REVIEW PAPER

Microfluidics-Based Nanobiosensors for Healthcare Monitoring

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Abstract
Efficient healthcare management demands prompt decision-making based on fast diagnostics tools, astute data analysis, and informatics analysis. The rapid detection of analytes at the point of care is ensured using microfluidics in synergy with nanotechnology and biotechnology. The nanobiosensors use nanotechnology for testing, rapid disease diagnosis, monitoring, and management. In essence, nanobiosensors detect biomolecules through bioreceptors by modulating the physicochemical signals generating an optical and electrical signal as an outcome of the binding of a biomolecule with the help of a transducer. The nanobiosensors are sensitive and selective and play a significant role in the early identification of diseases. This article reviews the detection method used with the microfluidics platform for nanobiosensors and illustrates the benefits of combining microfluidics and nanobiosensing techniques by various examples. The fundamental aspects, and their application are discussed to illustrate the advancement in the development of microfluidics-based nanobiosensors and the current trends of these nano-sized sensors for point-of-care diagnosis of various diseases and their function in healthcare monitoring.

Keywords Microfluidics · Nanobiosensors · Lab-on-a-chip · Biomedical diagnosis

Abbreviations

ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverables to end users
μPADs	Microfluidic paper-based analytical devices
BNP	B-type natriuretic peptide
CRP	C-reactive protein
DMF	Digital microfluidics
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
GMR	Guided mode resonance
HIV	Human immunodeficiency virus
LAMP	Loop-mediated isothermal amplification
LDL	Low-density lipoprotein
LFA	Lateral flow assays
LOC	Lab-on-a-chip
LSPs	Localized surface plasmons
MALDI	Matrix-assisted laser desorption/ionization
MEMS	Microelectromechanical systems
MF	Microfluidics
MFS	Microfluidic systems
MOF	Metal–Organic Frameworks
MS	Mass spectrometry
NATs	Nucleic acid-based tests
NEMS	Nano Electro Mechanical Systems
NMR	Nuclear magnetic resonance
PMMA	Poly-methyl-methacrylate
PoC	Proof-of-concept
POC	Point-of-care
RT-PCR	Reverse transcriptase polymerase chain reaction
SARS	Severe acute respiratory syndrome virus
SERS	Surface enhanced Raman scattering
SNPs	Single nucleotide polymorphisms
SPR	Surface plasmon resonance
WCRS	Waveguide Confined Raman Spectroscopy
WNV	West Nile Virus

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Introduction

The rising worldwide attention on healthcare challenges (non-communicable and communicable diseases) has gained focus on microfluidics-based nanobiosensors and their

potential to provide effective solutions to the wide range of unmet healthcare demands. With an aim to deliver point-of-care (POC) solutions, microfluidics(MF)-based nanobiosensors provide healthcare professionals with the information necessary to manage patient healthcare while they are still with the patient. However, the majority of commercially available diagnostic methods are traditional thereby do not meet the growing needs and global standards, such as limited-resource settings and basic healthcare infrastructure [1]. The shortcomings include the prolonged testing time which frequently causes a delay in the data availability and the eventual diagnosis. The role of diagnosis is essential in effective health management therefore, there is a need to establish novel solutions for POC applications. The development and adoption of microfluidics-based nanobiosensors for health monitoring can have significant positive societal impacts, including improved health outcomes, increased access to healthcare, cost savings, personalized medicine, and advancements in medical research [2].

Microfluidics-based nanobiosensors utilize microscale fluid flow and nanotechnology principles to detect and quantify biological molecules such as proteins, DNA, and cells. These sensors typically use a combination of biomolecules (e.g., antibodies and aptamers) and nanomaterials (e.g., carbon nanotubes and gold nanoparticles) to aid highly specific and sensitive detection of targeted molecules [3]. This type of nanobiosensor provides rapid and accurate information thus facilitating early detection and treatment of the disease. Moreover, Microfluidics based nanobiosensors offer real-time biomarker monitoring, minimal biomolecule concentration along with high sensitivity and specificity thus reducing the possibility of false positives and negatives in the testing process [4]. These nanobiosensors can also detect biomarkers in non-invasive samples like urine or saliva, thus eliminating the need of invasive procedures and the risk of infection and other complications [4, 5]. The various benefits, including ease of fabrication, reduced reagent consumption, quick reaction times, enhanced sensing parameters, and continuous monitoring of desired analytes, microfluidics-based POC offers robust platforms for desired biochemical and chemical analysis [5]. Some of the challenges connected to the microfabrication and miniaturization of POC devices include the need for small-volume samples, the mixing of different fluids, the separation of suspension fluids, detection, and the integration of all the components onto a single platform. The development of microfluidics-based nanobiosensors for health care monitoring requires a multidisciplinary approach that integrates engineering, chemistry, biology, and clinical research. Addressing these scientific issues will be critical for the successful translation of this technology from the lab to clinical practice [6].

These sensors are becoming increasingly versatile, able to detect multiple analytes simultaneously and to be integrated with other technologies such as microfluidic pumps and valves. As a result, these sensors have the potential to be used in a wide range of healthcare applications, from disease diagnosis to drug discovery and monitoring. For controlling and moving the fluid at this scale, a microfluidic–nanofluidic device is made up of parts including valves, pumps, and mixers. Based on various kinds of microfluidics flows, three different forms of microfluidics are classified: closed/confined channel, open, and two-phase (droplet) microfluidics. Closed/confined channel microfluidics are classified into creeping microflows and inertial microfluidics. Open microfluidics is categorized into three parts: paper-based microfluidics, Rail-based/suspended microfluidics, digital microfluidics (DMF), and Droplet microfluidics is classified into two categories encapsulation and two-phase microfluidics [7]. Microfluidics, which can be active or passive, can separate the desired suspension fluids from a difficult and diverse sample. While passive techniques manage the suspension fluids using channel architecture, inherent hydrodynamic forces, and steric hindrances, active methods use external fields such active pumps, electric, magnetic, acoustic, and optical to drive suspension fluids for separation [8]. The significant advantages of the technology are its lesser analysis time, lower consumption of chemical/biochemical reagents, efficient control over the reaction conditions, affordable costs for its use and disposal, portability, high analytical performance and unique functionality largely unknown in conventional techniques [9].

The combination of microfluidics and nanobiosensors has led to the development of microfluidics-based nanobiosensors, which have several advantages over conventional biosensors. Microfluidics-based nanobiosensors can detect biomolecules and cells at extremely low concentrations and analyze multiple analytes in a single sample. They are also portable and easy to use, making them suitable for POC applications. Healthcare monitoring involves continuously monitoring physiological parameters, such as glucose levels, blood pressure, heart rate, and detecting biomarkers for diseases such as cancer and infectious diseases. Microfluidics-based nanobiosensors have the potential to enable early disease diagnosis, real-time monitoring of disease progression, and personalized medicine [9, 10]. The catalytic affinity of the biological factor to be probed determines the quality of the nanobiosensor. An important aspect of the technology is the fabrication of the microfluidic chip. The new paradigm for building diagnostic tests for disease diagnosis is nanotechnology on a chip. The capacity to quantitatively quantify chemical and biological information in a timely and cost-effective manner will have an influence on molecular diagnostics and health care [11]. This interaction of the analyte with the concerned biological factor in the microfluidic system (MFS) is worthwhile in health-care monitoring and disease diagnosis [11, 12].

Overall, the study illustrates the recent developments in microfluidics-based nanobiosensors focusing on various detection techniques. There is a substantial gap in the research and development of nanobiosensors and their commercialization efforts. In addition, there is a need to unravel their potential in terms of healthcare applications, suitability in regular health monitoring, wearability and durability on the human body, compactness and cost effectiveness [5]. This review aims to provide an overview of microfluidic-based nanobiosensors and their potential application in the bio-analysis and POC diagnostics. Developing microfluidic-based nano biosensors for health care monitoring is motivated primarily by the desire to incorporate the expertise of multiple field approaches to make more effective detection and monitoring strategies for diseases and health conditions. We also summarize the challenges and future aspects to realize its potential as a technology.

Microfluidics

A "microfluidics" refers to the behavior, control, and manipulation of fluids at the sub-millimeter scale, either separately or in combination. Throughput processing is made possible by microfluidic devices, which also improve transport for regulating flow conditions, raise the pace at which various reagents are mixed, lower sample and reagent volumes (to the nanoliter scale), and boost detection sensitivity. In light of these benefits, microfluidics integration into biosensor technology opens up new possibilities for future biosensing applications, including portability, real-time detection, improved accuracy, increased sensitivity and selectivity, and simultaneous analysis of multiple analytes in a single device. Compared to conventional sensing techniques, MFSs have emerged as a potent tool for biosensing over

the past 20 years, especially in the purification and detection of biological analyte. The microfluidic chip has various components and compartments that allow various activities within the passing liquid, like mixing, chemical or physical reactions [13, 14]. These devices are made using a variety of different fabrication materials and techniques and have revolutionized analytical approach for detection [15]. Microfluidic devices have two forms of flows shown on Fig. 1a continuous or discrete, regulated by external factors [16]. Continuous flow microfluidics as shown in Fig. 1b makes it possible to control the continuous flow of liquid through microchannel devices by integrated external pressure pumps or mechanical micropumps. Continuous flow processes are used in a wide variety of applications such as bioanalytical, chemical, energy and environmental fields [14]. Droplet microfluidic device uses discrete water droplets formed by an immiscible oil and stabilized by surfactants, as microscale sample containers.

