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Miniaturized Biosensing Devices

Fabrication and Applications

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ISBN 978-981-16-9896-5 ISBN 978-981-16-9897-2 (eBook)
https://doi.org/10.1007/978-981-16-9897-2

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Advances in biosensing strategies and their fabrication techniques have paved the development of several biosensors as rapid, robust, and miniaturized modules. These advanced biosensors not only deliver the sensitive and selective detection of clinically important targets but also can identify the causative markers related to food safety and environmental surveillance. This book covers the fundamental concepts and various state-of-the-art biosensing strategies on all fronts, viz. healthcare, food safety, biosecurity, etc. For a better understanding of the contents to the budding scientist and the professional from the diverse fields, an evolution of biosensors is provided in Chap. 1, which introduces the fundamentals as well as the elaborative timeline of chemical and biological sensors. This chapter elaborates on the important aspects of the (bio)markers, and state-of-the-art miniaturized technologies adopted for making the biosensors better performing and futuristic. Extending the fundamentals of the most used formats of the biosensors, the next chapter (Chap. 2) describes the importance of carbon-based nanomaterials for achieving sensitive detection in the most widely used electrochemical formats. The properties of the nano-dimensional carbon materials have been highlighted, which not only helped to achieve miniaturization but also made the developed modules efficient towards the detection of the targets. This chapter also discusses the various technologies for the fabrication of the carbon-based electrode to mold the futuristic farm factors, viz. screen printing, 3D printing, etc. For the miniaturization, the sampling from real biological fluids relied on the fluidic units. Chapter 3 introduces the usage of micro-fluidics and nano-fluidics in the development of various formats of the sensors and their fundamental aspects, which eventually give the readers to their applications by integrating the fluidics platform for facilitating the real-time assessments. Chapters 4–10 are dedicated to the clinical biosensor's developments and their state-of-the-art advancements. These chapters comprehensively summarize the evolution of clinical biosensors for complementing the lab-based conventional techniques. Chapter 4 describes the various considerations for developing biosensors for clinical and biomedical applications. It also orients the reader to think about the technicalities of the biosensor developments which are highly interdisciplinary. Thereby, the next chapter assesses the area where conventional physical sensors are being supplements by the biosensors in futuristic clinical applications. Chapter 5 is dedicated to the in vitro detection of pathogens based on microfluidics, which is

essential for combatting the infection in broader populations. Chapter 6 summarizes the advancements in viral detection using various biosensing modules. Chapter 7 discusses one of the most discussed and studied conditions for the clinical history, i.e., diabetes. This chapter highlights the state-of-the-art biosensing modules for diabetes management using continuous monitoring. The continuous monitoring of the molecules and targets has opened the gate of next-generation biosensors, which have in situ applications. These include wearable, implantable, and ingestible (bio)-sensors for studying the complex physiological changes in the body not only to get clinical information but also for elevated performances. Chapters 8 and 10 describe various such advancements of the biosensors highlighting the possibilities of the futuristic medicine of digital health. These chapters also describe the importance of simultaneous multiplexed detection which are not only essential for clinical diagnoses but also crucial for environmental monitoring and food quality assurances. Chapter 9 emphasizes such miniaturized biosensing modules for detecting the various clinical markers simultaneously. These chapters will be highly beneficial not only to the scientists but also to the clinicians. These chapters are coherently composed with lucid and optimized terminologies, which will help the readers form diversified fields to conceptualize the state-of-the-art technologies in clinical and biomedical diagnoses.

Health and wellness are interlinked to the nutrition we regularly eat, which makes food safety and quality of major concern in this current era of commercialization. The emergence of various safe-keeping and quality preservation techniques has been preventing enormous spoilage of the food, and thus the consumers. However, certain microbial invasions and preservatives used for food compromise the health status when consumed in excess. This has led to the concept of onsite-quality controls, which are essentially supported by cost-effective biosensing modules. Chapter 11 describes various such advancements in the food safety and quality assessment frontier encompassing commercial modules and proposed prototypes. The food grown in the spoiled soil is contaminated with hazardous chemicals, thus evaluating the environment is important. The sensing/biosensing modules have also been employed for detecting the environmental hazards not only to save the food but also to protect the people from the direct exposure. Chapter 12 summarizes the advancements of the miniaturized biosensing modules for the onsite metal toxins detection from the environment, whereas Chap. 13 describes the challenges associated and advancements in the field of nitrogen detection in the environment, which is crucial for soil health. Biosecurity and biohazardous are other environmental concerns in a global context. Chemical and biochemical sensors have been used for their detection. Chapter 14 describes various types of biohazardous compounds and the biosensing module technologies for their detections. Chapter 15 concludes the advancements in the developments of biosensors by assessing these with their commercial feasibility. This chapter also highlights the challenges and commercial potentials of the developed biosensing prototypes. Thus, the latter half of this book will be highly beneficial to the environmentalists to develop their customized miniaturized tool for their study. More importantly, this part will greatly help the food scientist, agriculturists, and farmers to understand the recent technologies for

food safety and quality controls assessments. This book would be much helpful as a handbook for developing cost-effective sensing modules in research and development units at the manufacturing plants and the onsite quality control centers.

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About the Editors

Pranjal Chandra is an assistant professor at the School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India. He earned his Ph. D. from Pusan National University, South Korea, and did post-doctoral training at Technion-Israel Institute of Technology, Israel. His research focus is highly inter-disciplinary, spanning a wide range in biotechnology, nanobiosensors, material engineering, and nanomedicine. He has designed several commercially viable biosensing prototypes that can be operated for onsite analysis for biomedical diagnostics. He is an associate editor and editorial board member of various international journals, including Scientific Reports, Sensors International, Frontiers in Bioengineering and Biotechnology, Molecules, Frontiers in Sensors, and Green Analytical Chemistry. He has also been appointed as Advisor for the Biomedical Sensors Domain and Sensor Networks Systems, Institution of Engineering and Technology (IET), Michael Faraday House, London, United Kingdom. Prof. Chandra is author of over 110 high impact publications, including research/reviews papers and invited book chapter. He has published 13 books on various aspects of biosensors/medical diagnostics/material engineering. Prof. Chandra is the recipient of many prestigious awards, coveted honours, and fellowships such as DST Ramanujan fellowship (Government of India); Early Career Research Award (ECRA) (DST, Government of India); BK -21 and NRF fellowship, South Korea; Technion Post-Doctoral Fellowship, Israel; Nano Molecular Society India Young Scientist Award; Bio-tech Research Society India (BRSI) Young Scientist Award; Young Engineers Award 2018; Highly Cited

Corresponding authors in The Royal Society of Chemistry (RSC), Cambridge, London; Top 10% cited article in the General Chemistry Section RSC Journal, Cambridge, London; and Gandhian Young Technology Innovation Award (GYTI) 2020. Prof. Chandra is also listed among the world's top 2% scientist in the year 2020 and 2021 by Stanford University, USA.

Kuldeep Mahato is currently working at the Department of NanoEngineering, University of California San Diego, USA, as a post-doctoral scholar. He received his Ph.D. from the Indian Institute of Technology Guwahati, Assam, India. His research interests are majorly focused on the development of sensors/biosensors for clinical, food safety, and (bio)security applications. His interests extend to the self-powered biofuel-cell-based technologies for the development of wearable, implantable, and ingestible clinical and biomedical applications using multidisciplinary approaches where he has been solving various challenges associated with the development of commercially viable prototypes. He has authored more than 30 research articles in journals of high repute. He has received the prestigious national award "Gandhian Young Technological Innovation—2020" for his research innovation. Further, he has served as guest editor in several special issues of sensor journals. He has also been a reviewer for multiple peer-reviewed international journals in the field of analytical chemistry, sensors, and biosensors.

1 Sensor-Assisted Next-Generation Diagnostics: Emerging Concepts, Biomarkers, Technologies, and Challenges

Weiqiu Jin, Qisheng Yang, Shangjian Liu, Changzi Dong, and Tian-ling Ren

Abstract

The evolution of medical diagnosis has continued to broaden our understanding of diseases. The development of various omics technologies and the continuous advancement of medical sensor technology are pushing medical diagnosis to a more accurate and sensitive next-generation diagnosis (NGD). In this chapter, we begin with the emerging concept of next-generation diagnostics and give concise summaries concerning the construction and application of the hardware and novel technology relied on the next-generation medical diagnosis, including in-vitro diagnosis technologies (liquid biopsy, gas biopsy, etc.), in-vivo diagnosis technologies (sensor implantation, MEMS technologies, etc.) and other health monitoring technologies (wearable or ingestible electronics, Internet of things (IoT), Health Cloud, etc.), and decision-making assistants (information fusion, artificial intelligence (AI), data-mining, etc.). In the end, we briefly introduce the

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_1

new challenges faced by the next generation medical diagnosis on the following aspects: device performance reliability, miniaturization and system integration, ethics-related issues, and expense and patient compliance.

Keywords

Next-generation diagnostics · Biomarkers · Healthcare · Miniaturization

1.1 Emerging Concepts and Connotations of Next-Generation Diagnostics

Early detection and diagnosis of diseases are vital to the lives of patients. However, the previous medical diagnosis is subject to the limited clinical data and the experience of the attending physician, which is prone to misdiagnosis. Emerging sensing detection equipment and information technology provide a wealth of diagnostic tools, making medical diagnosis in the direction of precision, intelligence, and personalized development; thus, the next generation of diagnosis is also considered as a manifestation of precision medicine (Council 2011). Specifically, as shown in Fig. 1.1, while traditional in vitro testing devices can detect pathological markers in body fluids, some emerging wearable and implantable sensors can cover a much wider range of indicators, including nucleic acids, proteins, microorganisms, and even images. In-vitro detection equipment is also constantly updated in iterations, and powerful biochips can achieve high-throughput, real-time detection and point-of-care applications (Azizipour et al. 2020). The multidimensional data collected by these sensors, together with electronic medical records, capture many of the physical health characteristics of the patient and, aided by appropriate algorithms, provide more reliable decision-making. These sensing devices and information processing technologies are closely linked to the diagnosis, prognosis, and treatment of diseases and together map the blueprint for the next generation of diagnostics.

Based on the concept mentioned above, new ideas and technologies would be included to further conclude the kernels and the pursuits of the next-generation diagnosis:

1. **Personalized/precision diagnosis.** It is necessary for the next-generation medical diagnosis to scrutinize the genetic background and disease characteristics of the patients and apply precision medicine to the individual cases, striving to serve the personalized diagnosis and treatment.
2. **Integrated patient records for evidence-based medicine.** The next-generation diagnosis will comprehensively integrate the patient information with genetic information, imaging, patient record, biochemical examination, physical examination, auxiliary examination, etc. These inclusive data underpin the next-generation diagnosis by providing more evidence-based medicine indicators, assisting physicians' decision-making.

