three biomolecules were used: (i) An antibody that interacts with *E. coli*

O157:H7 specifically; (ii) Rabbit pre-immune serum as a negative control; and (iii) 0.5% bovine serum albumin solution in water as a second negative control. The chip's SPR at the bottom and epifluorescence at the top provides imaging. According to research, the chip's capacity to successfully catch *E. coli* O157:H7 was 97.7% effective. In a different study, vertically aligned carbon nanofibers embedded at the base of a microfluidic chip were used to combine nanostructured dielectrophoresis with nanotag-labeled SERS to monitor pathogen concentration, detection, and kinetics (Madiyar et al., 2015). Nanotag-labeled *E. coli* DH5 α cells were successfully collected by a nanoelectrode array. The core-shell nano-ovals in SERS nanotags are around 50 nm in size, covered with a QSY21 Raman reporter, and attached to *E. coli* by particular immunochemistry. At a concentration as low as 210 cfu/mL and in less than 50 seconds, SERS tests are sensitive enough to identify a single bacterial cell.

An SPR nanosensor was developed for *Brucella melitensis* (*B. melitensis*) detection in milk samples, addressing the global concern of brucellosis, a zoonotic disease often transmitted through contaminated milk products. Through a whole bacteria-SELEX procedure, specific aptamers recognizing *B. melitensis* were selected. Two aptamers, chosen for their remarkable specificity and affinity, played distinct roles in the detection process. The high-affinity B70 aptamer was modified on magnetic silica core-shell nanoparticles to initially purify *B. melitensis* cells from milk matrices. Meanwhile, a B46 aptamer, known for its exceptional specificity to *B. melitensis* cells, was employed to fabricate an SPR nanosensor. These nanosensor chips enabled highly sensitive *Brucella* detection in eluted samples obtained post-magnetic purification. This integrated approach combines efficient magnetic isolation with instant SPR-based detection, achieving an impressively low detection limit of 27 ± 11 cells (Dursun et al., 2022).

A low-noise and affordable SPR nanosensor, utilizing extraordinary optical transmission (EOT) in nanoholes, has shown quantification of a wide range of antibody-ligand binding kinetics. These kinetics encompass equilibrium dissociation constants spanning from 200 pM to 40 nM. This SPR nanosensor is constructed around a standard microscope and a portable fiber-optic spectrometer, making it straightforward to assemble and operate. Remarkably, it achieves a refractive index resolution of 3×10^{-6} , even without on-chip cooling, representing a notable achievement in EOT-based SPR nanosensors. This impressive performance is made possible by the rapid acquisition of full-spectrum data in just 10 ms, after the frame averaging of EOT spectra. This efficiency is facilitated via producing template-stripped gold nanoholes characterized by optical properties across extensive surfaces. To extend its utility, the study conducted sequential SPR measurements employing a 12-channel microfluidic flow cell. These experiments involved meticulous optimization of surface modification procedures and antibody injection protocols to minimize mass-transport artifacts. In the real-time monitoring of the immobilization process, the protective antigen of anthrax, on the gold surface, yielded a remarkable signal-to-noise ratio of approximately 860. After that, the research ventured into quantitatively measuring real-time binding kinetics between antigen and a selection of compact, 25 kDa single-chain antibodies, even at concentrations as low as 1 nM. These findings underscore the vast potential of nanohole-based SPR nanosensors, not only for

quantitative antibody screening but also as a versatile platform, seamlessly integrating SPR nanosensors with a wide array of bioanalytical tools (Im et al., 2012).

The necessity to battle upcoming epidemics and bioterrorism risks cannot be stressed enough, hence the search for speedy and sensitive virus detection technologies that are adaptable across multiple regions. The novel optofluidic label-free nanosensor described in this chapter may detect intact viruses in biological samples at clinically relevant concentrations without sample pretreatment. The nanosensor platform's extraordinary light transmission phenomenon within plasmonic nanoholes serves as its core. Additionally, it uses a large range of rapidly evolving viral strains to target group-specific antibodies. The similar size of the pathogens and the depth of surface plasmon polaritons' penetration, however, raised concerns about the method's possible limitations for detecting viruses. They showed the nanosensor's ability to detect and distinguish between small enveloped RNA viruses, such vesicular stomatitis virus and pseudotyped Ebola, and large enveloped DNA viruses, like vaccinia virus, in order to allay these worries. Their platform has an impressive dynamic detection range of three orders of magnitude. Additionally, it produces data with a high signal-to-noise ratio without the need for mechanical or optical separation, opening up new possibilities for pathogen identification in conventional biology laboratory settings (Yanik et al., 2010).