However, the discrete flow may have further categories (i) segmented-flow microfluidics, and (ii) DMF [14, 17]. The Micro-droplet methodology is a high-throughput technology using very low amount of reagents, user-friendly and with least cross contaminations between reagents [18]. Pang et al. [19] reported a droplet-based MFS and its effects on flow using a continuous stream of two or more immiscible fluids producing droplets at the T-junction. The T-junction is one of the most commonly used geometries. A T-junction channel as shown in Fig. 1c is considered in microscale having two inlets and one outlet for microfluidic droplet formation. The channel geometries have been found to play an important role in the droplet formation process. If the parameters are varied there are three key guidelines can be illustrated for drop formation: dripping, squeezing and parallel flowing stream. In the dripping regime, droplet breakup occurs when the viscous shear
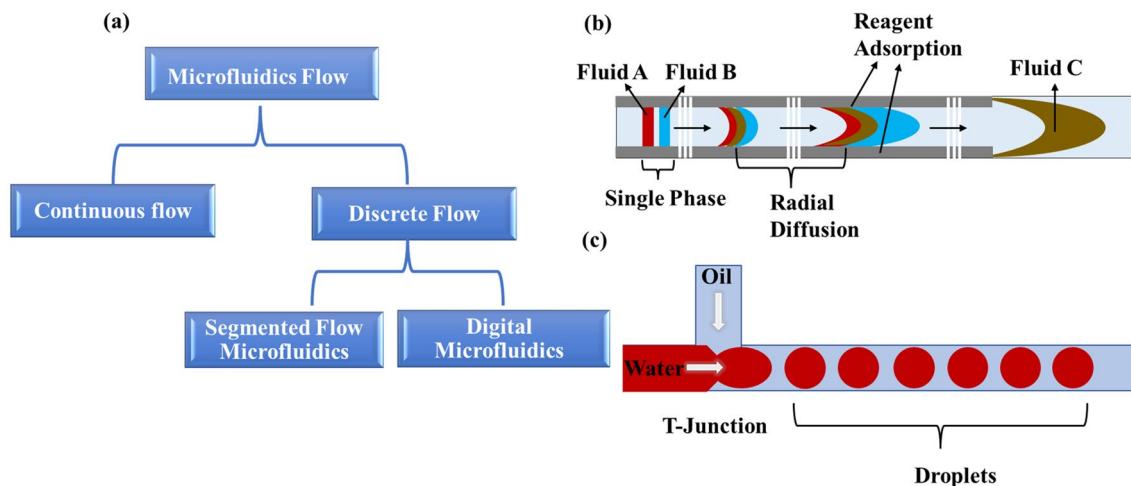

Fig. 1 Schematic illustration **a** Microfluidics Flow, **b** Continuous flow of liquid and **c** Droplet generation at T Junction in discrete flow

stress overcomes the interfacial tension, analogous to the breakup of spherical droplets. If the capillary number is chosen large enough, the droplets are emitted before they can block the channel. Alternatively, if the capillary number is low, the formed droplets will hinder the channel and later restrict the continuous phase. This causes a dramatic increase of the hydrodynamic pressure in the upstream part, which in turn induces the pinch-off of droplets. Flow-focusing techniques for the fabrication of droplets were widely used, (e.g., the preparation of the ferrofluid droplets and the droplets with colored polystyrene beads). The flow-focusing devices generally consist of a nozzle which was coaxially aligned with a downstream orifice. The fluid in the orifice was used to provides shearing force, and produce droplets [20, 21]. One of the main objectives of the microfluidics community are to create technologies that enhance the skills of biologists and medical researchers. Proof-of-concept (PoC) studies are frequently conducted in microfluidic research to show the viability of innovative approaches to replace conventional macroscale assays [22]. As required by ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverables to end users), microfluidic technologies are becoming essential tools. Since infectious diseases only require a tiny amount of biological fluid, microfluidics-based devices can be used at the POC for diagnosing and real-time monitoring infectious diseases [23]. The newly established wearable electronics using microfluidics, which combines flexible electronics with microfluidic devices, have attracted much interest in medical diagnosis. A MFS can precisely control the conductive fluid, electrical connections and the biological system in a single platform thus developing into a hand-held self-sustaining entity [4].

Nanobiosensor

Biosensors are a measuring system for analyte detection that combines a biological component with physicochemical detectors. Nanobiosensors are essentially sensors that are composed of nanomaterials, and interestingly, they are specific sensors that can detect events at the nanoscale. A nanobiosensor detects biological agents such as antibodies, nucleic acids, pathogens, and metabolites. The working principle consists of binding bio-analytes of interest onto bio-receptors, which modulates the physicochemical sign associated with the binding [24–26]. A typical Nanobiosensor is represented in Fig. 2. It consists following components: Analyte, Bio-receptors, Transducers, and Displays. *Analyte* is referred as a material of interest that needs detection, e.g., cholesterol is an 'analyte' in a biosensor designed to detect the cholesterol levels. *Bio-receptors* are the molecule that primarily recognizes the analyte is known as a bio-receptor such as enzymes, cells, aptamers, DNA, RNA, and antibodies. The interaction of the bio-receptor with the analyte generates a signal in the form of light, heat, pH, charge, or mass change, etc., which helps in the process of bio-recognition. *Transducer* is a component of a biosensor that mainly converts one form of energy into another form. The role of the transducer is to convert the bio-recognition event into a signal which can be measured easily, the process
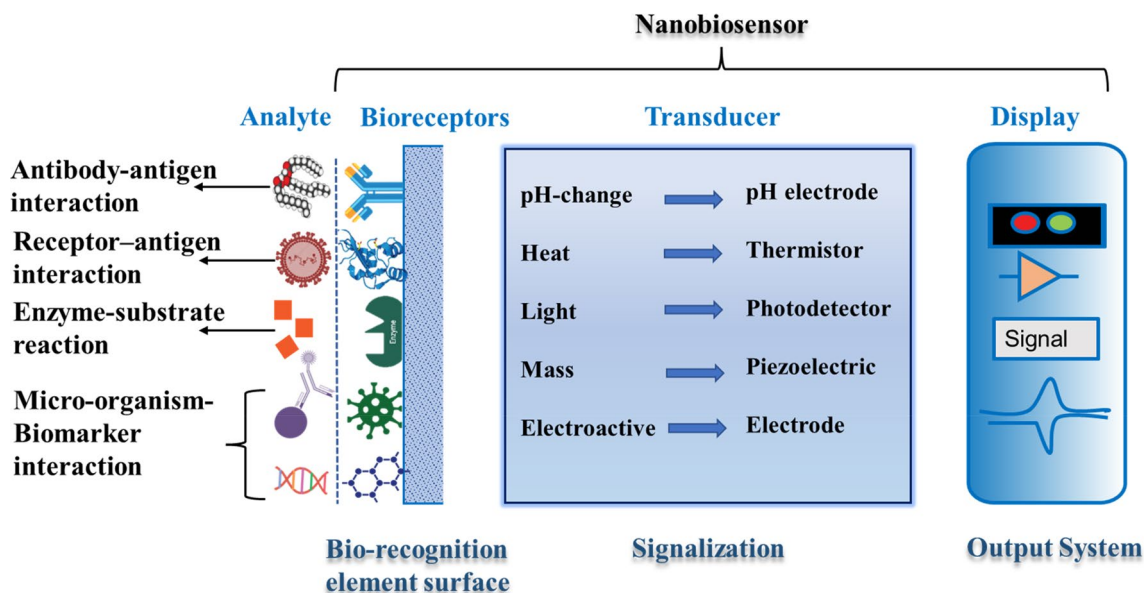

Fig. 2 Schematic illustration of Nanobiosensors, there interaction and its components

is known as *signalization*. This is the part of a biosensor that processes the signal from the transduced analyte–bioreceptor interaction and prepares it for display on the device. The display unit of the biosensor then evaluates the processed signal. *Display* constitutes an interface system (e.g., a liquid crystal display of a computer or a direct printer) that generates measurable values or curves easily comprehendible by the user [26, 27].

Nanobiosensors are utilized for infectious disease monitoring, forensic investigation, biological testing, health monitoring, diagnostic, and mental patient management. In addition to delivering healthcare and therapy, nanobiosensors can follow patients' states and discover illnesses more rapidly [28]. In addition to monitoring infectious illnesses, biosensors are employed in forensic investigation, biological testing, health monitoring, diagnostics, and treating psychiatric disorders. In addition to offering treatment and healthcare, biosensors can more swiftly identify ailments and monitor patients' situations [29].

Nanobiosensors can revolutionize healthcare management and enable real-time, sensitive, and specific detection of biomolecules. However, their development and application in healthcare also face several challenges. Some of these challenges are *s*pecificity and sensitivity related to biomolecules such as proteins, nucleic acids, and small molecules (living and non-living) have diverse structures and properties. Therefore, designing of the nanobiosensor which can detect them with high accuracy are challenging [30]. Nanobiosensors must be biocompatible and non-toxic to be used in in vivo applications. The materials used in the fabrication must not cause any damage to the body and should not induce an immune response [31]. Stability and reproducibility of nanobiosensors are essential to ensure a consistent result in performance. Dissimilarities in the interpretation of result outcomes can be caused by variations in the environment condition and due to the presence of interfering substances. The cost of developing and manufacturing nanobiosensor is high, which can limit their widespread usage in healthcare. The cost-effectiveness must be carefully considered, especially in low-resource settings [28, 32]. Nanobiosensors are subject to regulatory approval (e.g., FDA) before being practiced in healthcare monitoring. The regulatory approval process is long and complex, requiring extensive testing and documentation to demonstrate the safety and efficacy of the nanobiosensors [4, 5].

Overcoming these challenges requires interdisciplinary collaborations between materials science, biology, and medical researchers and clinicians. Advances in technology, such as the development of new materials and manufacturing techniques will also be critical to the widespread adoption of nanobiosensors in healthcare management [33].