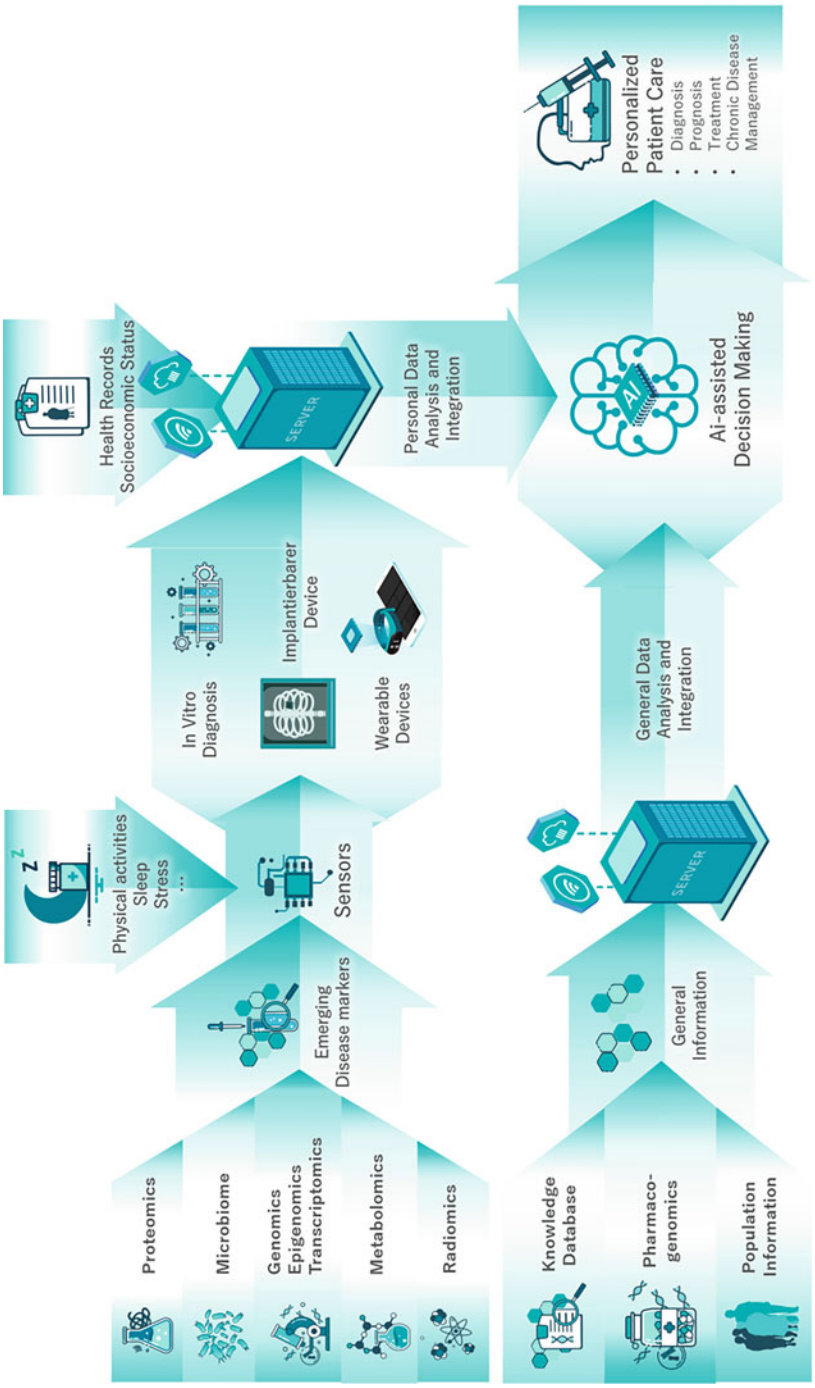

Fig. 1.1 Connotations and compositions of the next-generation diagnosis

3. **Emerging of disease markers and pathogen characteristics.** In order to discover more disease markers, the next-generation diagnosis needs both the underpinning supports of metabolomics, genomics, epigenetics, transcriptomics, radiomics, and proteomics and the technological support of novel advanced sensors. This exploration will consequentially be beneficial for the realization of the high-precision, low-cost in vitro/vivo diagnosis.
4. **Early intervention in the occurrence and development of the disease.** The next-generation diagnosis will incorporate chronic disease management and thus can discover risk factors at an early stage. The sequent intervention will prevent the occurrence or the escalation of the disease.

Several proof-of-concept studies have been conducted and applied to precise/personalized diagnostics, disease risk monitoring, and chronic disease management, demonstrating the superiority of next-generation diagnostics (Guzik et al. 2020). Taking a multimodal fusion technology as an example, multimodal image fusion technology (such as radiological image + ultrasonic image) can overcome the deficiency of single-mode information, which has become a research hotspot in recent years (Antropova et al. 2017). Data mining methods can also extract semantics from previous examination reports and obtain valuable disease information (Kirshners et al. 2012; Mahmoodi et al. 2016).

However, cutting-edge and effective ideas such as precision medicine, personalization, and information fusion have yet to take hold among more doctors, largely because of the unforeseen complexity of next-generation diagnostic technologies. In the case of polygenic and complex diseases such as mental illness and diabetes (Jaffee and Price 2007; Nassief Beshay et al. 2018), the occurrence of diseases is the result of a combination of genetics and environment. Therefore, precision medicine for complex diseases requires comprehensive consideration of multiple levels of information such as protein, metabolite, or epigenetics, so as to make more accurate judgment and assessment of the result of environment–gene interaction and then effectively guide the prevention, diagnosis, and treatment of diseases. Therefore, metabolomics, genomics, epigenetics, transcriptomics, radiomics, and proteomics are expected to be applied in multimodal diagnosis, thus covering early, long-term, and real-time monitoring of the disease. For this initiative, it is necessary to systematically review the emerging biomarkers, detection technologies, and information technologies to assist diagnostic decision-making in order to accelerate the implementation of the next generation of diagnostics.

1.2 Emerging Biomarkers

To discover and utilize more disease markers, the next-generation diagnosis needs both the underpinning supports of metabolomics, genomics, epigenetics, transcriptomics, radiomics, and proteomics and the technological support of advanced sensors. This section focuses on emerging biomarkers associated with disease. According to the report of the World Health Organization (WHO), four

main kinds of noncommunicable chronic diseases are cancers, chronic respiratory diseases, cardiovascular diseases, and diabetes mellitus (World Health Organization 2020). Additionally, acute diseases such as contagion, septicemia, organ failure, etc. are also included in this section. We dissected potential detectable biomarkers for these diseases from the perspective of omics, which will be considered for inclusion in the next-generation of diagnostic criteria.

1.2.1 Biomarkers for Cancer

With the advent of the omics era, a wealth of biomarkers has been discovered with high-throughput analysis. From classic enzymes and antibodies to noncoding RNA, the types of biomarkers have increased while their combinations have also become increasingly diverse. As shown in Fig. 1.2, the genomics, epigenomics, transcriptomics, proteomics, metabolomics, and even fluxomics are rapidly developing, which produce multiple emerging biomarkers; among them, DNA methylation, circRNA, miRNA, and CTCs are famous representatives.

1.2.1.1 DNA Methylation

DNA methylation may take an active role in malignant progression by promoting the expression of oncogenes, suppressing tumor suppressor genes (TSGs), or changing the stability of chromosomes. At the same time, DNA is more stable than other biological molecules, such as protein or RNA, making it more conducive to detection (Li et al. 2012). DNA methylation plays an important role in cancer detection, such as gastric cancer (Tahara and Arisawa 2015), lung cancer (Kneip et al. 2011), and prostate cancer (Litovkin et al. 2014). For the detection of lung cancer, methylation has satisfactory sensitivity (60%) and high selectivity (90%) using blood plasma samples. An even higher sensitivity was achieved when detecting squamous cell carcinoma (SCC) and small cell lung cancer (SCLC) with a sensitivity of 80 and 63%, respectively (Kneip et al. 2011; Pan et al. 2018). The use of DNA methylation as a tumor marker in detection faces concerns such as lack of standard and reproducible detection schemes and difficulties in the DNA purification methods.

1.2.1.2 circRNA

CircRNA, which exists in most cells and plays an important role in the promotion and progression of cancer, is a novel kind of noncoding RNA having covalently closed loop structures. Existing evidence has shown that some circRNA will be enriched in exosomes, which bring new ideas for circRNA detection (Wang et al. 2019b; Li et al. 2021b). Exosomes are also called important mediators of intercellular communication by regulating a variety of biological processes (Zhang et al. 2019b). As shown in Fig. 1.3, previous studies have shown the role of exosomal circRNA in the proliferation of cancer (Zhang et al. 2019a), cancer metastasis (Li et al. 2020), and mediating drug resistance in a variety of cancers (Luo and Gui 2020). Zhang et al. reported the role of circular RNA ciRS-7 (CDR1as) in non-small cell lung cancer (NSCLC) (Zhang et al. 2018). The mechanism is through

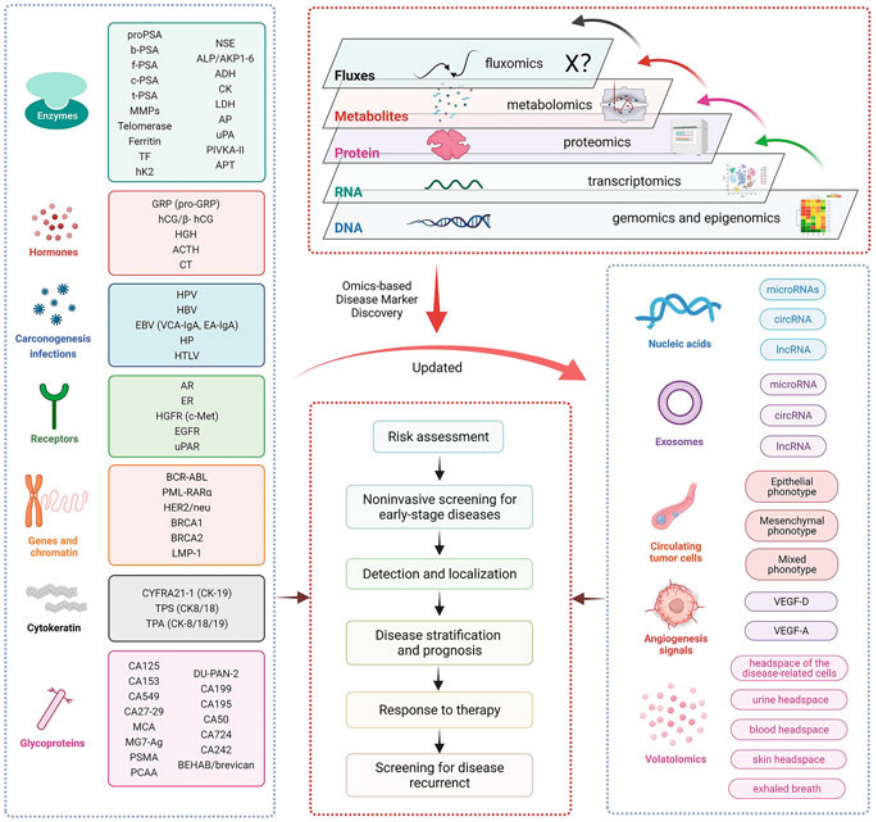

Fig. 1.2 The evolution of disease biomarkers (cancer) (Created with Biorender.com)

downregulation of tumor suppresser miR-7 to upregulation target gene of miR-7, including proto-oncogenes like Ki-67, EGFR, CCNE1, and PIK3CD.15 (Sun et al. 2016). As shown in Fig. 1.3, circRNAs are closely related to the hallmarks of cancer. For example, CDR1as and circHIPK3 can regulate the EGFR, thus activating cell proliferation, and circ-ITCH, circ-ZFR, etc. could intervene the expression or the activation of tumor suppressor genes (TSGs) (such as PTEN and CDK) and, in this way, assist cancer cells in evading antigrowth signals (Su et al. 2019). Meanwhile, cellular apoptosis or autophagy can be resisted by cancerous cells with the help of Hsa_circ_0007534 (Su et al. 2019). Moreover, Has-circ-0020397, CircRNA-MYLK, and Dirc-10,720 could regulate the TERT, VEGF, and the EMT process, respectively, thus influencing the proliferation potential, angiogenesis, and tumor metastasis (Su et al. 2019).