There has been a continual requirement for quick and visible detection, which has made it a difficult task thus far, given the terrifying potential of ricin-based bioweapon threats. This study presents a method for qualitative ricin analysis and recognition utilizing a gold nanopin-based colorimetric nanosensor that supports multicolor variants. The unique reflected hues produced by the activation of narrow-bandwidth standing-wave resonances in the visible spectrum are attributable to the plasmonic metasurfaces constructed around nanopin-cavity resonators, which are the basis for this nanosensor. As a result of the reflected color amalgamation resulting from various resonant wavelengths, a wide spectrum of colors can be seen. Additionally, these colored metasurfaces display clear fluctuations over a constrained refractive index range, making them particularly well-suited for a variety of applications. As a result, this biosensor, which has nanopin-cavities that have been functionalized with antibodies, has extraordinary sensitivity and quick response times, making it possible to detect ricin visually and quantitatively in a concentration range of 10–120 ng/mL. This method has a lot of potential thanks to its outstanding detection limit of 10 ng/mL and typical measurement duration of under 10 minutes. Furthermore, the on-chip integration of these nanopin metasurfaces into transportable colorimetric microfluidic devices opens doors for quantitative studies spanning a wide range of biological compounds (Fan et al., 2017).

It is extremely difficult to quantify botulinum neurotoxin A (BoNT/A), a bacterial toxin with extraordinary toxicity and medicinal potential in neurological therapies and cosmetics. Accurate measurement is challenging due to its great physiological potency and the requirement for three essential functionalities: Receptor binding, catalytic activity, and internalization-translocation. The mouse bioassay, a long-standing industrial standard that was formerly universally recognized, has become obsolete because of ethical considerations. As a potential solution to this conundrum, cell-based tests were made available. Their labor-intensive nature, however,

has prevented their broad use. This study proposes a novel *in vitro* method that combines microfluidic technology with a nanosensor made with nerve cell-inspired nanoreactors. This nanosensor can assess all three essential functions, making it a useful instrument for estimating the amount of physiologically active BoNT/A. This technique detects and quantifies BoNT/A levels quickly when integrated into a microfluidic device, taking much less time than the mouse bioassay (less than 10 hours versus 2–4 days). Additionally, this approach shows its effectiveness in accurately measuring physiologically active BoNT/A in a straightforward pharmaceutical formulation. This strategy represents a significant ethical advance over *in vivo* methods by providing a thorough *in vitro* testing platform that combines a highly sensitive nanosensor with microfluidic technology. It also offers a workable substitute for time-consuming cell-based *in vitro* detection methods (Weingart et al., 2019).

These state-of-the-art sensing technologies provide a number of critical issues that call for careful evaluation. Making sure that these nanosensors operate dependably and consistently in a variety of environmental scenarios, especially field settings with limited resources, is one of the biggest problems. Assuring that these nanosensors are capable of detecting a variety of biological threat substances with the highest sensitivity and accuracy is another crucial component. Continuous research and development efforts are devoted to improving these technologies to handle these difficulties. The nanosensors' sensitivity and specificity are now being improved by scientists, making them better able to respond to the constantly changing landscape of biological threats. Efforts are also being made to make these technologies easier to use, with the aim of making it possible for anyone to use them effectively in emergency response situations. Wearable technology and artificial intelligence (AI) integration are two notable trends in this area. By aiding in data processing, pattern identification, and real-time decision-making, artificial intelligence, in particular, has the potential to significantly improve the capabilities of these nanosensors. The benefit of continuous monitoring provided by wearables with these nanosensors allows for the early identification of biological risks and prompt notification of users. It is imperative to remain aware of these technologies' ethical implications as they develop. To guarantee responsible implementation, considerations for privacy, data security, and potential for abuse must be taken into account. Furthermore, regulatory frameworks and policies are crucial in regulating the creation and application of these technologies, ensuring that they are ethically acceptable, accurate, and safe. In conclusion, there is constant development taking place in the field of biological hazard detecting technology. Conquering obstacles, enhancing capabilities, and making these nanosensors more useful for actual applications are all ongoing goals. The integration of AI and wearable technology offers the potential for sensors that are more sensitive, accurate, and user-friendly in the future. But it also calls for a watchful attitude to moral considerations and legal compliance.