Nanobiosensors that are based on nanotechnology and nanomaterials such as nanoparticles, nanotubes, and quantum dots. These materials, usually known as nanomaterials or nanoparticles, are reshaping the scientific world mainly due to their exceptional physical, chemical, and biological properties compared to their bulk counterparts. They have found many applications, especially in biomedicine and optics. medical imaging, catalysis, and electronics [34, 35]. Since particles in this size range have distinctive characteristics, developing sensors at the nanoscale has various benefits. It has been shown that nanoprobes may spontaneously absorb by cells and have a high penetration efficiency. Nanomaterials can be used as chemical probe carriers to increase the stability and half-life of the probes. Due to their small size, nanoparticles have a high surface-to-volume ratio, making them amenable to detection at femto, atto, and zeptoscales. Additionally, nanoparticles have exceptional electrical conductivity and reactivity and may take various forms [36]. The healthcare sector has made substantial use of nanosensors, some of which are employed for detecting and diagnosing infectious and non-infectious diseases. Additionally, since target-oriented distribution is made feasible by numerous nanoparticles; it is widely utilized in precisely delivering genes and drugs. Metal or ion detection is helpful for diagnostic purposes. Biosensing has greatly benefited from the quick advancement of nanoscience and nanotechnology. Integrated nano-materials with electrical systems are referred as Nano Electro Mechanical Systems (NEMS), which are quite active in the electrical transduction mechanism. Based on the electrical and mechanical properties, several nano-materials like nanotubes, nanowires, nano-rods, nanocomposites, and thin films made up of nano-crystalline materials have been explored for their use in improved biological signaling and transduction mechanisms [37]. Among these, the use of nanoparticles is studied extensively in most of the bio-sensing systems e.g., Amperometric technique used for enzymatic detection of glucose using quantum dots as fluorescent agents and bio-conjugated nano-materials for specific biomolecular detection. Nanobiosensors have a wide range of applications in health-care as shown in Fig. 3 [38, 39]. These materials, including metal-based nanoparticles can be used in the microscopic detections, nucleic acid sequences etc., by exploiting their optoelectronic properties [40]. A recent study by Obisesan et al. [32] demonstrated the use of gold electrodes modified with metal nanoparticles, to develop electrochemical sensor devices for the detection of β-hematin in blood samples from mice. The nanoparticles used were specifically CuO, Al_2O_3, and Fe_2O_3, each synthesized using chemical and microwave methods. Each of the nanoparticle-modified electrode surfaces acted as platforms on which electrocatalytic reduction of β-hematin in the blood sample occurred. The authors found a more favorable electrocatalytic reduction of β-hematin on CuO-modified gold electrodes, both chemically synthesized and microwave synthesized. Furthermore, the CuO-modified
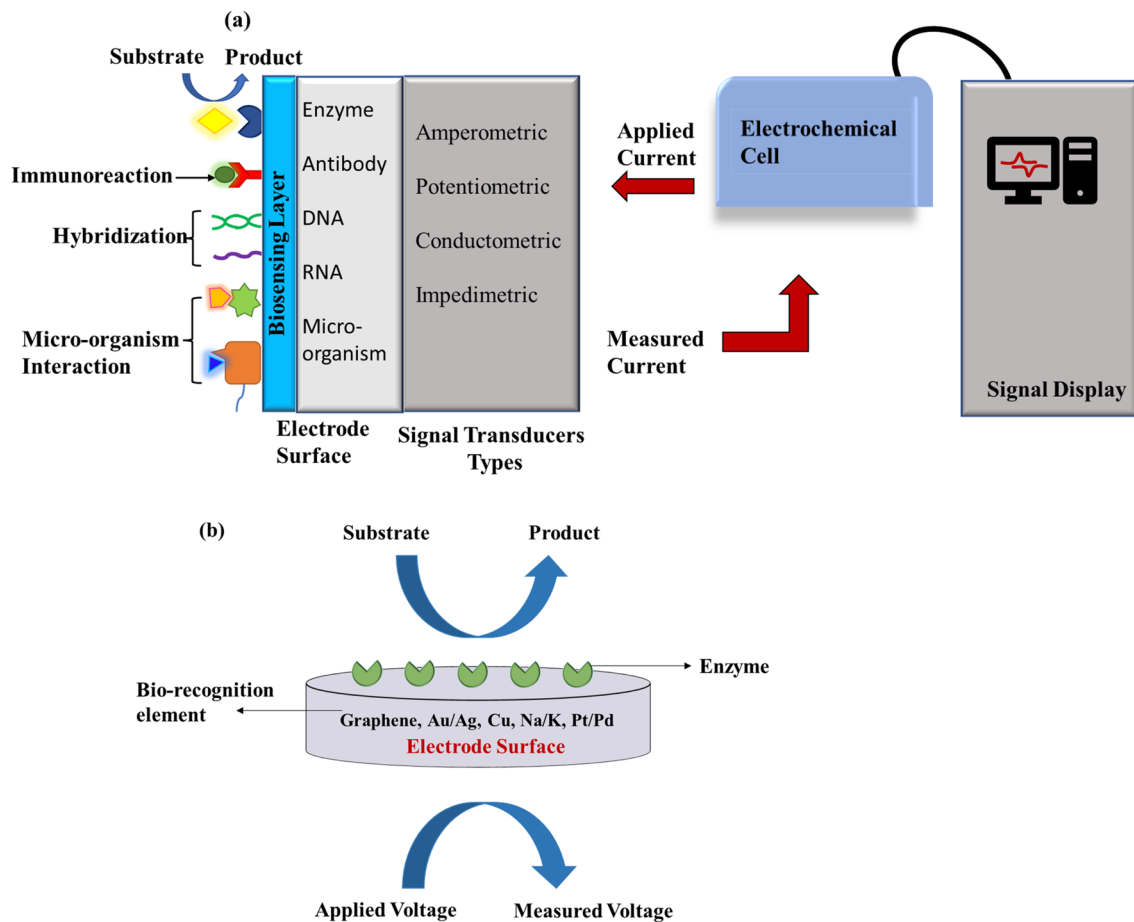
Fig. 3 **a** Detailed explanation of electrochemical biosensors components and **b** Surface modifications of electrode surface

gold electrode exhibited high stability and good selectivity to the β-hematin compared to *Salmonella typhi* antiserum VI typhoid biomarkers. Specifically, the nanoparticles used were CuO, Al_2O_3, and Fe_2O_3, each synthesized using chemical and microwave methods. Each of the nanoparticle-modified electrode surfaces acted as platforms for the electrocatalytic reduction of β-hematin in the blood sample. The authors found a more favorable electrocatalytic reduction of β-hematin on CuO-modified gold electrodes. In addition, the CuO-modified gold electrode exhibited high stability and good selectivity to β-hematin compared to *S. typhi* antiserum VI typhoid biomarkers. In another study, Chung and colleagues [41] designed a dual probe-nanoparticle system capable of detecting the phenotyping of common human pathogens. They prepared a nanoparticle-based sandwich hybridization technique which includes two distinct oligonucleotide probes targeting bacterial 16S rRNA. They were designed to detect amplified target DNAs using a miniaturized nuclear magnetic resonance (NMR) device. The test was found to be robust and rapid, simultaneously diagnosing 13 bacterial species in clinical samples within 2 h.

Nanobiosensors are designed either to reduce the detection limits or to make them detect a number of analytes in the same sample called multiplexing. Multiplexing is essential in the diagnosis of a disease which requires detection and quantitation of number of molecular markers in a particular body fluid for early detection of the health disorder [42]. Metallic nanoparticles, such as silver (Ag), gold (Au), platinum (Pt) and palladium (Pd) have fascinated researchers to construct nanobiosensors for their unique physicochemical properties. These particles offer various advantages, such as a large surface-to-volume ratio, high surface reaction activity and strong adsorption ability to immobilize the desired biomolecules. Predominantly, gold nanoparticles have been widely used to construct nanobiosensor due to its significant potential to immobilize biomolecules [43]. Nano-materials can further facilitate the reuse of expensive enzymes and improve sensitivity and accuracy. This usage of Nanoelectro-mechanical systems (NEMS) and Microelectromechanical systems (MEMS) has led to the development of lab-on-a-chip (LOC)-based assays, biochips and microarrays [44].

The future prospects for nanobiosensors in healthcare management are promising. Here are some potential prospects in this area, POC devices that can be used in hospitals, clinics and at the personal level are becoming increasingly popular. Development of new sensing platforms and recognition elements will allow more sensitive, specific, and affordable system for healthcare monitoring. Nanobiosensors can be integrated with digital health technologies, such as mobile applications and cloud computing, to enable real-time monitoring of the user's health. The use of immense data analytics such as artificial intelligence (AI) and Internet-of-Things (IoT) can also provide insights into the population health and initiate government interventions in public healthcare policies [45, 46]. Implantable nanobiosensors can provide continuous monitoring of health. Advancements in materials science, such as the development of biocompatible and non-toxic materials that facilitates better health and avoid immune response need to be investigated. The fundamental understanding in this direction can expand the patient outcomes by reducing the risk of adverse drug reactions and enabling the use of more effective treatments [28, 45]. Nanobiosensors have remarkable potential for the detection of various microbes, disease biomarkers, environmental pollutants, and drug delivery (e.g., pathogen in food, blood pathogen biomarkers in blood) [47, 48]. They also have the potential to detect early cancer in body fluids (e.g., breast cancer) [49]. Based on research progress in this area, nanobiosensor devices could move from the laboratory to the commercial level. Although the road has several limitations such as few biomaterials are unstable and can undergo denaturation under stressful conditions, cells in sensors can show intoxication by the other molecules, and heat sterilization due to the possibility of denaturation. In a way, proper scaling efforts are required for the commercialization of nanobiosensors to meet the public demand [50].