However, there are still some problems in the clinical application of these tests. First, some RNA tests require living tissue. In addition, due to the wide variety of circRNA and the relatively complex functions, its reliability in detection needs to be further confirmed (Meng et al. 2017).

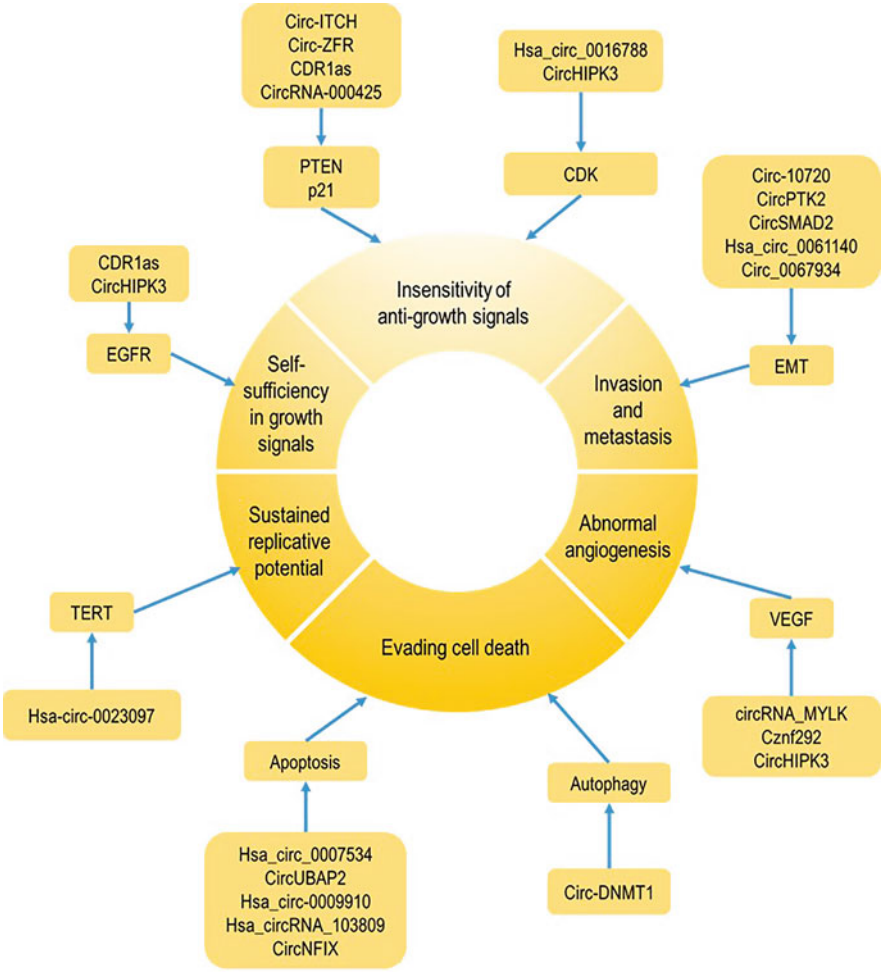

Fig. 1.3 CircRNAs with the hallmarks of cancer. Reprinted from Su et al. (2019)

1.2.1.3 miRNA

miRNA is involved in almost every cellular process, which is essential for organisms' development and homeostasis. Many diseases are related to dysregulation of miRNA, especially cancer, for mature miRNA can combine with some proteins to form RNA-induced silencing complex (RISC), which further regulates gene expression (Calin et al. 2004). Some of them can be used as a site for diagnosis or prognosis or therapeutics target.

1.2.1.4 Circulating Tumor Cells (CTCs)

CTC has an important role in early cancer diagnosis and prognosis. It provides a less invasive means to detect the molecular and genetic profile of metastatic cells and

give a prediction of tumor metastasis before clinically detectable metastasis occurs (Bankó et al. 2019). Because of the heterogeneity of cancer in different regions, the CTC can provide a more comprehensive result than tissue biopsy in several sites (Sundaresan et al. 2016). However, several limits remain, such as insufficient proof grounded in clinical utility (Alix-Panabières and Pantel 2016).

In addition to CTC, some molecular markers such as cell-free DNA (cfDNA) can be detected (Zhang et al. 2017). When apoptosis or necrosis happens, CfDNA can be released from cells as important signals of inflammation and malignant cell activity. Tumor cells can also actively get into or passively be pushed into the bloodstream by external forces ranging from tumor growth kinetics or mechanical forces during surgical operations, and these tumor cells can be shielded by platelets. Moreover, circulating exosomes can be generated by many cell types, including tumor cells, normal cells, and blood cells (even platelets), which provide another way to analyze the tumor burden, thus evaluating the treatment effect and predicting the patient prognosis.

1.2.2 Chronic Respiratory Diseases

Chronic respiratory diseases are a group of diseases including lung cancer, chronic obstructive pulmonary disease (COPD), and asthma, which are serious health issues worldwide (Chen and Wang 2012). Many biomarkers have been discovered, such as serum microRNA (Pattarayan et al. 2018), circulatory cell epigenetics, genome (Gruzieva et al. 2014; Saco et al. 2018), and exhaled breath molecules (Kharitonov and Barnes 2002; Bregy et al. 2018).

1.2.2.1 Exhaled Breath Molecules

Both volatile molecules and nonvolatile molecules, including nucleic acids, proteins, lipids, etc., can exit in exhaled breath. These molecules contain a lot of information about diseases, which makes them potential biomarkers. Compared with traditional imaging, endoscopy, immunology, and other technologies, the detection of exhaled gas is rapid, noninvasive, and relatively simple (Karnon et al. 2007; Broza et al. 2018). At present, the detection method of exhaled gas markers is mainly mass spectrometry based (Bregy et al. 2018; Gaugg 2018). The current development of nanotechnology provides a potential low-cost and rapid detection method for the biomarker detection of exhaled breath (Broza et al. 2018). However, some challenges remain: lack of sensitivity due to the interference of other chemical compounds in exhaled breath, sometimes use of toxic chemicals, and lack of standardization (Khan and Hegde 2020).

1.2.2.2 MicroRNA

Extracellular microRNA is an important biomarker for respiratory diseases. Some existing studies have shown its role in acute lung injury (Guo et al. 2014; Yang et al. 2015b), idiopathic pulmonary fibrosis (Li et al. 2014; Yang et al. 2015a), chronic obstructive pulmonary disease (COPD) (Van Pottelberge et al. 2011; Ellis et al.

2013), and asthma (Liu et al. 2012; Panganiban et al. 2012). For example, research showed that several miRNAs including miR-223, miR-1274a, miR-18a, miR-106a, and miR-146 have been involved in biological pathways that may be relevant to the progress of COPD, including the transforming growth factor β and wnt signal pathways (Ezzie et al. 2012; Salimian et al. 2018).

1.2.3 Cerebrovascular Diseases

1.2.3.1 Trimethylamine-N-Oxide (TMAO)

TMAO produced by gut microbes can be a potential biomarker for cerebrovascular disease. Research has demonstrated the positive dose-dependent association between serum TMAO level and increased cardiovascular risk and mortality (Schiattarella et al. 2017). There may be multiple mechanisms that cause this adverse effect: first, it can promote macrophage foam cell formation in the artery wall (Wang et al. 2011); second, it is related to the reduction of "reverse cholesterol transport" together with general defection of cholesterol metabolic pathway. Exiting methods available for the detection of TMAO include mass spectrometry, ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), and nuclear magnetic resonance (Garcia et al. 2017), and other new pathways are shown in Fig. 1.4.

1.2.3.2 Urine Biomarkers

Protein or peptide markers in urine also have broad clinical prospects (Röthlisberger and Pedroza-Diaz 2017). Urine sample is an ultrafiltrate of blood, so it will reduce interference from nonspecific proteins such as albumins, which were originally present with a large amount in the blood. At the same time, the sample can be

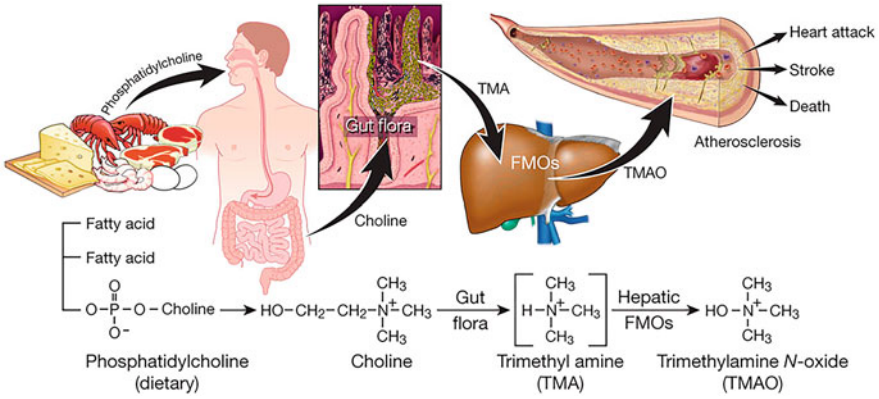

Fig. 1.4 Recently reported pathway for gut-flora-mediated generation of the pro-atherosclerotic metabolite from dietary PC. Reprinted from Wang et al. (2011)

obtained noninvasively, and less pressure is given on the patient. Polypeptides in urine can be used in coronary artery disease (CAD) and heart failure (HF).

1.2.4 Diabetes

Depending on the prediction by the World health organization, diabetes patients will continue to grow and reach at least 366 million by 2030 (Smyth and Heron 2006). It is necessary to recognize and control the progress of diabetes to induce both the incidence of the disease and its complications (Narayan et al. 2011). There are two main kinds of diabetes mellitus. Type 1 diabetes is due to deficient insulin production and needs daily insulin replenishment. Type 2 diabetes results from the body's ineffective use of insulin (Gavin et al. 1997).

There are many markers that can predict the diabetes process before abnormal blood sugar concentration appears. For type 1 diabetes, the correlation between disease and many factors has been found, such as immune response to islet autoantigen, gene expression profile of whole blood, proteomics of serum, circulating-miRNA (Gavin et al. 1997), proinsulin/C-peptide ratio (Truyen et al. 2005; Schölin et al. 2011; Sims et al. 2016), and DNA methylation pattern (Mirmira et al. 2016). DNA methylation can be an alternative for early diagnosis because islet β-cells express many genes in a nearly exclusive way. One example is the gene encoding preproinsulin (INS in humans and Ins1 and Ins2 in mice); this gene is usually hypomethylated in mouse and human islet β-cells at selective sites in promoter and coding regions than in other cells (Fisher et al. 2015; Lehmann-Werman et al. 2016). The hypomethylation form of cell-free circulating DNA (cfDNA) may reflect both autoimmune and alloimmune destruction of β cells (Lebastchi et al. 2013; Lehmann-Werman et al. 2016).