5.3.4 DETECTION OF CHEMICAL THREATS

Chemical threat agents are substances that can cause harm to humans, animals, and the environment when intentionally released and/or deployed with harmful intent (Saylan et al., 2020). These agents are typically toxic chemicals that can have a

wide range of effects, from causing injury or illness to death, based on the type and concentration of chemical and the exposure duration (Kumar et al., 2023). Chemical threat agents can be categorized into several classes, including blister agents, nerve agents, choking agents, blood agents, vesicant agents, and toxic industrial chemicals (Chen et al., 2018). Chemical threat agents are prohibited by international conventions, including the Chemical Weapons Convention, which aims to eliminate the production, stockpiling, and usage of chemical weapons. The use of chemical threat agents is considered a violation of international law and is widely condemned (Gupta, 2015). Preparedness, detection, and response measures are in place in many countries to mitigate the impact of potential chemical threats, whether they result from intentional acts or accidental releases. The detection of chemical threat agents is of paramount importance for several critical reasons, such as protection of public health, prevention of mass casualties, environmental protection, public safety and security, international security, emergency response planning, public awareness and preparedness, prevention of accidental releases, supporting law enforcement and investigations, and scientific research and monitoring (Black, 2016).

There are some recent studies about microfluidic-based plasmonic nanosensors for chemical threat agents. For instance, Wang et al. introduced an approach to material fabrication, centered around a fluid-based construction method to produce three-dimensional (3D) nanostructures with microchannels (Wang et al., 2016). These nanostructures are precisely positioned to form an integrated microfluidic system designed for the SERS method (Figure 5.5A). This fluid-based construction method

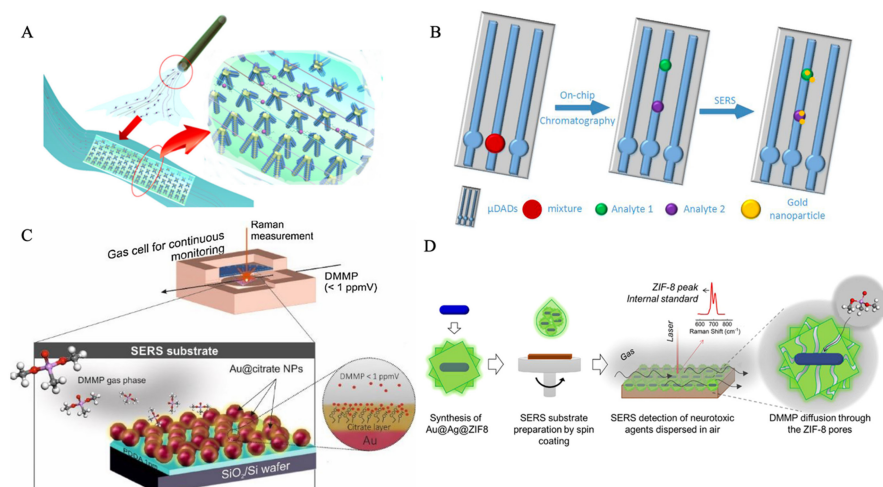


FIGURE 5.5 Scheme of 3D clustered nanostructures-based microfluidic system (A), on-chip chromatography-SERS sensing with DADs (B), SERS substrate and experimental set-up (C), and fabrication of SERS substrates for neurotoxic agent detection (D). Reproduced from Wang et al. (2016) with the permission of ACS. Reproduced from Kong et al. (2018) with the permission of Elsevier. Reproduced from Lafuente et al. (2018) with the permission of Elsevier. Reproduced from Lafuente et al. (2021) with the permission of ACS.

simplifies the creation of nanostructured substrates, which have been demonstrated to incorporate a significant number of hot spots essential for supporting an SERS response. Finite-difference time-domain experiments have indicated that these 3D clustered arrangements of the nanostructures actively promote hot spots formation. The performance of the SERS microfluidic device was assessed and revealed high sensitivity and efficient recyclability when tested with 4-mercaptobenzoic acid and Rhodamine 6G and 4-mercaptobenzoic acid mixture, both serving as target organic pollutants. Furthermore, these 3D clustered nanostructures exhibited effectiveness in detecting a representative nerve agent. The outcomes of this study present a materials and manufacturing approach for creating the necessary nanostructures, paving the way for the detection of organic pollutants, real-time monitoring of environmental threats, and personal health screening devices.