Detection Technique

Microfluidics-based nanobiosensors offer many advantages for healthcare monitoring, such as high sensitivity, specificity, and real-time detection capabilities. We review some of the detection methods commonly used in microfluidics-based nanobiosensors used in healthcare monitoring in this study described below. Optical detection methods are widely used in microfluidics-based nanobiosensors. These include absorbance, fluorescence, and surface plasmon resonance (SPR) spectroscopy. Optical detection methods can be used to detect a wide range of biomolecules, including DNA, proteins, and viruses. Electrochemical detection methods are also commonly used in microfluidics-based nanobiosensors. These include amperometry, potentiometry, and impedance spectroscopy. Electrochemical detection methods offer high sensitivity and specificity and can be used to detect a wide range of biomolecules, including glucose, cholesterol, and proteins. Raman detection methods used to detect and quantify biomolecules such as proteins. Mass spectrometry (MS) is a powerful analytical technique that can be used to detect and quantify biomolecules. In microfluidics-based nanobiosensors, MS is often used in conjunction with other detection methods to provide additional information about the biomolecules being detected. Overall, the choice of detection method in microfluidics-based nanobiosensors depends on the specific application and the biomolecule being detected.

Electrochemical Technique

The integration of an electrochemical detection system together with microfluidic technology is an attractive choice for the construction of miniaturized components on a single platform [51]. Electrochemistry plays an important role in transforming chemical or biological changes into measurable signals [52]. Electrochemical nanobiosensors typically consist of an analyte, receptors, a signal, a transducer, and a data analysis system (Fig. 3a). Each of these functions has a different responsibility within the biosensor. An analyte can be described as any biological element or chemical element, also known as a target, which differs from viruses, bacteria, chemical components, and others. Generally, electrochemical sensors can be divided into two categories, potentiometric and amperometric. Sensors can be distinguished according to the way the analyte is measured: while the response to the analyte for amperometric measurements measures differences in electric current and the potentiometric type is measured by differences created in voltage. A variety of different configurations of electrodes materials can be integrated (Fig. 3b) into microfluidic chips [44] to perform the above function by coupling electrochemistry with the microfluidic platform for the POC applications. Graphene, a carbon-based nanomaterial, has emerged as an attractive nanomaterial in the field of electrochemical applications primarily due to its excellent electrical and thermal conductivity [53]. With another nanomaterial sensors, electrochemical nanobiosensors based on rGO nanocomposites have unique advantages. There are several biomolecules those are specific for pathogenic bacteria and other healthcare monitoring, such as nucleic acids, antibodies, peptides, etc. Integrating these biomolecules with electrochemical nanobiosensors based on rGO nanocomposites not only increase the biocompatibility of rGO nanocomposites, but also protects them from environmental interference [54].

The electrochemical nanobiosensor translates chemical response into the electrical response [55] to detect various biomolecules in the human body such as glucose, cholesterol, uric acid, lactate, DNA, hemoglobin, blood ketones, to name a few [56, 57]. In essence, the

electrochemical-based nanobiosensors have wider potential for the identification of imbalances in the biomolecules in the body with applications in biosensing–drug delivery. Multiplex microfluidic electrochemical nanobiosensors have been shown for various applications in the biomedical field [58], therapeutic drugs [59], detection of metabolites in bacteria [60, 61] and viruses [62].

Raman Spectroscopy Technique

Raman spectroscopy is the analytical method for investigating structural properties of a compound. It's an optical detection technique that can detect a molecular concentration in a label-free and non-invasive manner [63] Localized surface plasmons (LSPs) in metallic nanostructures and their interactions with the electromagnetic field scattered by the sample have been exploited to develop surface enhanced Raman scattering (SERS), which can enhance the Raman signal to several order of magnitude($\sim 10^6$–10^7) and help in probing low concentration analytes in metallic nanostructures [64, 65]. For nanobiosensor fabrication, In SERS MFSs, analyte liquids are controlled and analyzed in a microchannel in which they interact with externally added nanoparticles or embedded in noble metal micro- or nanostructures. these structures are capable of reproducing SERS signals (Fig. 4a) [66]. Further, multiplexing in identification of target analytes and precluding cross-contamination are added advantages [67, 68].

Raman spectroscopy has a very high chemical specificity and generates lots of information which has made is very popular amongst the researchers [69]. It can perform multi-component detections from an analyte giving separate fingerprint bands for each component in the spectrum, which makes it very unique for simultaneous monitoring of various components in a single microfluidic run [70–72]. The synergic combination of optical SERS sensors and microfluidic technologies are the future for SERS optical analysis [73]. Ashok et al. has reported the use of Waveguide Confined Raman Spectroscopy (WCRS) based on microfluidic Raman spectroscopic detection [74]. The advantage of WCRS is that it can be downscaled to micrometer dimensions enabling it to combine with several microfluidic devices for on-chip geometry helping to collect the maximum Raman signals in a microfluidic chip with alignment-free Raman spectroscopic detection scheme. WCRS is a generic technique capable of adapting to various microfluidic platforms to exploit the advantages of Raman spectroscopy. The main advantage of WCRS is its scalability. WCRS results in the evolution of efficient, alignment-free, portable MRS devices suitable for field applications [74]. Therefore, the Raman spectroscopy

opens an information window of biomarkers and provide the qualitative analysis with low volume sample.

Optical Spectroscopy

Optical spectroscopy deals with the measurement of absorption, reflection and emission of light in the near ultraviolet (~ 250 nm) through the mid-infrared (~ 3000 nm) part of the spectrum [75]. Non-invasive optical detection has evolved in the last decade from fluorescence spectroscopy to grating, SPR, SERS, GMR (guided mode resonance), etc. (Fig. 4b). Fundamental light emission or light—these optical sensing methods use interactions matter, such as the emission of fluorescent light from the target analyte, optical diffraction due to light device structure interactions, and optical resonance in the optical cavity or designated structure [76]. Opto-fluidics, a term coined in 2003, is the fusion of optics and microfluidics to extract information from microfluidic devices [77]. Opto-fluidics devices integrate optical detection techniques (photonics) into microfluidics and nano-fluidics, an important role in the advancement of LOC technology [71]. In a nano-fluidic chip, the nano-space can be used to characterize the behavior of individual molecules. Nanoscale devices can be used to manipulate individual cells or to deliver chemicals into cells in a controlled manner. Nano-fabricated structures are mainly used in the analysis of biological polymers such as DNA molecules. In principle, single-molecule DNA analysis with nanostructures could analyze the genome from single-cell nanostructures and can be used to optically analyze the length of DNA [78]. Optical imaging technique provides microscopic images of samples in microfluidic channels, especially suited for LOC, containing more information than other conventional detection methods [79]. Thus, a new field of optofluidic for LOC applications has been developed, integrating optics with microfluidics in a single chip [80–82]. However, fluorescence-based sensing method has been very efficiently used for biological, chemical, and medical applications at ultra-low detection limits by integrating the active and passive optical constituents on a planar layer of micro and nano-fabrication technologies [83–85]. Researchers are currently using in-plane microfluidic lenses, printed-board microfluidics, Anti-Resonant Reflecting Optical Waveguide and on-chip integration of Solid-State Light Emitters [86]. Integrated optical nanobiosensors have many advantages. First, integration miniaturizes the components that construct optical nanobiosensors. Second, sensitivity and accuracy can be further improved by incorporating additional physical, mechanical, and electrical sensing technologies. Many optical nanobiosensors facilitate microfluidic delivery of the target analyte, miniaturization, and performance

Fig. 4 Schematics illustration of **a** Raman scattering at microfluidics platform, **b** Optical detection of analytes in microfluidic confinement, and **c** Quantitative analysis by mass spectroscopy
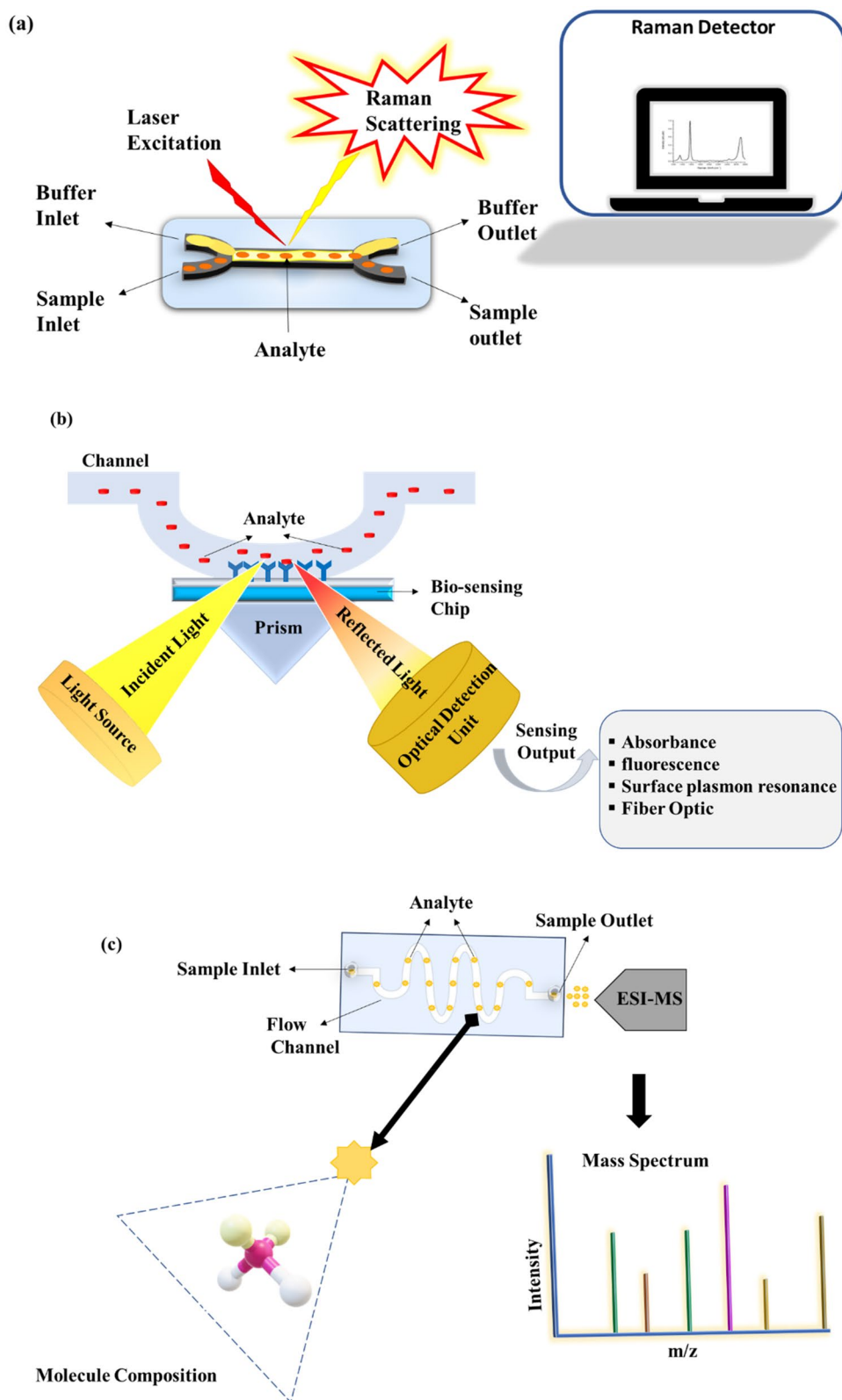
improvement. On the other hand, the integration of optical biosensors with electric charge sensing with field effect is rare [86]. Therefore, integrated optical nanobiosensors have many advantages. First, integration miniaturizes the components that construct optical nanobiosensors. Second, sensitivity and accuracy can be further improved by incorporating additional physical, mechanical, and electrical sensing technologies. Many optical nanobiosensors facilitate microfluidic delivery of the target analyte, miniaturization and performance improvement [76].