For type 2 diabetes, emerging biomarkers include organokines (Chung and Choi 2020), circulating miRNA (Zhu and Leung 2015), and metabolic markers (e.g., mannose (Lee et al. 2016) and α-hydroxybutyric acid (Cobb et al. 2016)). Adipokines are a wide range of cytokines secreted by adipose tissue. Abnormal secretion of adipokines is associated with insulin resistance and obesities, which make them an effective biomarker for diabetes screening and diagnosis (Jung and Choi 2014). Adiponectin is a kind of adipokine involved in glucose and lipid metabolism and also takes part in insulin resistance (Thanakun et al. 2014; Ojeda et al. 2015). Ojeda and his colleague have reported an electrochemical immunosensor that uses special electrodes (SPCEs) modified with functionalized double-walled carbon nanotubes (DWCNTs) and uses DWCNTs as platforms for further immobilization of specific antibodies (Ojeda et al. 2015).

1.2.5 Acute Diseases

Apart from chronic diseases, acute diseases are also a major killer of human health. Their disease duration is short, and patients' physiology can deteriorate rapidly in a

short time, a well-known example of which is the inflammatory storm during the COVID-19 pandemic (Russell et al. 2020). Usually, acute diseases include most of the severe infectious diseases, as well as the complications of septicemia, myocardial infarction, organ failure, etc. In addition to the pathogens that cause infection, some substances increase with complications. For example, the sharply increased inflammatory factors include interleukin, histamine, reactive oxygen metabolites, etc. However, it is important to note that the content anomalies of these nonspecific markers exist in other diseases; therefore, a combination of other markers for integrated diagnosis is required. At present, the detection of disease markers is relatively mature in terms of infectious diseases (shown in Table 1.1).

1.3 Emerging Technologies

Although current medical testing equipment can provide reliable test results, it often fails to meet the need for point-of-care applications and requires complex laboratory operations and personnel requirements. Over the past decade, the fusion of technologies such as microchannels, flexible electronics, and networked information has made diagnostic tools more portable, enabling rapid, real-time detection of a wider range of markers with high accuracy. Based on a quick review of the fundamentals of general biosensors, this section introduces the emerging trends of diagnostic tools from four aspects: in vitro diagnostic technology, in vivo diagnostic technology, wearable health testing devices, and decision assistance technology.

1.3.1 Fundamentals of Biosensors

1.3.1.1 Basic Mechanisms of Biosensors

Medical history, symptoms, signs, and auxiliary examinations are the core of medical diagnosis. Medical sensing technology uses signal conversion, amplification, and signal post-processions to provide clinicians and computers with original reference materials to enrich the evidence of medical diagnosis and predict the prognosis of patients' diseases (Fig. 1.5).

As shown in Fig. 1.5., there are various types of indicators that could be regarded as the inputs of healthcare biosensors (Table 1.2a). They could be captured by sensing elements, and subsequently, the signals produced by sensing elements could be amplificated (or sometimes transferred) to measurable electrical, optical, and physical signals (Table 1.2b). After necessary post-procession, the quality of signals could be further improved.

1.3.1.2 Evolution of Biosensors: From the Classical to the Next Generation

As shown in Fig. 1.6, the concept of next-generation diagnosis is a revolution that is developing extensively in the medical field. From the perspective of the medical world, the concept of next-generation diagnosis is not limited to molecular

Table 1.1 The biomarkers of most common infectious diseases

Pathogenic factors	Diseases	Biomarkers	Detection methods	Performances	
				Sensitivity	Specificity
Mycobacterium tuberculosis	Tuberculosis	mRNA (cytokine which responses to Mtb antigens: IFN-γ, IL-12β, IL-9, FOXP3, IL-10, IL-6, IL-8)	q-PCR (Li et al. 2015)	74.36%	84%
		DNA (special site)	PCR (Zhou et al. 2019) (detect NTM or MTBC)	100%	100%
		Protein (heparin-binding hemagglutinin adhesion–specific antibody)	ELISA (Sun et al. 2011)	77.08%	87.5%
Staphylococcus aureus	Various	Protein	Data-independent acquisition mass spectrometry (DIA-MS) (Liu et al. 2021c)	93.3%	88%
		Breath VOC	Gas chromatography–mass spectrometry (GC-MS) (Neerincx et al. 2016)	100%	80%
Salmonella typhi	Typhoid fever	Antibodies (blood or saliva)	Dot-EIA (Mohd Redhuan et al. 2017)	90.9% (salivary IgA), 90.9% (serum IgG)	N/A
Bacillus anthracis	Anthrax	Peptide (fingerprint)	Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Wei et al. 2020)	100%	100%
		Surface array protein	Immunochromatography test (ICT) (Puranik et al. 2020)	/	/
		Calcium dipicolinate (CaDPA)	ZnO quantum dots Functioned with europium ions (Eu3+) (Zhou et al. 2017)	/	/

Vibrio cholerae	Cholera	OmpW	Surface plasmon resonance (Taheri et al. 2016)	/	/
		D-amino acids (d-methionine and d-leucine)	Enzyme-based microfluidic chip (Batalla et al. 2015)	/	/
HIV	AIDS	miRNA	qRT-PCR (Munshi et al. 2014)	/	/
SARS-CoV-2	COVID-19	Antibody	IgM/IgG (Bin et al. 2020)	95.8%	98.5%
		RNA	RT-PCR (Chu et al. 2020; Corman et al. 2020)	/	
Influenza A virus	Influenza	Protein	ELISA (Jian-umpunkul et al. 2012)	/	
		RNA	Real-time PCR (Ilinykh et al. 2010)	/	
Hepatitis B virus	Hepatitis B	DNA	Structure-enhanced fluorescence polarization biosensor (Chen et al. 2015)	/	
		Hepatitis B surface antigen (HBsAg)	Automated fluorescent lateral flow immunoassay (FLIA) (Ryu et al. 2018)	99.8%	99.3%
Hepatitis C virus	Hepatitis C	antibody to HCV (anti-HCV)	Automated fluorescent lateral flow immunoassay (FLIA) (Ryu et al. 2018)	98.8%	99.1%
Dengue virus	Dengue	NS1 antibody	ELISA (Ahmed and Broor 2014)	73.5%	100%
		RNA	Real-time RT-PCR (Ahmed and Broor 2014)	79.4%	100%
Plasmodium vivax	Malaria	*P. vivax* lactate Dehydrogenase (LDH)	ELISA (Sousa et al. 2014)	/	/
Plasmodium falciparum		Plasmodium falciparum histidine-rich protein II (PfHRPII)	Magnetic bead-based ELISA (Markwalter et al. 2016)	/	/

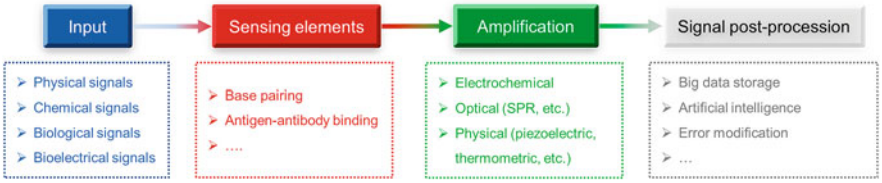

Fig. 1.5 Basic units of a medical sensing system

diagnostic tools brought by next-generation sequencing (NGS) but goes deeper into data storage, intelligent diagnosis, rapid disease screening, health monitoring, and more.

1.3.2 In Vitro Diagnostics

1.3.2.1 Liquid Biopsy

Liquid biopsy is the sampling and analysis of the nonsolid biological sample. It gives us noninvasive access to disease diagnosis and dynamic monitoring. In particular, in cancer, it contains detection, isolation, and further operation to circulating tumor cells, circulating tumor DNA/RNA, exosomes, and other biomarkers, which brings us genomic and epigenetic information about cancer. This information can help us in "precision medicine" and "point-of-care" treatment (Crowley et al. 2013; Soiza et al. 2018).

Biochips (e.g., Gene Chip, protein biochips, electrochemical biochips, microfluidic chips, and nanotechnology-based biochips) are playing an important role in liquidoid molecular diagnostics, and some of them are applied in point-of-care diagnosis, which potentially helps the development of personalized medicine. Many biochips with different properties have been developed, such as electrical biochips, to achieve rapid and multiplexing detection. DNA chips provide high throughput or easy access for sequencing and detection. Another trend in bio-chips is programmable bio-nano-chip (p-BNC), which often includes bio-sensitive elements such as field-effect transistors. p-BMC is a versatile multiplexed and multiclass bio- and chemical detection system for clinical and research use (McRae et al. 2015). This trend is generated by the need to deal with much information about potential biomarkers generated by today's advanced "omics" such as genomics, proteomics, and metabolomics (Hanash et al. 2008; Hunter et al. 2008) and the demand for a portable, inexpensive, and sensitive detection system (McRae et al. 2015).

Isolation of target cells from a liquid sample is usually the first step in a liquid biopsy; one of the core challenges is the usual need to deal with a large volume of samples with rare targets in complex backgrounds. Filtration-based micro-device brings us a solution for its high-throughput, label-free, and high recovery rate of target (Liu et al. 2021b). For example, a microcavity array for rapid and highly

Table 1.2 Main inputs and amplification approaches of biosensors

(a) Biosensors with different indicators

Indicators	Detection targets	Basic principles	Application
Physical indicators	Temperature	Common temperature sensors utilize the thermoelectric effect to convert heat to electrical characteristics such as conductor resistance by using thermocouples or thermistors	Versey et al. (2011)
	Pressure	Pressure sensors use a force collector to scale the strain, and its methods can be categorized into piezoresistive strain sensor, capacitive, electromagnetic, etc.	Graphene et al. (2020); Tao et al. (2020)
	Blood flow	Blood flow measurement utilizes laser Doppler effect or ultrasound Doppler effect. The frequency of the reflected light (or ultrasound) varies according to the blood-flow velocity	Ruiz-vargas and Arkwright (2019); Min et al. (2021); Surkova et al. (2021)
	Heart sound	Heart sound sensors generally are electronic stethoscopes, which use an acoustic transducer to convert the sound wave to an electrical signal. Electronic stethoscopes can be divided into air conduction and contact conduction	Pei et al. (2019); Liu et al. (2021a); Wang et al. (2021)
	Pulse	Heartbeat sensors utilize photoplethysmography, which measures changes in the intensity of light caused by the variance of the blood amount passing through the organ	Kwak et al. (2017)
	Respiration	Respiration can be measured with the chest strap that monitors the chest expansion during breathing or with a thermal mass flow sensor or pressure sensor to detect the respiratory air volume flow	Kanaparthi (2017); Yu et al. (2017)
Chemical indicators	Electrolyte	Ion concentration detection method varies. It can be detected via an ion-selective electrode (ISE), which converts the activity of the dissolved specific ion into an electrical potential	Shinwari et al. (2007); Melzer et al. (2014); Wang et al. (2019a)
	Saccharide	Saccharide sensor is usually based on the electrochemical method monitoring redox	Huang et al. (2009); Jina et al. (2014)