Kong et al. exemplified microfluidic paper-based plasmonic nanosensor-based diatomite analytical devices (DADs) for illicit drugs detection (Kong et al., 2018). The DADs are characterized by highly porous photonic crystal biosilica channels. The manufacturing process of these DADs involves the application of diatomaceous earth onto standard glass slides using spin-coating and tape-stripping techniques, resulting in microfluidic channels. A distinctive attribute of the DADs is their ability to simultaneously execute on-chip chromatography, effectively separating small molecules from complex samples. Additionally, they enable the acquisition of SERS with exceptional specificity for the target chemicals (Figure 5.5B). Leveraging the extremely small dimensions of microfluidic channels and harnessing the photonic crystal effects, they showcased an unprecedented level of sensitivity, determining pyrene (1 ppb) in a mixture of Raman dye and cocaine (10 ppb) in plasma. This research underscores the distinctive advantages of DADs as an emerging microfluidic platform for sensing, particularly in the context of drug screening.

Lafuente et al. crafted a tailor-made SERS-based nanosensor for the detection of dimethyl methylphosphonate (DMMP) (Lafuente et al., 2018). DMMP serves as a substitute molecule for nerve agents, a group of chemicals of significant concern because of their exceptionally high toxicity, historical deployment, and persistence. The platform was meticulously designed utilizing straightforward components, primarily employing AuNPs coated with a citrate layer (Figure 5.5C). The citrate coating plays a pivotal role by effectively capturing the targets in the immediate surroundings of the AuNPs surface through mutual hydrogen bonding effects. These marks detected DMMP in the gas phase at ppm concentrations, specifically as low as 130 ppb. Such levels of sensitivity are indicative of the potential for real-world deployment, particularly in emergency scenarios. Moreover, the SERS platforms developed in this study offer advantages in terms of ease of preparation and reusability, presenting a promising avenue for the urgent detection of chemical threat agents in practical and realistic settings.

Lafuente et al. also presented a plasmonic-sorbent based thin-film nanosensor, incorporating a Raman, in the detection of chemical threat agents (Lafuente et al., 2021). This film is composed of densely packed core-shell Ag and Au nanorods, each meticulously enclosed with a ZIF-8 framework, in trapping 2-chloroethyl ethyl sulfide (CEES) of DMMP from the gas phase (Figure 5.5D). They delved into the

underlying adsorption mechanisms responsible for capturing molecules with the framework, as well as interactions between DMMP and the Ag surface, through computational calculations. The thin films exhibit remarkable SERS sensing capabilities. For DMMP, they reported a limit of detection as low as 0.2 ppbV. Furthermore, experiments conducted with the Raman system successfully detected 2.5 ppmV for DMMP in air and 76 ppbV for CEES in a nitrogen atmosphere, with reply times of 21 and 54 seconds, respectively. This system heralds the advent of handheld SERS-based sensing at extraordinarily low concentrations, with vast applications encompassing home security, safeguarding infrastructure, chemical process monitoring, and personalized medicine.

Saito et al. presented an advanced self-governing air sampling and detection system designed to assess the existence of chemical threat agents (Saito et al., 2018). This developed system incorporates a mist generator-assisted air collection mechanism, capable of sampling at a rate of 338 L/min. It harnesses cutting-edge sensing technologies, including electrochemical measurement, AuNPs-based localized surface plasmon resonance, and microfluidic platform PCR. These technologies enable the system to detect minute concentrations of hazardous substances, even below the mean lethal dose (LD_{50}), encompassing nerve gases like sarin and VX, toxic proteins such as BTX/A/Hc and ricin, and pathogens like an anthrax simulant. One of the key advantages of this system is its remarkable operational efficiency, with a collection and detection process requiring just 5 to 15 minutes. Additionally, the system integrates sample preparation seamlessly, eliminating the requirement for direct human mediation. Beyond its sensitivity and user-friendliness, the system's portability stands out as a significant asset, particularly for first responders. It facilitates immediate risk assessment and on-site event mitigation, underscoring its potential to be a valuable tool in critical situations.