Mass Spectroscopy

MS is first reporting in 1990s a well-known analytical approach to distinguish molecules by their mass-to-charge ratio. MS a powerful label-free technique that can provide molecular weight information, is one of the most widely used analytical tools used in various fields. This information can be used to monitor disease progression and treatment response. Compared to other molecular diagnostic approaches such as polymerase chain reaction (PCR), MS provides several distinct advantages for bacterial identification, such as low cost, high mass resolution, and multicomponent analysis. A MS generally consists of an ionization source, ion optics, and a detector. As MS technology advanced, the advent of two "soft" ionizations methods, namely electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) MS analysis. In both methods, remove molecules they are ionized with minimal fragmentation [87]. By detecting specific mass labels using MS multiplex pathogens can be easily distinguished. Although MS is a good method for analysis, for real complex samples clean isolation is necessary to reduce interference before introducing the sample. Microfluidics is a promising and flexible technique that can integrate on-chip sample pretreatment operations to easily implement simple processing procedures to enhance high throughput and achieve automation. Combining microfluidics with MS can help improve the overall analytical performance of MS and expand their potential application MS is a powerful analytical tool that can be used in microfabrication-based nanobiosensors for healthcare monitoring. Some of the uses of MS can be used to identify and quantify proteins in biological samples, detect specific biomarkers, screen for drugs and their metabolites and analyze the size, composition, and surface properties of nanoparticles [88]. MS has been exploited for a variety of biomolecular analyses by combining it with microfluidic devices via ESI, MALDI, conventional capillary electrophoresis or high-performance liquid chromatography and micro/nano-ESI interfaces. Besides, the integration of microfluidic chips to MS can greatly expand the potential of MS analysis for applications requiring faster analysis time, increased sensitivity, and throughput. Soon development of microfluidic chips aimed at ESI–MS interface. MS-based biosensors rely on the ability to accurately measure the mass-to-charge ratio (m/z) of ions generated from biological samples. Microfluidics can be used to control the ionization process and guide ions to the mass spectrometer, improving the efficiency and sensitivity of the analysis. Furthermore, microfluidics can be used to perform label-free detection, which eliminates the need for expensive reagents and reduces the time and cost of the analysis [89, 90]. When coupled with microfluidic devices (Fig. 4c), MS detection is helpful for quantitative analyses [91]. As a result, ionization methods for on-line microfluidic-MS analysis are becoming more popular, particularly with the advancement in microfabrication technology [92]. Therefore, MS-based analysis is attractive due to its ability to detect through the absence of labeling, which offers chemical and spatial information of the analyte.

Microfluidics-Based Nanobiosensors

Microfluidics is considered a multidisciplinary technology that brings together several different sciences, including chemistry, biochemistry, engineering, physics, microtechnology, nanotechnology, and biotechnology. Microfluidic devices and biosensor technology are combined to provide new choices for biosensing applications, including mobility, real-time detection, increased sensitivity, and selectivity. Numerous microfluidic devices have been developed for use with biosensors. Enzyme-based microfluidic nanobiosensors, DNA-based microfluidic nanobiosensors, and microfluidic immunosensors are the three most significant microfluidic-based nanobiosensors [93]. In the case of DNA-based nanobiosensors, the recognition element is a single strand of DNA. The basis of this attribute is the hybridization process in which the principle relies on the affinity of single-strand DNA (i.e., DNA probe) with the complementary DNA strand. It has great potential in diagnosis of cancer, detecting specific DNA mutations, and in pathogen identification responsible for the diseased state. Precise analysis of specific sequences is of utmost importance. DNA-based biosensors have excellent predispositions and hold significant promise to become a powerful tool in disease prevention [94]. Nanobiosensor devices are integrated with a microfluidics-based system to measure a biochemical or biological event using optical, electronic, or magnetic technology through a compact probe [95]. Recently, there has been substantial demand and effort in integrating nanobiosensors into LOC technology employing microfluidics systems, which adds numerous biosensor technology applications [96]. Combining nanobiosensors with MFSs provides an integrated and miniaturized alternative to typical repetitive laboratory processes, significantly reducing space, energy consumption, reagent, sample, and waste output [97]. In order to maximize their value, nanotechnology-based biosensors must be combined with appropriate micro/nanofluidic devices that can deliver suitable specimens to the sensing platform. Micro/

nanofluidic techniques are among the most promising ways to link nanoengineered biosensors in a range of clinical and biological applications. The sensors must be combined with efficient fluid handling equipment since the bulk of biological samples are frequently in a liquid environment. Inappropriate microfluidic delivery techniques can be used to shorten analysis times, eliminate contamination, and enable portable devices [98]. Microfluidic diagnostic equipment can be used to evaluate clinical samples such as blood, urine, and oral fluid or saliva. Disposable equipment can be made that eliminates the need for washing processes between sample separations and makes them user-friendly even at remote locations. One of the most effective disposable devices to date is the immune-chromatographic strip, which is now used in underdeveloped countries to identify bacterial, viral, and parasite antigens. Miniaturization, mass production, and quality control can all help to bring down the cost of disposable microfluidic devices [99]. Furthermore, microfluidic nanobiosensors can reduce costs compared to traditional detection methods while increasing specificity and detection sensitivity limits. Because of the tiny size of microsystems, a single microfluidic nanobiosensor may perform an entire analysis, including continuous sampling, sample separation, mixing, and sensor pre-concentration and treatment [100]. Additionally, these microfluidic nanobiosensor provide high throughput, analytical performance, quick reaction rates, real-time detection, mobility, and permitting detection at POC applications. Combining biosensors with MFSs produce a robust analytical tool that will significantly advance the POC solutions [101].

Paper-Based Nanobiosensors

The paper-based platform uses the simple capillary passive force instead of the active external power sources in the conventional methods. Paper-based nanobiosensor protocols are not only flexible, simple, portable but also inexpensive and very efficient for detection of biomarkers in environmental and clinical samples [102, 103]. The primary source of support for POC systems nowadays is the technical difficulty in microfluidic paper-based analytical devices (μPADs). LOC, which Whiteside's' group suggested in 2007, is another name for PADs. The primary function of chemistry, biology, and other laboratories is reduced to a miniaturized paper area. It is an analytical platform that, by constructing hydrophilic and hydrophobic channels, integrates the functions of injection, reaction, separation, and detection onto a paper. There are various techniques available for fabrication of paper (e.g., photo etching, inkjet printing, wax printing, laser processing, plasma processing, cutting one-step plotting technique, flexographic printing, and stereoscopic printing) have all been used to manufacture PADs [104].

Because POC requires a quick reaction rate and great sensitivity, the detecting system is critical for signal capture. Electrochemistry, electrochemiluminescence, colorimetry, fluorescence, SERS, and chemiluminescence have all been constructed on PADs thus far [105]. The paper-based nanosensor technologies are also expanding beyond the more traditional classes of biomarkers—nucleic acids and proteins to metabolites and direct detection of pathogens. In one of the research the extremely selective paper-based amperometric biosensor have been developed which can analyze choline in clinical samples from patients suffering from renal diseases and receiving repetitive haemo-dialysis treatment [106]. These nanobiosensors have been classified as dipstick, lateral flow assays (LFA), and μPAD based on their utility [107–110]. However, the only constraints associated with these biomarkers are their thermal instability and requiring specialized storage [111].

Many micro- and nano-materials with distinct signal transduction processes, such as metal nanoparticles, metal oxide, graphene or graphene oxide, quantum dots, hybrid materials, and metal–organic frameworks (MOFs), have brought significant advancements in the creation of innovative paper-based sensors. Furthermore, signal scanners are becoming more compact. Portable electronics, such as smartphones and electric watches, can read optical and electric signals. As a result, the POC was implemented on PADs (Fig. 5a) and utilized in illness analysis (e.g., biological fluids such as whole blood, serum, sweat, tears, urine, saliva, cells, and viruses), environmental monitoring (e.g., water, gas, soil), and food control (Table 1) [112].