(continued)

Table 1.2 (continued)

(a) Biosensors with different indicators

Indicators	Detection targets	Basic principles	Application
		reaction generated by the hydrogen peroxide with the saccharide at the electrode	
Biological indicators	Protein	Protein can be targeted by various molecular probes. Aptamer-based biosensors (aptasensors) and antibody-based biosensors (immunosensors) can use those biological molecules bound with physically detectable signals (for example, fluorescence) for the detection of the target	Resende et al. (2018); Villalonga et al. (2020); Li and Lillehoj (2021)
	Nucleic acid	Electrochemical sensors can detect the electrical activities (change in resistance, capacitance, inductance, etc.) during the hybridization of the targeted DNA with tagged short DNA probes	Tabata and Miyahara (2019); Santhanam et al. 2020; Wu et al. (2020)
		Optical sensors can detect the optical activities during the hybridization of the targeted DNA with tagged short DNA probes via fluorescence, surface-enhanced Raman scattering, colorimetric assays, surface plasmon resonance, etc.	Aydın et al. (2020); Julker et al. (2021)
Bioelectrical signals (biophysical mechanism)	EEG/EMG/ECG	Electrophysiology detects the electrical properties of biological cells and tissues. It usually involves measurements of voltage or current	Onose et al. (2012); Lin and Jiang (2017); Level et al. (2019)

(b) Main technology types of biosensors

Types of biosensors	Detection device or principles	Brief descriptions
Electrochemical biosensors	Amperometric biosensor	Current produced by the oxidation could quantitatively reflect the amount of some certain substances
	Potentiometric biosensor (mostly field-effect transistors, FET)	By replacing the gate terminal in FET with an antibody or an enzyme, the physical and electrochemical changes happen in FET due to the change of pH, ionic force hydration, and redox response that will produce a change of the source flow in the drain

(continued)

Table 1.2 (continued)

(b) Main technology types of biosensors		
Types of biosensors	Detection device or principles	Brief descriptions
	Impedimetric detection	Impedimetric biosensors combine the analysis of both the R (resistance) and C (capacitance) of material, namely the analysis of the electrochemical impedance spectroscopy (EIS) by perturbating a small signal produced into a system at its equilibrium, and this small signal, when EIS is applied in biosensors, is generally produced by the biochemical activity that occurs when the detected substance meets the enzymes, nucleic acids, antibodies, and cells immobilized onto the electrodes' surface
	Voltammetric test	Voltammetry is an electrochemical technique in which a varying potential is applied to a working electrode in an electrochemical system, and the corresponding current is measured
Optical biosensors	Plasmonic resonance biosensors (including surface plasmon resonance, localized surface plasmon resonance, SPR imaging, etc.)	Plasmonic resonance occurs in free carrier-rich metal conductors or heavily doped semiconductor materials. The plasmonic resonance frequency is closely coupled with the external refractive index distribution, so small changes in concentration can affect the resonance mode. Sensitive signal conversion can be obtained by detecting information about incoming or transmitted light
	Evanescent wave biosensors (waveguide or optical fiber)	The evanescent wave arises from the manner in which light behaves when confined in an optical waveguide or fibers. It is sensitive to near-surface (less than 100 nm to a wavelength) fluorescent molecular excitation or biological binding events. For optical fibers, flexible mechanics and temperature measurement can be realized
	Spectroscopic analysis	Surface-enhanced Raman spectroscopy and fluorescence spectroscopy can reflect the concentration and fingerprint information of substances, which

(continued)

Table 1.2 (continued)

(b) Main technology types of biosensors		
Types of biosensors	Detection device or principles	Brief descriptions
		are widely used in high-throughput and high-precision material inspection. The spectral signal is received by a CCD or photodiode for further analysis
Magnetic biosensors	Alternating current (AC) susceptometry, Hall effect, or giant magnetoresistance effect	Utilizing paramagnetic or supra-paramagnetic particles, or crystals, which are bound with bioreceptors, to detect biological interactions. The binding of the bioreceptors will affect the magnetic particle properties that can be measured
Micro-electromechanical systems (MEMS)	Surface acoustic wave (SAW), cantilevers, micro-accelerometers, etc.	The resonant frequency of surface acoustic wave/cantilever or other mechanical vibration modes are very sensitive to the bonding events near the sensor side. Specific conversion of concentration information can be achieved by fixing biometric elements on the sensor surface. In addition, micromechanical sensors are also widely used in wearable biomechanical signal detections
	Piezoelectric effect, piezoresistive effect, thermo-electric effect, capacitive sensors, etc.	Microelectronic devices cover most bioelectrical signals' acquisition, stress, temperature, gas, and analyte measurement applications. For example, the piezoelectric effect and thermoelectric effect can directly convert physical quantities into electrical signals, which is very convenient for back-end signal processing

efficient tumor cell detection from blood was developed (Fig. 1.7a, d), which is mainly based on the differences in size and deformability between tumor cells and other cells (Hosokawa et al. 2010). A more complicated separable bilayer was reported to further reduce the mechanical stress on CTCs, which retains the ability to establish culture after capture (Fig. 1.7b). Some points may need further investigation in this area like purity increment, finding more cost-effective fabrication, and release of the cells from filter (Liu et al. 2021b).

Circulating free DNA (cfDNA) was another feasible biomarker for liquid biopsy. Due to the active and increasing cell proliferation, patients who have malignant tumors usually have a higher concentration of cfDNA (Schwarzenbach et al. 2008). Meanwhile, DNA is a stable chemical compound and has already been researched

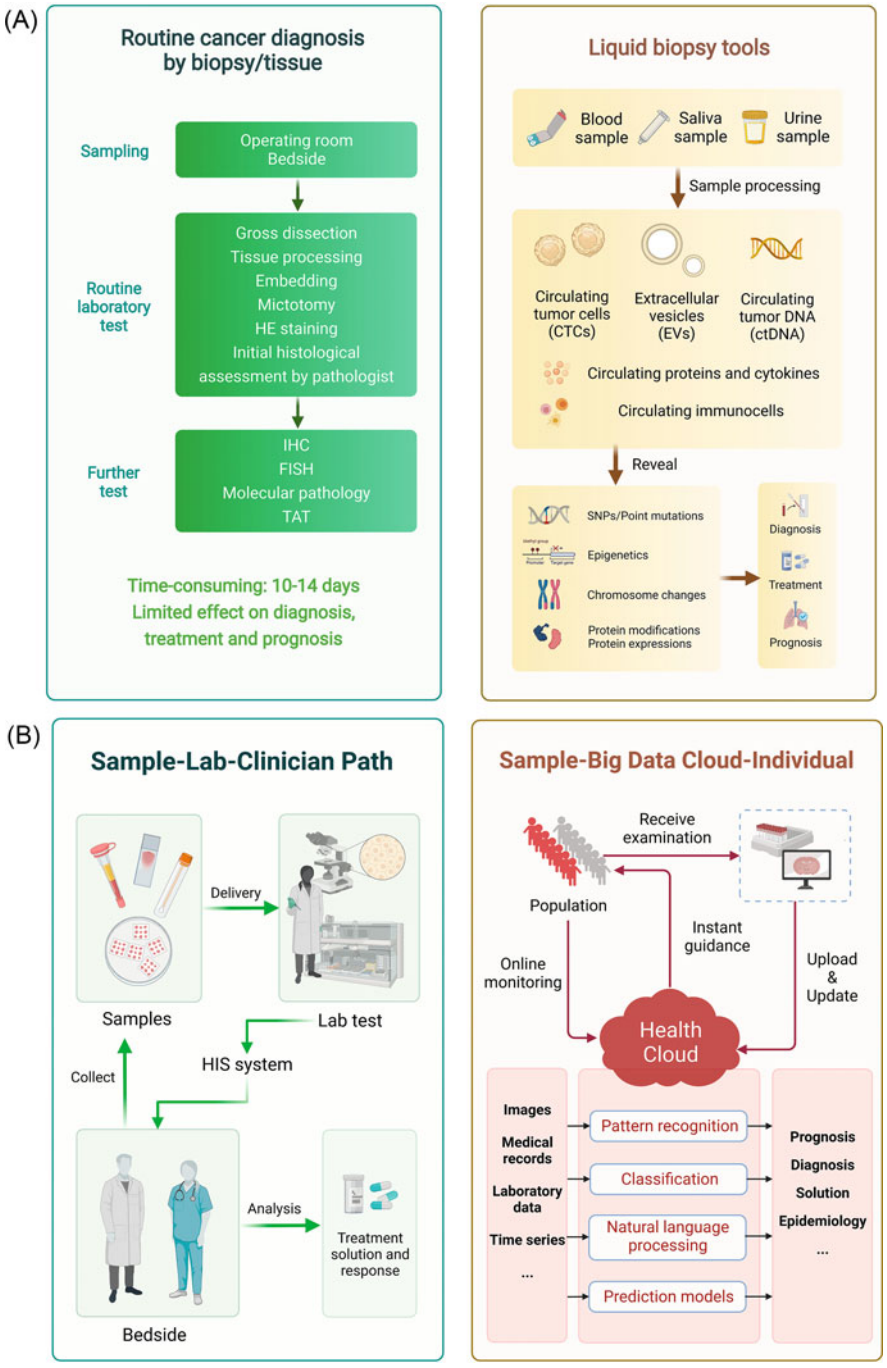

Fig. 1.6 Evolution from conventional diagnostics to next-generation diagnostics (NGD). (**a**) Liquid biopsy is changing the speed of diagnostics and the abundance of information by revealing the SNPs, epigenetics, chromosome changes, and the expression/modification of protein in a

for many years, which gives us lots of existing techniques for analysis. Some quantitative methods have been used to establish the relationship between cfDNA concentration and cancer, such as quantitative polymerase chain reaction methods (qPCR) (Spindler et al. 2014, 2015), DNA BEAMing, and digital droplet polymerase chain reaction (Sefrioui et al. 2015). Although these methods are advancing very well, the normal cfDNA level still needs to be tested in health cohorts, and consensus for the normalization strategy prevents clinical applications.

The exosome is essential to intracellular communication and metabolism in both physiological and pathological conditions. Some research shows that RNA and protein transport by exosomes can be a potential biomarker (Fig. 1.8). The complex informative composition of exosomes (DNA, RNA, protein, lipid, oligosaccharides, etc.) makes it suitable for multi-analyte testing (Yu et al. 2021). For example, researchers demonstrated a higher sensitivity in the detection of mutation in cancer patients by RT-PCR-based methods, which combine DNA and RNA detection. A new on-chip analysis target to exosomal miR-550, which suggests to be an indicator for cancer development, has been reported (Xu et al. 2020). This method includes rapid lysis of exosomes to release mi-RNA based on the surface acoustic wave (SAW), and the detection of this mi-RNA is achieved by ion-exchange nanomembrane. This detection can be performed in about 1.5 h with about 100-μL samples, which may provide a rapid, sensitive, and noninvasive approach to cancer diagnosis and prognosis (Taller et al. 2015).