5.4 CONCLUSION AND FUTURE PERSPECTIVES

Microfluidic-based plasmonic nanosensors have emerged as a pioneering platform at the crossroads of microfluidics and plasmonic nanotechnology, holding immense promise for the rapid and precise detection of biological and chemical threat agents. This chapter has illuminated the synergistic potential of integrating these two technologies, showcasing their ability to address critical challenges in threat detection. The case studies provided demonstrate the wide applicability of microfluidic-based plasmonic nanosensors, not only in terms of the threats they can detect but also in the versatility of their sensing mechanisms. Looking ahead, the future of microfluidic-based plasmonic nanosensors in threat detection is both exciting and challenging. As researchers continue to innovate, the integration of microfluidic-based plasmonic nanosensors with miniaturized and portable devices is expected to enable field-deployable sensing solutions. Advances in nanofabrication techniques and material science will further enhance the sensitivity, selectivity, and stability of plasmonic nanosensors. The incorporation of artificial intelligence and machine learning algorithms will contribute to real-time data analysis and decision-making, expediting the identification of threats. However, the journey is not without

obstacles. The translation of microfluidic-based plasmonic nanosensors from the laboratory to practical applications demands addressing issues related to sample preparation, long-term stability, and cost-effectiveness. Standardization protocols and regulatory considerations will play a pivotal role in establishing the credibility and widespread adoption of microfluidic-based plasmonic nanosensors-based detection systems. In conclusion, the marriage of microfluidics and plasmonic nanosensors has opened new frontiers in the realm of threat detection. Microfluidic-based plasmonic nanosensors offer a transformative approach that combines precision, speed, and accuracy, paving the way for advancements in security, healthcare, and environmental monitoring. As this technology matures, interdisciplinary collaboration, innovative research, and a keen awareness of practical implementation will be crucial in harnessing the full potential of microfluidic-based plasmonic nanosensors for the betterment of society.

The future of microfluidic-based plasmonic nanosensors holds tremendous potential for revolutionizing threat detection and sensing technologies across various domains. As researchers continue to push the boundaries of knowledge and innovation, several exciting future perspectives emerge:

- **Multimodal Sensing:** Integrating multiple sensing modalities, such as electrical, optical, and mechanical, could enable microfluidic-based plasmonic nanosensors to provide more comprehensive and accurate threat detection. Combining different sensing mechanisms can enhance sensitivity and reduce false positives.
- **Real-Time Monitoring:** The development of real-time monitoring systems that can continuously track changes in complex samples will be crucial for early threat detection. This could be achieved through the integration of microfluidic-based plasmonic nanosensors with automated sampling, data analysis, and decision-making algorithms.
- **Nanomaterial Innovations:** Advances in nanomaterials, including novel plasmonic materials and nanostructures, will enhance the performance of microfluidic-based plasmonic nanosensors. Tailoring the properties of these nanomaterials can lead to improvements in sensitivity, specificity, and stability.
- **Point-of-Care Diagnostics:** Microfluidic-based plasmonic nanosensors integrated into portable and handheld devices could bring threat detection capabilities to the point of need, whether in the field, clinical settings, or remote locations. This would democratize access to advanced detection technologies.
- **Environmental Monitoring:** Beyond security, microfluidic-based plasmonic nanosensors have significant potential for monitoring environmental pollutants, water quality, and air contaminants. Their ability to operate in complex samples makes them valuable tools for safeguarding ecosystems and public health.
- **Bioinformatics Integration:** Incorporating bioinformatics and machine learning algorithms will enable the interpretation of complex sensor data.

This integration can improve the accuracy of threat identification and facilitate data-driven decision-making.

- **Global Collaborations:** Collaboration between researchers, industries, and policymakers will accelerate the development, validation, and deployment of microfluidic-based plasmonic nanosensors-dependent technologies. Open dialogue and partnerships are essential to overcoming technical and regulatory challenges.

In conclusion, the future of microfluidic-based plasmonic nanosensors is marked by groundbreaking advancements that will reshape threat detection and sensing paradigms. With sustained innovation, interdisciplinary collaboration, and a commitment to addressing real-world challenges, microfluidic-based plasmonic nanosensors have the potential to become pivotal tools in ensuring global security, health, and sustainability.

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