The LFA is a paper-based (bio) analytical (Fig. 5b) approach for on-site detection of target compounds, in which a sample is put to a standalone device and the result is acquired in a short amount of time. LFA can contribute significantly to screening, diagnosis, prognosis, monitoring, and surveillance. The rapid clinical evaluation might have a significant influence on disease management by reducing burden, improving workflow, increasing clinical treatment and patient outcomes, and perhaps lowering costs. The use of LFAs can allow patients to obtain both the diagnosis and the particular treatment within the same session, minimizing the number of clinical visits and avoiding referral and difficulties associated with delayed therapy initiation [112, 123]. Microfluidic paper-based analytical devices are being developed to meet the rising need for ASSURED diagnostics for infectious (e.g., COVID-19, viral hepatitis, HIV/AIDS, dengue, TB, typhoid, malaria) and noncommunicable illnesses (e.g., diabetes, obesity, hypertension, cardiovascular disorders and cancer) [124]. Because cellulose fibers occupied a large part of the electrode surface in the early iterations of electrochemical PADs, their sensitivity was reduced by up to 40% [125]. The μPAD was then used to detect West
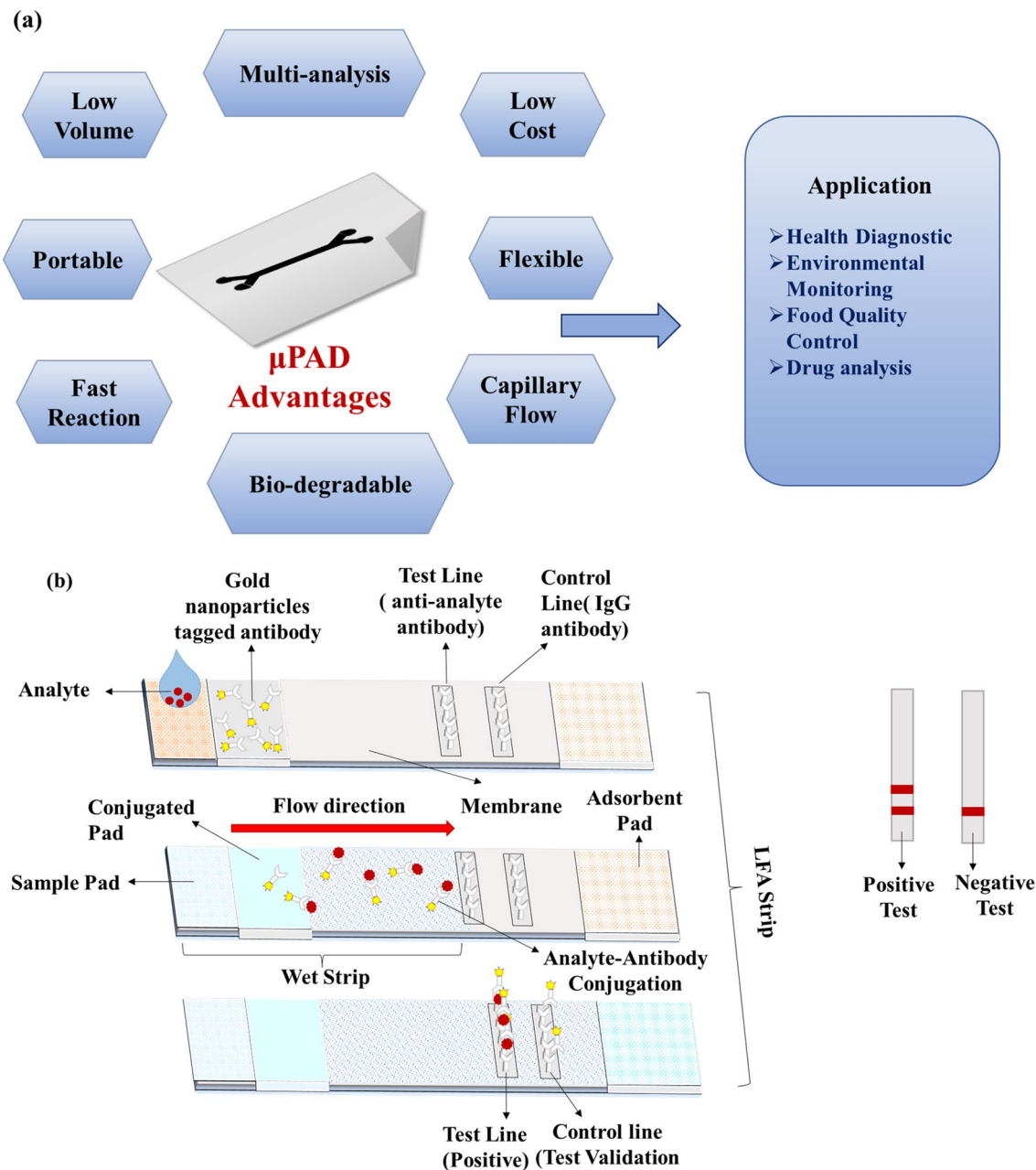
Fig. 5 a Important applications and advantages of analytical microfluidic devices based on paper and **b** Schematic representation of the LFA test mechanism. Top: the sample is deposited on the sample pad and migrates towards the conjugate. Middle: conjugated antibodies bind to the target analyte and (bottom) migrate to the test line where the bound target analyte is captured

Nile Virus (WNV) particles with a detection limit of 2.04 * 103 WNV particles/mL, or approximately 10 WNV particles/50 L of sample. This detection limit is comparable to that of Reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA and is in the clinically relevant range of 1.0 * 102 to 1.0 * 106 genomic equivalents/mL. The clinically relevant range varies according to sample type (tissue location and blood/urine) and infection stage. The µPAD established in this study illustrates what we think to be a promising direction in µPAD research in terms of providing POC biomedical diagnostics that are rapid, simple, resilient, economical, specific, and highly sensitive [125, 126].

Table 1 Recent studies on paper microfluidics technologies in the diagnosis of biomarkers

Target	Matrix	Biomarkers	Detection platform	Limit of detection/Sensitivity	Reference
Alzheimer's disease (AD)	Blood	ADAM10	Electrochemical immunosensors	0.35 fg/mL	[113]
Vitamin-D	Blood	25-Hydroxyvitamin D	Colorimetric immunosensors	15 nM	[114]
Malaria	Blood	*Plasmodium vivax*	Electrochemical immunosensors	~40 Vivax infected RBCs/10 μL	[115]
Rotavirus A infection	stool samples	Rotavirus A	Nucleic acid test	1×10^3 Copies/mL	[116]
Zika virus	Tap water, urine, and plasma samples	Zika virus	RT-LAMP	1 Copy/μL	[117]
Jaundice	Blood	Bilirubin	Paper-based SERS	7 μM in 1 μL	[118]
Acute myocardial infarction	Blood	GPBB, CK—MB and cTnT	SERS-based μPAD	8, 10, and 1 pg/mL	[119]
pH sensor	Aqueous sample	pH	Colorimetric	pH range 1.00–13.60 with a high resolution of 0.20 pH unit	[120]
Pancreatic cancer	Blood	PEAK1	Electrochemical immunosensor	10 pg/mL	[121]
Cancer	Serum	Cancer antigen 125	Electrochemical immunosensor	0.01 U/mL	[122]

Application of Nanobiosensors in Healthcare Monitoring

Microfluidic-integrated nanobiosensors technology has brought some relief in this direction in the area of personalized health-care in general. The applications of this emerging technology are vigorously exploited in the areas of screening of infectious organisms, including viruses for their early detection, analysis and cure of chronic diseases, general health management, retinal prostheses, cardiovascular diseases, diabetes, maternity and paternity testing, contrast imaging during MRIs, heart diagnosis, medical mycology, and overall health monitoring to name a few [28, 127]. However, this nanobiosensor-based technology is on a regular up gradation mode to uplift health-care in the light of new challenges in the diagnostic revolution being posed in society [29, 103]. Following are the most important applications of nanobiosensors used in health-care sector [38, 39].

Tracking of Biological Abnormalities

Nanobiosensors are being extensively used for early detection of various biological abnormalities in patients, which helps in reducing the health-care costs and solve many health-care challenges [102, 128–130]. With the evolution of microfluidic technology, integrated nanoscale systems can now carry out high-sensitivity and selectivity sequencing,

genetic genotyping, and DNA fingerprinting automatically. These microsystems can also be used to describe certain nucleic acids that have previously been linked to specific illnesses or hereditary abnormalities. A variety of microfluidic devices for the quick study of patterns linked with genetic illnesses have also been developed recently [131]. Among them, the detection of single nucleotide polymorphisms (SNPs) via PCR-based microfluidic devices holds the promise of identifying prospectively genetic genes in individuals, particularly in pharmacogenomics for personalizing individual medication dose and efficacy. Many micro-scale temperature-control modules have been successfully put into these microfluidic devices as a design upgrade for fast amplification and detection of SNP [132]. The microchip was combined with a cover plate using hot-embossed polymethyl-methacrylate (PMMA) microfluidic channels and a syringe pump to improve kinetics during DNA hybridization. Within minutes, a point mutation in a K-Ras oncogene was discovered at a level of 1 mutant DNA in 10,000 wild-type sequences. The kinetics of hybridization have also been explored by lowering the size of the microfluidic channels [133].