These emerging liquid biopsy methods enable rapid and simple clinical diagnosis. However, some challenges remained in this area: for example, exosome production by cells and molecular content of exosome seem to be influenced by many factors. Also, the lack of standardization makes it difficult to get a promising conclusion in using exosomes.

1.3.2.2 Volatolomics

Volatolomics is an emerging frontier for rapid, noninvasive, and cost-effective diagnosis methods with many applications. The so-called volatile organic compounds (VOCs) are emitted from cells and their microenvironment, including, but not confined to, the VOCs from blood, breath, skin, feces, urine, and saliva

Fig. 1.6 (continued) molecular level, which assists the analysis of the diagnostic criteria, treatment solution, and prognosis prediction to a great extent. (**b**) The NGD is revolutionizing the clinical route in daily practice by turning the classic sample–laboratory–clinician path into an individual sample–big data cloud–individual analysis path, where the pattern recognition and classification algorithms, data storage system, natural language processing solutions, and image analysis are playing increasingly important roles. (**c**) The in vivo diagnostics will challenge the classical in vitro diagnostics since the continuous monitoring provided by wearable sensors, the delicate view captured by ingestible sensors, and the abundant biochemical information detected by implantable sensors could become a good supplement for discrete, invasive, and low-throughput in vitro testing. (**d**) After entering the omics era, high-throughput analysis methods and a wide range of biological sample sources (including volatolomics) provide more analysis methods and more comprehensive biomarkers. (Created with Biorender.com)

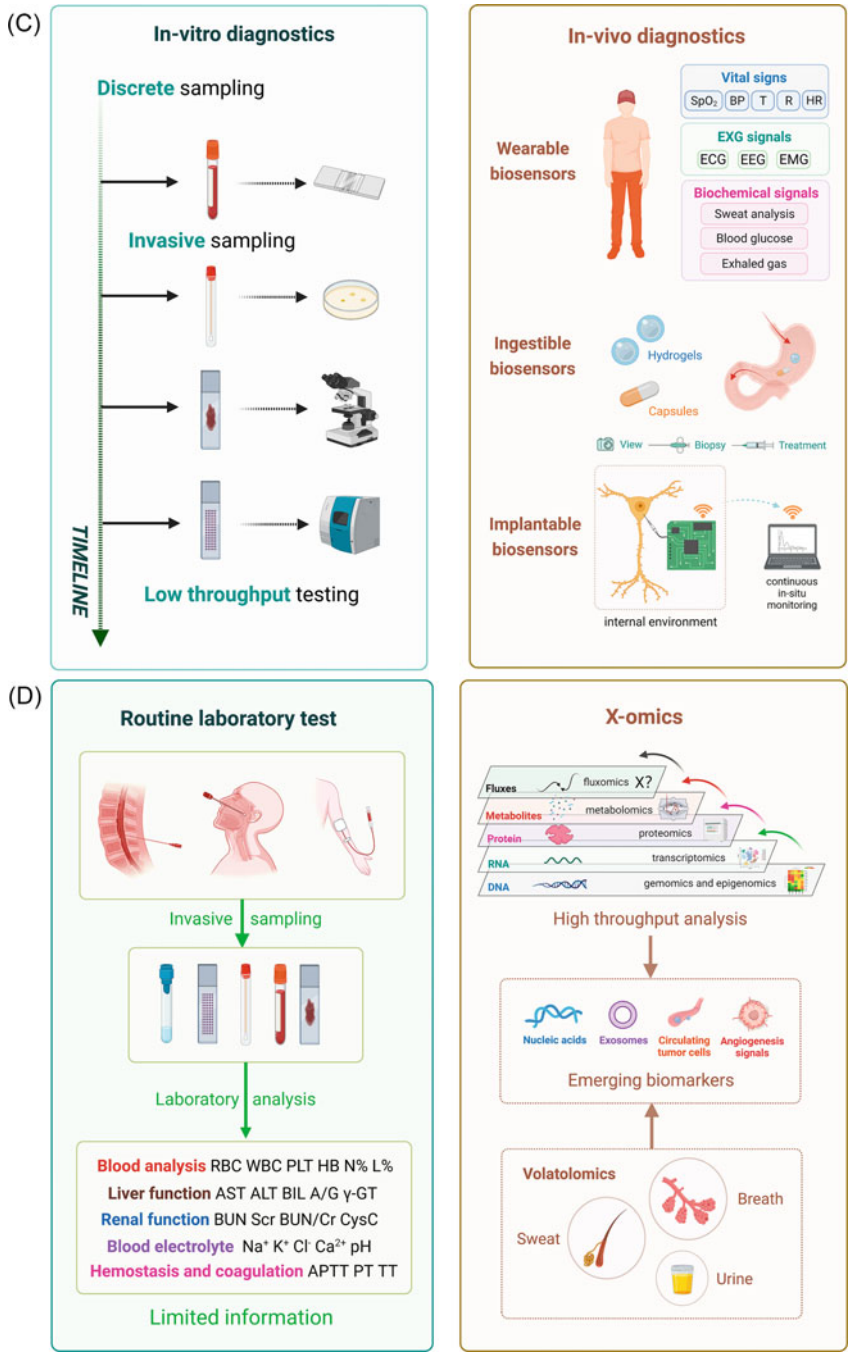

Fig. 1.6 (continued)

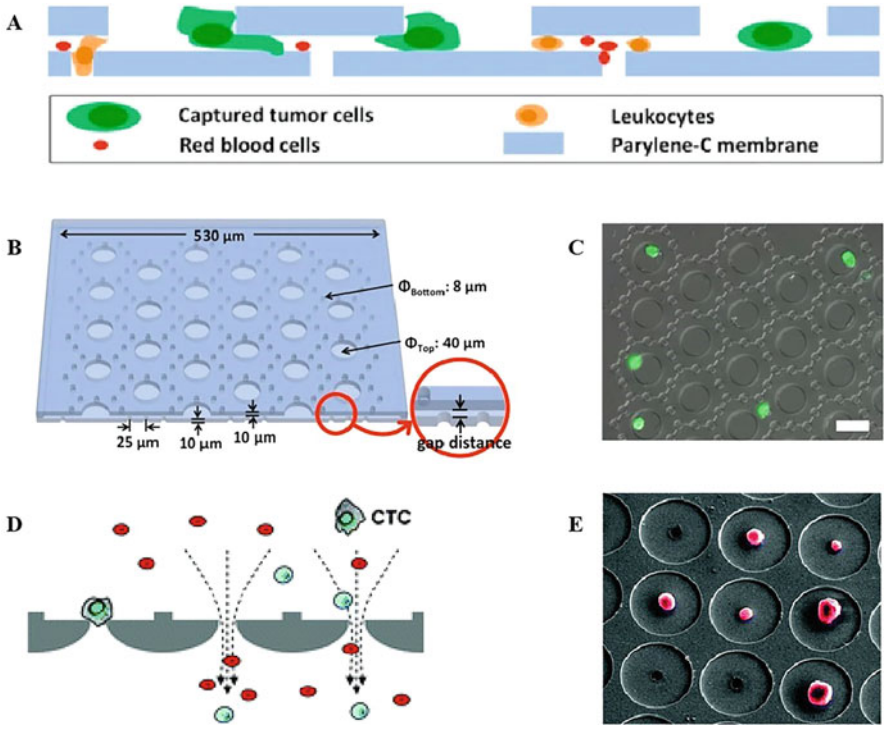

Fig. 1.7 A separable bilayer (SB) microfiltration device. (**a**) Schematic of device's cross-sectional view (the large top parylene-C pores form the edges, and the tumor cells are captured along them). (**b**) 3D view of an elemental unit model, including the gap distance in the inset. (**c**) Representative areas of the SB microfilter after filtration. MCF-7 breast cancer cells were GFP-expressing, which led to a green signal point on the filter (Da et al. 2014). (**d**, **e**) CTC recovery device equipped with the size-selective microcavity array. (**d**) Schematic of CTC recovery using the size-selective microcavity array. (**e**) SEM image of MCF-7 cells trapped on the microcavity array (Hosokawa et al. 2010)

(Broza et al. 2014, 2015; Vishinkin and Haick 2015). Volatolomics has been reported to have potential diagnosis value in myeloid leukemia (Dutta et al. 2018), cancer (Di Lena et al. 2016; Oguma et al. 2017; Chin et al. 2018; Guest et al. 2020), and numerous pathogenic bacteria (Djago et al. 2021). An induced volatolomics was reported in the diagnosis of respiratory disease. Researchers generated an engineered breath biomarker by local delivery of protease-sensing nanoparticles to the lung. The cleavage of the protease-sensing region will be accomplished by neutrophil elastase in pulmonary tissue, a protease with elevated activity in lung disease. After cleavage, the volatile reporters are released and are detected in exhaled breath (Chan et al. 2020). Notably, some obstacles remain in this area, including different confounding factors and lack of a standardized experimental procedure making it difficult to get an overview of the results (Fig. 1.9) (Di Lena et al. 2016; Djago et al. 2021).

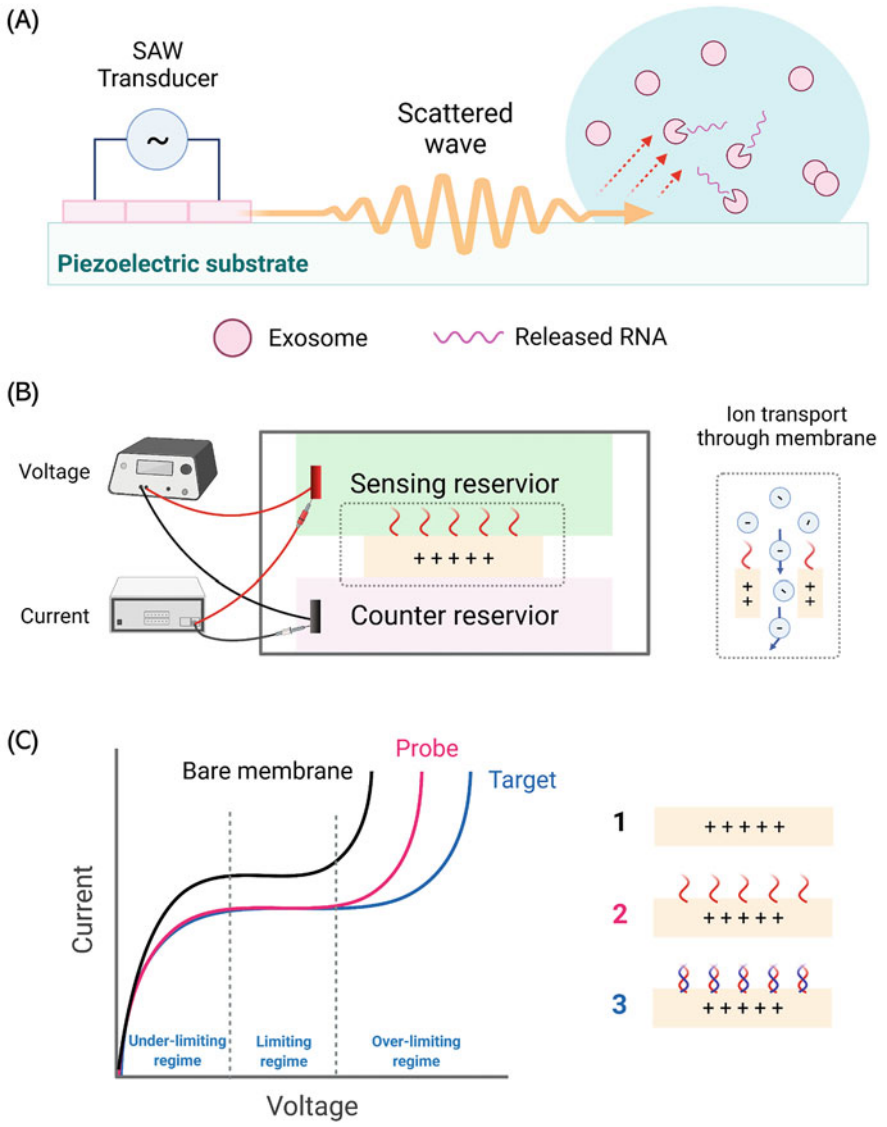

Fig. 1.8 A detector for exosomal RNA. (**a**) A side view of surface acoustic wave (SAW) device (SAW can lyse the exosomes, and then the exosomal RNA will be released and detected). (**b**) An ion-exchange nanomembrane sensor consists of two reservoirs separated by the membrane. RNA in the sensing reservoir hybridizes to complementary oligos immobilized on the surface of the membrane. (**c**) A characteristic current–voltage curve illustrating the under-limiting, limiting, and over-limiting regimes (Taller et al. 2015) (Redesigned with Biorender.com)