Cardiac Health Tracking

A nanobiosensor, such as a smartwatch or athletic band, has become very popular to track heart rates and transmits

physiological information to the concerned continuously [94]. Advanced sensors are being upgraded regularly using electrochemical and optical information to monitor the diseases continuously clinically [37, 134]. The microchip was combined with a cover plate using hot-embossed PMMA microfluidic channels and a syringe pump to improve kinetics during DNA hybridization. Within minutes, a point mutation in a K-Ras oncogene was discovered at a level of 1 mutant DNA in 10,000 wild-type sequences. The kinetics of hybridization have also been explored by lowering the size of the microfluidic channels [135]. Microfluidics has also been used to develop in vitro cardiac models by manipulating heart tissue to generate morphological, electrophysiological, and contractile microenvironments that are more like physiological conditions. The heart-on-a-chip produced can be utilized as an in vitro platform to study the pathophysiology of cardiac disorders [136]. C-reactive protein (CRP), B-type natriuretic peptide (BNP), low-density lipoprotein (LDL), and hydrogen peroxide are examples of representative biomarkers that should have parameters (e.g., high specificity, simple accessibility, high stability, and a long plasma half-life). An L012@PAni-PAAm hydrogel composite-based electrochemiluminescence biosensor for in situ detection of H_2O_2 emitted from cardiomyocytes [31]

Tracking of Body Chemistry and Detection of Signs in the Patient

Implantable nanobiosensors inserted under the human skin have an important role in providing vital chemical information about the biomarkers patient's biomarkers in real-time mode [106]. This technology is being used to continuously track life threatening diseases like diabetes, cardiovascular diseases and exercise habits of chronic patients and monitored by medical professionals in a real-time basis [107, 108]. Nanobiosensors enable clinicians to detect early signs and symptoms of life threatening diseases in patients more quickly than the conventional approaches [45, 137].

Glucose Monitoring

Electrochemical test strips are being used to monitor glucose in diabetic patients for glucose surveillance and adjust the dose of the medicine accordingly by the clinician [138]. Due to the benefits of high throughput, complicated fluid manipulation, multiplexed analytical testing, and parallel sample distribution, three-dimensional microfluidic paper-based analytical devices (3D-PADs) constitute an emerging platform development trend [139]. Because glucose is a key measure of metabolic activity, quick and simple glucose testing has become crucial in impoverished and emerging nations. Since the Harvard group introduced a microfluidic paper-based analytical device in 2007, it has gotten a lot of attention in a variety of applications. Several ways for making μPADs have been

devised, as have several detecting systems for glucose testing. The most essential approaches are without a doubt colorimetric and electrochemical detection. Colorimetric detection is more extensively employed than electrochemical detection, although having a lower sensitivity. The development of POC devices is predicted to create carry-on paper-based analytical equipment [140].

Patient Status in the Healthcare Unit and Management of Disease

Wearable nanobiosensors another important development of the nanobiosensor technology which is helping children, athletes, older people, and many others in monitoring their vital health parameters, particularly heart rate, sleep apnea, peripheral artery disorders, blood oxygen concentration and pulmonary obstruction etc., on a continuous mode [141–143]. Wearable devices are micro- and nano-fabricated systems that offer a suitable mechanical match to the biosensor with the surrounding tissue and therefore decrease the human biological response. For example, skin-integrated biosensors, sweat sensors, bio-potential sensors, tattoo-like sensors and implantable biosensors which utilize a polymer layer as a flexible support, combined with a functional material to generate, transmit and process the obtained signal [144, 145]. Nanobiosensors are very useful for the detection and management of chronic diseases e.g., clinical disorders like bacterial, fungal or viral infections and various cancers with high sensitivity and accuracy at low costs. Such vital information is very useful for disease management by the clinicians [145]. In case of cancer therapy, designing an ideal drug delivery system is a multidimensional challenge in biomedical applications, Bharath et al. (2019) report a novel hydrothermal technique with calcium carboxymethyl cellulose template for the synthesis of biocompatible and pH-sensitive biodegradable hydroxyapatite with mesoporous nanoplates for nanoscale drug delivery applications [146]. Likewise, Bharath et al. (2019) synthesis methods of Ni–Co nanocomposites have been reported, leading to different shapes of ferromagnetic nanoparticles such as 3D hedgehog, icosahedron, flower, needle, nanowires, and nanorods. However, the preparation of ferromagnetic alloy nanoparticles with controlled morphology (shape and size) coupled with synthetic properties of better productivity, cost-effectiveness and higher production speed is challenging. This simple microwave synthesis method is recommended for cost-effective, rapid synthesis and large-scale industrial production for multifunctional applications [147]. Nanobiosensors also enhance the treatment process after surgery with the use of dissolved pressure sensors for the brain and control implants infection and inflammation [148, 149].

Tracking Cell Protein

A nanobiosensor is capable of tracking live cell unlabeled proteins on a real-timescale. This MFS consists of a cell module and a nanobiosensor module centered on a single zigzag channel [150]. Spectral changes help directly monitoring the dynamics of the cell secretions. The updated platform versions have capacities for multiplex and label-free detections that could be miniaturized and integrated into LOC equipment's [151].

Biomolecular Detection and Measurement

Nanobiosensors can analyze biomolecular detection and measurement which permits researchers to monitor oxygen levels in systems in real-time. It enables the equipment to stimulate and replicate the functioning of actual organs more closely helping the clinicians in the development of new medicines [152, 153].

Pathogen Detection

Pathogens detection through microfluidic chips is an upcoming technology with flexibility for automation, integration, miniaturization, and multiplexing. The reaction chambers are usually at micro- or nano-scale, miniaturized and portable, suitable for POC testing. The technology also has the capability of Nucleic acid-based pathogen detection of bacteria, viruses, and fungi [154–156]. Commercial chip capability is being developed based on PCR or real-time PCR and using integrated microfluidic Loop-mediated isothermal amplification (LAMP) systems for large scale detection of pathogens for population screening [157, 158]. Bacterial detection is one of the most essential applications in medicine since the selection of medicines is often contingent on identifying the causative agent. Microfluidics-based devices have detected a wide range of microorganisms, (e.g., *Escherichia coli*, *Mycobacterium tuberculosis*, *Salmonella* spp., and *Vibrio cholerae*) are all examples of pathogens [159]. MFSs are a new generation of traditional detection technologies that rely on phases such as specimen preparation, reagent manipulation, bioreaction, and detection, all of which may be combined onto a single platform. Rapid detection, ease of use, cost effectiveness, and high accuracy in the diagnosis of infectious illnesses, including the most serious pathogens such as HIV, HBV, and ZIKV, are all advantages of microfluidic devices for diagnostic purposes [160, 161]. For microbial detection, PCR and LAMP on microfluidic chips, microfluidics in conjunction with MS, fluorescence spectrometry, and electrochemistry are used [162]. Enzyme-linked immunosorbent assay (ELISA) is a conventional cell culture-based method used for the detection of microbes.

Different from new generation technologies such as microarrays and MFSs, in ELISA a wide range of antibodies each with specific antigen bio-affinity for pathogens are used. Microorganisms like *Listeria* spp., *Listeria monocytogenes*, *Salmonella*, *Shigella* and *Staphylococcal enterotoxins* can be detected. HIV causes one of the most dangerous chronic infections, and is detected by ELISA. The HIV ELISA detects the presence of host antibodies, which have been produced as a result of the infection. The micrometer scale of MFSs is ideally suited for ELISA-based assays. Antibodies against other important viruses like hepatitis B and Zika viruses can be also detected by ELISA. Another technique within the non-culture category is microfluidic PCR-based pathogen identification [161].

Identification of Multiple Bacteria on the Microchip

Bacteria can cause serious infections which leads to severe diseases. The microfluidic technique has developed a series of miniature devices for rapid detection of bacteria [163–165] and antibiotic susceptibility testing [166–168]. Microfluidic chips have shown immense promise as diagnostic tools to identify bacteria in resources with limiting niches [169]. Besides, bacteria can also be identified on chip using chromogenic media and monitoring the change in color due to the presence of specific metabolites [170]. It requires interaction between microbial metabolites and specific chromogenic substrates in the medium. Bacterial metabolites induce chromogenic substrate to release a specific color to appear in the medium, indicating a specific bacterial type. The microchip method takes less time than conventional methods to identify bacteria [171]. The microchip method can be scaled up to detect different types of bacteria using several types of chromogenic media [172].

Nucleic Acid Detection

There are various advantages in molecule-based diagnostic tests than immunoassays in terms of sensitivity and specificity. However, most of the diagnostic tests are based on immunoassay and conventional nucleic acid-based tests (NATs) require extensive sample processing, trained operators, and specialized equipment. To build well-suited NATs, specifically for on-site diagnosis and POC testing diagnostics, a simple plastic microfluidic cassette ("chip") has been developed for nucleic acid-based testing of blood, other clinical specimens, food, water, and environmental samples [159]. These protocols utilize the conventional PCR for detection of nucleic acids [173–178]. However, some modifications have been made to accommodate the PCR-based NAT in a microfluidic cassette e.g., thermoelectric elements have been used for temperature regulation, solenoid valves for regulation of flow and pumping and LED detectors for

the detection of the amplified DNA previously labeled using intercalating fluorescent dyes [179].
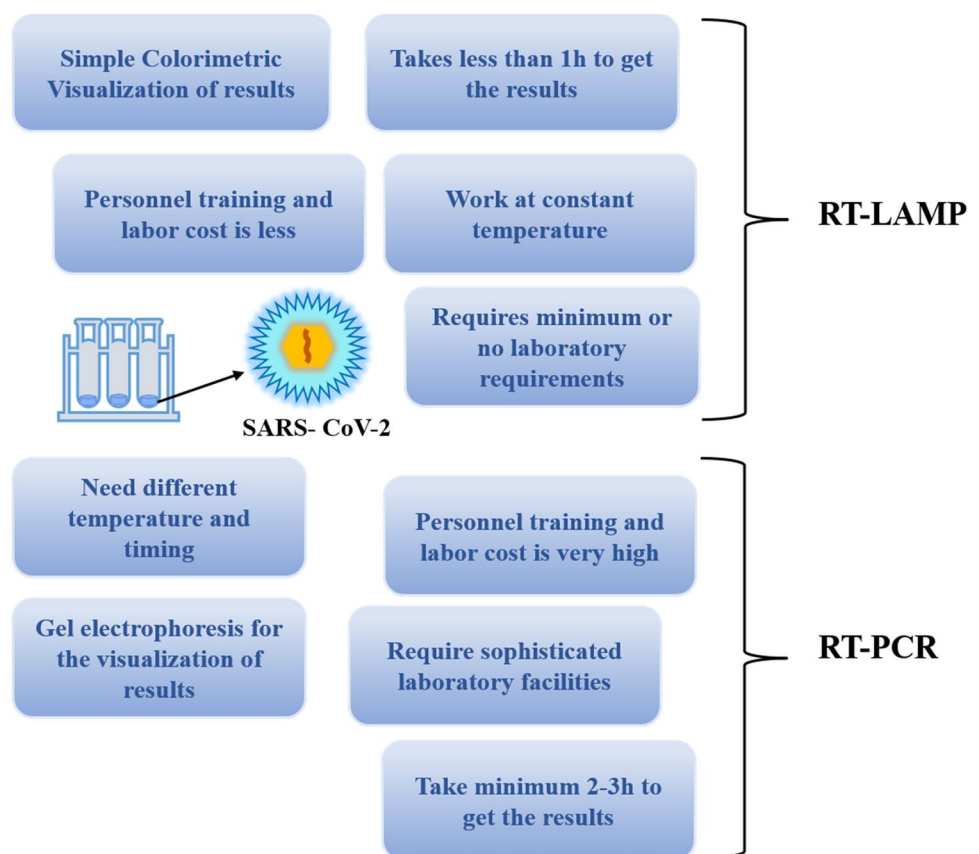
HIV Virus Detection

Viruses are also in the range of 10–100 nm, and nanostructures are suitable for their manipulation and analysis. The system could analyze the size distribution of different viral species, such as the human immunodeficiency virus (HIV) and the influenza virus. Nano-microfluidics has brought the attention of scientists to develop inexpensive, efficient and disposable diagnostic protocols for the detection of viruses [180]. This nano-optofluidic system demonstrated its sensitivity to detect single particles smaller than 10 nm, in which the nanofluidic structures confined the nanoparticle paths for continuous detection [181]. For HIV-1 diagnostics, scientists have already miniaturized the conventional ELISA for the detection of p24 antigen [182–184] and RT-PCR-based test to detect HIV-1 RNA [185]. However, these protocols have a major constraint that these do not fit onto on-chip designs [186]. Early detection is very important for any disease and the same applies to COVID-19 as well. Nanomaterial-based nanobiosensors and detectors have made this process quite simple and cost-effective. A recent study in this regard has been very thoroughly reviewed [187]. However, novel protocols are being designed for POC testing of viral loads in clinical samples [188]. Protocols for the on-chip detection of HIV-1 particles have also been optimized with automatic counting by ImageJ tool. Automatic virus counting significantly reduces the sample-to-answer time and manual labor. This novel method based on the quantification of virus particles paves a new way of developing POC viral load assays. Such devices have been applied to the diagnosis of several viruses like influenza, SARS corona virus, and smallpox [112].

SARS COVID-19 Detection

Many viruses have RNA as their genetic material e.g., Ebola virus, hepatitis C, influenza, severe acute respiratory syndrome virus (SARS), coronavirus and poliomyelitis virus etc., responsible for severe to fatal diseases [189, 190]. The latest world-wide epidemic is caused by coronavirus, a single-stranded RNA mainly affecting the respiratory system [190]. According to one of the estimates, more than 14 million people got infected by this virus (COVID-19) by the end of 2020, putting a lot of pressure on healthcare and non-healthcare facilities world-wide [191]. The study on COVID-19 has shown that it causes multisystem infectious disorders in co-morbid patients leading to large

Fig. 6 Comparison of RT-LAMP and RT-PCR techniques for the detection of COVID-19 virus

Simple Colorimetric Visualization of results

Takes less than 1h to get the results

Personnel training and labor cost is less

Work at constant temperature

Requires minimum or no laboratory requirements

SARS- CoV-2

RT-LAMP

Need different temperature and timing

Personnel training and labor cost is very high

Gel electrophoresis for the visualization of results

Require sophisticated laboratory facilities

Take minimum 2-3h to get the results

RT-PCR

scale fatalities in them [192, 193]. The most reliable gold standard at presenter-PCR [194]. The latest version of the same is Microfluidic nano-scale real time PCR which is ultra-sensitive in detecting SARS-CoV-2 and can detect as low as < 1 viral copies/μL of the sample. Such methods can preclude the frequency of false negatives [173]. The other reliable technique is reverse transcription loop mediated isothermal amplification (RT-LAMP), which is much faster and takes less than 30 min [174, 175] Fig. 6 developed a quick and cost effective method based on RT-LAMP as an alternative to real time RT-PCR, that gave the results in less than 30 min, and reverse transcription recombinase-aided amplification assay, which are much faster than the conventional real time RT-PCR.

El-Tholoth M., et al., has introduced another fast molecular test (Penn-RAMP) based on LAMP two-stage isothermal amplification to detect SARS-CoV-2 in closed tubes for high sensitivity and POC suitable for home-use [176]. T. Nguyen et al., reported a POC testing device, which is rapid, robust and affordable with minimal training for emergencies such as outbreaks [177]. This device uses a LAMP reaction with a LFA to detect the virus in less than 1 h. Another example of using LFS is the Bio-Medomics COVID-19 IgM/IgG Rapid test has also been reported recently. Yang, T., et al. invented an RNA-based POC testing device for diagnosing SARS-CoV-2 using both a LAMP assay and a paper-based POC testing diagnostic protocol and integrated it with a smartphone to provide a fast, sensitive, and more accessible tool [178]. Thus, microfluidic devices offer a wide range of methods, including RT-PCR, RT-LAMP, etc. for the detection of RNA viruses and can be optimized for detection of viruses like COVID-19, which has caused a worldwide fatal pandemic at present [179].

Very recently researchers have used nanomaterial-based sensors with multiplex capabilities for detecting COVID-19 virus from the exhaled breath of the patients. These gold nanoparticle-based nanobiosensors respond to the volatile organic acid compounds present in the breath of the patient in early stages of viral infection, thus very helpful in early diagnosis of the virus [195, 196].

Another study has reported the use of three-dimensional chemical structures like the MOFs as very useful in developing the sensors for early detection of viruses including the SARS-CoV-2 [186, 188]. MOFs are porous structures and work by quenching fluorescence of fluorescence-labeled probes [197, 198]. However, microfluidic approaches are also been exploited for rapid and inexpensive detection of viruses such as influenza, SARS-coronavirus, etc. [62]. Besides, nanotechnology-based affinity nanobiosensors have been reportedly being developed for the fast detection of SARS and COVID-19 viruses [43, 175]. These nanobiosensors are antibody-based combined with electrochemical, optical or FET-based transduction devices [30, 199]. However, validation of the protocol with clinical and environmental samples are expected shortly [200]. Another development in tackling the pandemic globally is by combining nanobiosensors, AI, information technology and dynamic networking devices to enable monitoring the data through long-distance communication thereby improving health care scenario [201, 202].

Protein Detection

Biomarkers of specific diseases are correlated with DNA and also proteins present in the organism. As the proteins are directly associated with the biological functions of an organism being the final product of the gene expression, proteins are considered as more authentic diagnostic source for POC testing diagnostic. Protein nanobiosensors are now used in portable/disposable POC testing applications facilitating the integration of the nanobiosensors with the microfluidics platform [203]. μPADs have demonstrated clinically relevant concentrations of glucose and proteins in artificial urine quantitatively. However, the application should be limited because the immobilization techniques on a paper are yet immature and dried bio-receptors could lose their activity with time [204, 205]. Protein microarrays for studying protein function are not frequently utilized, in part due to the difficulties in creating proteins that can be detected on the arrays. Printing complementary DNAs onto glass slides and then translating target proteins with mammalian reticulocyte lysate can be used to create protein microarrays. Epitope tags linked to the proteins enable for in situ immobilization. This approach eliminates the requirement for protein purification, avoids protein stability issues during storage, and collects enough protein for functional research [206].

Conclusion

High standards of health care management must be reached in order to deliver higher quality health care through the development of microfluidics-based nanobiosensors for POC diagnostics is an essential area of research. To efficiently identify diseases, simple, sensitive, and cost-effective diagnostic technologies such as biosensors must be developed. Microfluidics-based nanobiosensors are used in a wide range of medical applications, supporting doctors and patients with operations such as disease control, clinical care, preventative treatment, patient health information, and disease examinations. In this review, attempts have been made to update the applications in microfluidic-based biosensing, giving examples of the latest technologies developed in chemistry and biology. The future of microfluidics-based biosensing technology seems to be very bright, exploiting

the developments and breakthroughs in new biomaterials, electronics, cloud computing, and the manufacturing of affordable smart devices. Microfluidics-based technologies are an attractive platform for the detection of infectious pathogens because they offer short processing times, reduced sample consumption, and a miniaturized format with portability and simplicity. Moreover, the integration of multiple functions into a single unit with full automation remains a challenge. The reliability of assay readings produced by microfluidic devices must be proven, the external parts must not be complex, and the devices must be accessible to end-users. Much effort is needed in the early stages of technology and product development. The advanced design of POC diagnostic devices will require greater understanding of both the locations in which the technologies will be adopted and the end users. Affordability is of great importance to users in low-resource settings.

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