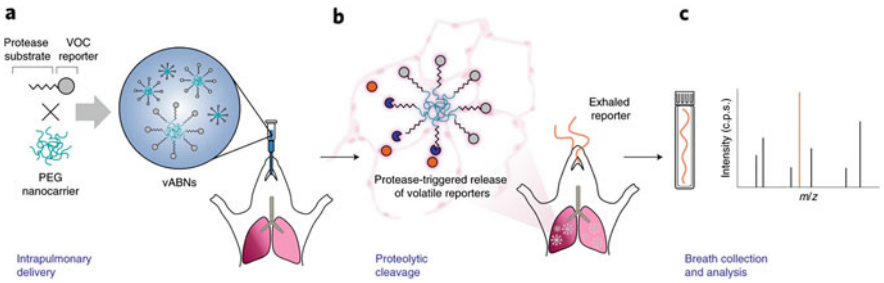

Fig. 1.9 An example of engineering synthetic breath biomarkers for respiratory disease. (**a**) Peptide substrates that are modified with VOCs are conjugated onto a multi-armed PEG nanocarrier, and then they are formulated into vABNs. Later, they are delivered into the lungs through intratracheal instillation. (**b**) VOC reporters are in a "gray" state when they are attached to the vABN, which means that they are undetectable and nonvolatile when the attachment remains. However, the extracellular proteases produced during respiratory disease could cleave surface-conjugated peptide substrates. In this way, the VOC reporters are released from vABNs, recover their "orange" state, namely of characteristic mass and volatility, and are exhaled. (**c**) Breath could be collected into a receptacle, and mass spectrometry could measure the VOC reporter concentration quantifiably (Chan et al. 2020)

1.3.3 In Vivo Diagnostics

One of the most common types of in vivo diagnosis is implantation devices, which can obtain physiological information in situ and rapidly. However, invasive operation, with a risk of infection together with usually higher fees, limited the use of these technologies (Li et al. 2021a). In some particular situations, in vivo diagnosis still has potential advantages, such as in intracranial pressure and electroencephalogram. To avoid the adverse effects of implanted sensors, some new technologies have been developed; many of these technologies have the same requirements as implantable therapeutic devices (Li et al. 2021a). For example, Kang et al. reported a new kind of bioresorbable silicon electronic sensor, which can continuously monitor the intracranial pressure and temperature. The signal was then transmitted by a biodegradable wire to a potentiostat for data transmission (Kang et al. 2016). Despite the fascinating promise of in vivo diagnostics, there are still some challenges that need to be solved for the further progress of this area. For instance, many of the bioresorbable devices may not have as complex functionalities as silicon-based chips. Also, further development of technologies that are fundamental in this area is necessary. These technologies include (1) in vivo biocompatibility that is more stringent than biocompatibility defined in wearable devices, and some of them are even fabricated by bioresorbable materials; (2) wireless communication, and this approach can help avoid discomfort and risk of infection when using wiring device; and (3) improved insertion strategies and mounting strategies. More details are covered in the chapter on "biologically compatible sensors" in this book (Fig. 1.10).

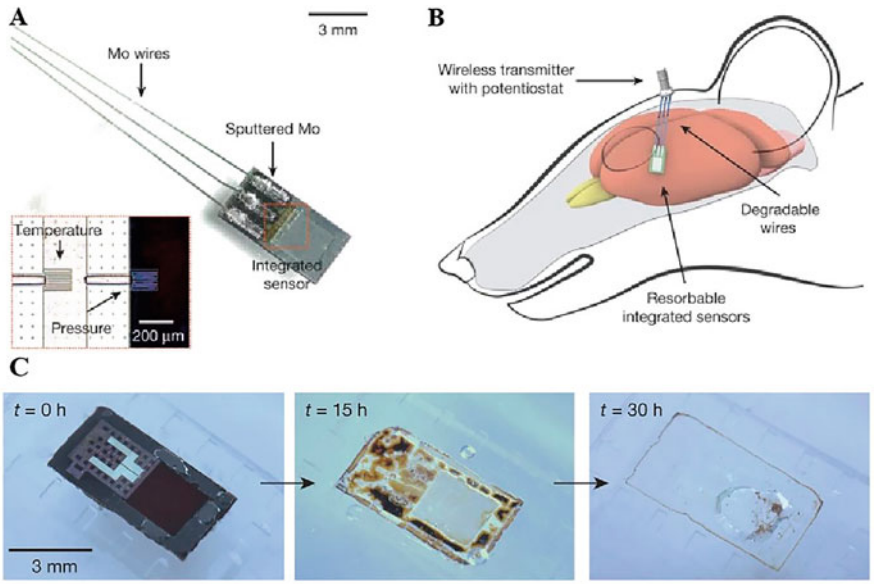

Fig. 1.10 Pictures of bioresorbable sensors detecting pressure and temperature. (**a**) The sensor was integrated with dissolvable metal interconnects and wires. As shown in the inset, the serpentine Si-NM structures form the sensing regions, where one that is not above the air cavity (left) responds only to temperature, and the one at the edge of the air cavity (right) responds primarily to pressure. (**b**) Diagram of a bioresorbable sensor system in a rat's intracranial space. For long-range detection and data transmission, there are electrical interconnects providing an interface to an external wireless data-transmission unit. (**c**) Pictures collected at several stages of accelerated dissolution of a bioresorbable pressure sensor upon insertion into an aqueous buffer solution (pH = 12) in a transparent PDMS enclosure at room temperature (Kang et al. 2016)

1.3.4 Wearable Health Monitoring Devices

In addition to in vitro and in vivo diagnosis, the skin, the body's largest organ, provides a valuable alternative diagnostic interface. Wearable devices can interact with them in a common way to obtain a variety of physiological health information. In contrast with traditional centralized healthcare services that require patients or samples to travel to the hospital, wearable sensing provides new insight into personalized medicine and point-of-care testing, as portable, wearable, remote, and timely features. Flexible sensors have become current trends in this area because of the mechanic mismatch and motion artifacts that occur between conventional rigid electronic materials and soft biological tissues. Technologies innovation in wearable sensors include, but not confined to, (1) new materials with special properties like flexible or stretchable, which can be used not only as substrate but also in sensors like some organic transistor (Kim et al. 2018; Cea et al. 2020); (2) sensor design, such as piezoelectric or piezoresistive; (3) device fabrication, like solution-based approaches or some mechanical-based printing; and (4) system integration not only

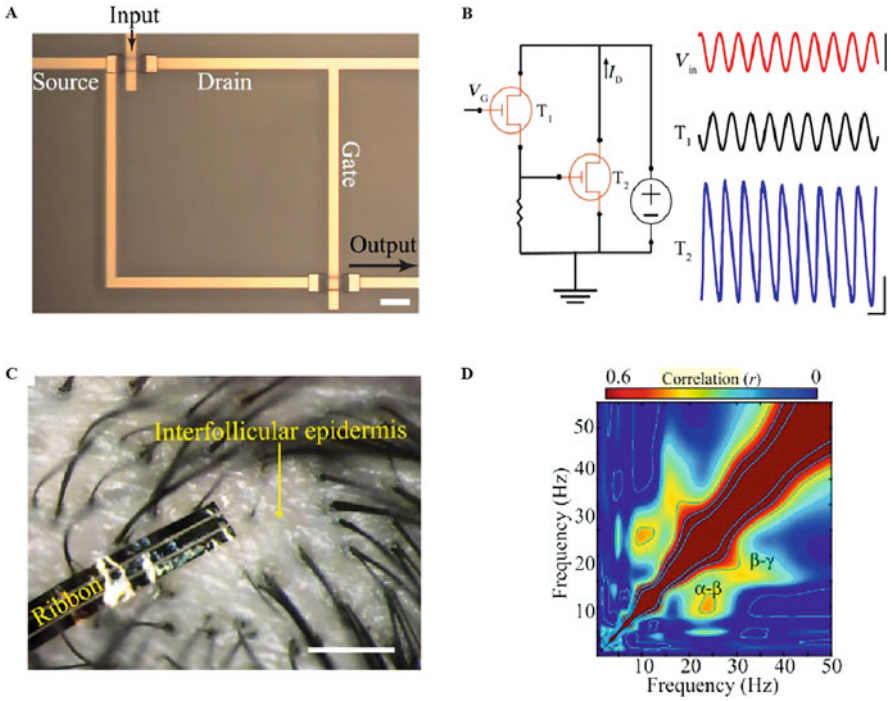

Fig. 1.11 An integrated ion-gated organic electrochemical transistor-based biosensor. (**a**) IGT-based NAND and NOR gates conform to the surface of orchid. (**b**) Temporal response of the output (O) drain current of a NOR (top) and a NAND (bottom) logic gate. (**c**) Picture of μ-EEG IGT conforming to human scalp, where devices were designed to fit the interfollicular epidermis (scale bar = 2 mm). (**d**) Comodulogram showing the cross-frequency coupling of the same recording epoch; significant coupling between α–β and β–γ is indicated (Spyropoulos et al. 2019)

with the form like an array, but some sensors also developed logic on the detection area. Spyropouls et al. reported an internal ion-gated organic electrochemical transistor that can be used in human electroencephalography (EEG). This device has a flexible substrate and gel-based biocompatible channel, which can be further integrated into amplifier logic (Spyropoulos et al. 2019). Some challenges still lie ahead before the daily use of wearable sensors in practical applications, such as the difficulty of distinguishing signals with physiological meaning from noise, the energy consumption of device, and the cost of device manufacturing (Wang et al. 2017). Further information on the features and design strategies of wearable devices can be found in the "biocompatible sensors" section of this book (Fig. 1.11).

1.3.5 Decision-Making Assistants

The development of Internet of things (IoTs) hardware promotes the fusion of multimodal information, especially represented by wearables and implantable devices. Data acquired by sensors need to be transmitted through wireless communication. These multimodal data constitute a health cloud concept that describes our physiological state. To minimize the probability of misdiagnosis, many algorithm strategies for decision fusion have been developed.

In general, decision fusion methods fall into three broad categories: probabilistic methods, evidential methods, and intelligent methods. Probabilistic methods represented by Bayesian theory use probabilistic and statistical foundations, which are effectively used in event fusion and are an efficient method for managing randomness (Antink et al. 2015). the theory of evidence relates to a combination of evidence to calculate the probability of an event. Fuzzy methods and artificial neural networks are examples of intelligent methods (Chowdhury et al. 2018; Köhler et al. 2015). Fuzzy-logic theory has been effectively used in fuzzy information processing and succeeds in making decisions and fusion systems for identifying and managing healthcare systems. The application of IoTs, information fusion, and artificial intelligence in multimodal sensing data-based diagnosis has initially been equipped with the function of a digital doctor. However, the biggest problem in the wide application of these decision assistants is still ethics and data norms.

1.4 Emerging Challenges

All of these emerging disease markers and detection technologies will be or are being used in the next generation of diagnostics, outlining a grand vision of medicine that enables precise diagnosis and disease intervention. However, we should also recognize that the next generation of diagnostics still faces challenges such as many scientific principles, engineering techniques, economic costs, and ethical challenges. Here we briefly analyze and look into some of these typical challenges:

1. **Sensitivity, accuracy, and reliability.** Although many studies have provided convincing experimental data, the actual clinical application is often faced with complex interference, and the accuracy and repeatability of the emerging sensors need to be compared with the reference technology and further cross-validation.
2. **Miniaturization and system integration.** Compared with the traditional diagnostic methods, the next generation of diagnostic technology inevitably requires direct contact with the human body to obtain physiological information in situ. But this poses a number of biocompatibility challenges. One of the most straightforward solutions is to implement a miniaturized device that integrates multiple sensing functions through appropriate packaging methods. However, this is a challenging system engineering problem, with difficulties such as power supply, signal transmission, and organizational rejection.

3. **Ethics-related issues.** The combination of IoTs technology and healthcare has given birth to the Health IoTs (H-IoTs). However, the application of H-IoTs technology also brings a series of ethical issues. For example, H-IoTs devices record personal health and activities with unprecedented scope and detail, leading to the emergence of ethical issues such as personal privacy in the H-IoTs scenario. In general, the main challenge facing H-IoT technology is how to scientifically design equipment and protocols, that is, to collect, share, process, and verify cross-domain data while achieving cost-effectiveness, advanced technology, scientific reliability, and ethics.
4. **Expense and patient compliance.** The cost of a single device is directly proportional to the intensity of technology, and the increasing cost of detection and deployment is also a challenge that cannot be ignored, although some optimized medical services can reduce administrative and operational costs. Some useful low-cost sensors have been developed in recent years (such as paper-based sensors), but there may be a trade-off between further cost reduction and more comprehensive and efficient diagnostic performance. Another challenge is patient compliance due to telemedicine. Because physicians cannot physically instruct patients to use specialized next-generation diagnostic equipment, the acquired data will be subject to motion artifacts and environmental interference or even completely ineffective. Standardized use is a viable solution to patient compliance.

Finally, we believe that driving basic research and development from the clinical application is also thought worthy of reference. Solving these problems for the next generation of diagnostics requires the collaboration of a variety of professionals, including electronic engineers, information scientists, and medical practitioners.

1.5 Conclusion

In this chapter, we systematically reviewed various concepts of next-generation diagnosis and sorted out some representative markers and sensor technologies in next-generation diagnosis. The most noteworthy points include the following two:

- **The core of next-generation diagnosis is precision medicine and individualized medicine.** The goal of next-generation diagnosis is to use omics technology and constantly update new discoveries in translational medicine to make the diagnosis and treatment of diseases individualized.
- **To truly realize the next generation of diagnosis, the advancement and innovation of sensor technology is an important foundation.** The basis of the next generation of diagnosis is the flourishing data technology, omics technology, and basic medicine. To make its concept fully close to the starting point and objective needs of clinical practice, it is necessary to have sensors to collect human signals. In order to improve patient compliance, this sensor-based collection must be efficient, accurate, low-cost, reliable, and ethical.

As witnesses in the medical–industrial interdisciplinary field, engineers and clinicians are looking forward to participating in the next-generation diagnostic revolution. The core of this revolution will be the close interaction of new sensor technologies and new disease markers. In this revolution, its participants are everyone who is interested in biosensing technology, and the beneficiaries will be a large number of people.

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Carbon Electrodes as Emerging Platforms for Miniaturization of Electrochemical Biosensors

2

Achi Fethi and Meskher Hicham

Abstract

Electrochemical biosensors are analytical tools converting a biochemical reaction into an output current signal and are generally composed with a transducer and biorecognition platform. Electrochemical sensors should be stable with highly active catalytic sites and a fast reaction–diffusion biosystem. Carbon nanomaterials display the opportunity to be fastly printed on the various structures to get flexible electrodes as low-cost disposable ready-to-use for controlling human chronic diseases. In this chapter, techniques for the preparation of the electrodes based on carbon materials with various forms, including paper-based electrodes, screen printed, and flexible electrodes, are described. Also, the most important carbon printing strategies and the miniaturization processes of biosensors using 3D-printed carbon electrodes are discussed. The effect of the 3D printing strategy on the analytical performance of biosensors is highlighted. Also, this chapter presents an overview of the progress in the field and discusses the opportunities, challenges, and limitations of using miniaturized electrochemical biosensors as analytical tools to monitor organic pollutants and for clinical and cancer treatment.

Keywords

Miniaturization · Electrochemical · Biosensors · Carbon electrodes · 3D printing strategies · Organic pollutants · Cancer treatment

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_2

2.1 Introduction

Electrochemical (bio) sensors are among the most analytical devices applied for monitoring compounds in aqueous media. Their construction is manageable using transducer and sensing platforms, which allows the selective determination of the target analyte. The analytes diffuses from the solution to the sensing platform, where it reacts to release electroactive product delivering an output current signal. This diffusion–reaction mechanism occurs in electrochemical cells with a three-electrode system. However, these electrodes are centimeter size and need a handset electrical connector and surface wash before being immersed in a cell containing electrolyte. Then, manufacturing an all-in-one device for monitoring analytes is of great urgency, and more recent strategies now focus on 3D printing materials for the miniaturization of electrochemical sensors. These strategies offer tailored-shaped analytical devices with small sizes using low-cost printable materials like carbon-reinforced polymers (Yang et al. 2021).

In this regard, 3D printing of carbon nanocomposites has received great attention because this material is largely available and highly biocompatible, offering low-cost sensing tools. In addition, carbons are synthesized with simple methods forming controllable pastes and 3D printable inks (Hyun et al. 2018; Secor and Hersam 2015). The use of carbons for 3D printing strain sensors displays a flexible structure using various functional nanocomposites (Abshirini et al. 2019), opening the door to various novel strategies to construct wearable and stretchable sensing platforms (Fethi 2020; Guo et al. 2017). Their large active surface area, as well as an effective electron transfer rate, enables large use of carbon nanomaterials as 3D printing materials for storage energy applications (Yu et al. 2017; Wang et al. 2018; Fu et al. 2017). Carbons are emerging nanomaterial inks for printing electrodes by inkjet, gravure, and screen-printed techniques (Secor and Hersam 2015). Recent papers review the progress made on miniaturized analytical devices prepared with 3D printing technologies using different methods for electrochemical sensing and biosensing applications (Manzanares Palenzuela and Pumera 2018; Zhang et al. 2020).

In this chapter, we focus on recent advances in the use of carbon nanomaterials for the synthesis of sensing platforms as a miniaturized tool for sensing applications. We first review the synthesis methods to prepare carbon pastes and printable ink carbon nanocomposites for miniaturized sensors. Recent progress in 3D printing strategies and the development of recognition interactions for miniaturized biosensing systems are then summarized. In addition, recent methods for the construction of sensors based on paper electrodes and flexible materials are described.

2.2 Experimental Insights to Conventional C-Based Electrodes

Electrochemical sensors are based on the signal variation under controlled conditions. For instance, amperometric detection measures the concentration of the target of interest under a fixed potential that was applied between two electrodes. The

first is called reference electrode and the second is a working electrode that is commonly constructed with different materials like platinum, gold, or carbon. Electrodes based on carbon materials are largely used because of their simple preparation and low-cost, offering a wide potential window (Liu et al. 2019; Li and Lin 2020).

Carbon paste electrodes are fabricated by mixing graphite powder with water and immiscible organic ligands to get a paste. This type of electrode is nongraphitizable with glassy and ceramic characteristics obtained by carbonization treatment using high temperature reaching 3000 °C. The electrode is very resistant to acidic corrosion, polishable, and biocompatible with solvents (Uslu and Ozkan 2007; Cruz-Navarro et al. 2020).

2.3 Preparation Methods of Carbon Electrodes

Carbons exhibit several benefits, which make them excellent modifiers for the fabrication of analytical biosensors. This type of material has efficient conductivity (Evtugyn et al. 2020). They have functional surface groups that improve the immobilization process of biological elements (Rashid et al. 2021; Quesada et al. 2020). On the other hand, such groups are obtained spontaneously in the mechanical and/or chemical synthesis of composites, functionalized carbon nanotubes (Balram et al. 2020). Carbon nanomaterials then could promote the electron transfer by increasing the active surface area of the sensing platform (Salvo-Comino et al. 2020; Wang et al. 2021; Evtugyn et al. 2020). Carbon catalysts are functionalized by chemical methods providing more biocompatibility of biosensing application (Pilan and Raicopol 2021; Tang et al. 2020; Prasad Aryal and Kyung Jeong 2020).

Graphene has become one of the most important carbon nanomaterials, and previous studies showed that graphene has a high active surface area (Bonaccorso et al. 2015). It can be synthesized by several methods, including mechanical cleaving, exfoliation technique, and chemical vapor deposition (CVD) (Son et al. 2020; Yang et al. 2020; Kitao et al. 2020; Gürünlü and Bayramoğlu 2020). The best method to synthesize graphene sheets is micromechanical cleaving as long as it is almost free of defects (Nawz et al. 2020).

To produce graphene at high temperatures, the chemical vapor deposition process is recommended (Schiller et al. 2020). Due to its simplicity and predictable synthesis results, graphene synthesis through graphite exfoliation has been widely used to produce highly sensitive and stable graphene. Here, in this method, graphite will be oxidized through the acidification process to oxide graphene and then reduced by thermal or chemical methods to get rGo (Casallas Caicedo et al. 2020). Research then focused on improving graphene electrocatalytic activity where graphene quantum dots has appeared. Several works studied graphene quantum dots that were produced through electrochemical (Mansuriya and Altintas 2020) or ultrasonication exfoliation (Hwang et al. 2021). Subsequently, carbon nanotubes (CNTs) have also been widely used recently in the form of one tube (SWCNTs) or in the form of

several concentric tubes, and here it is called multiwalled carbon nanotubes (MWCNTs) (Shah et al. 2020). CNTs are produced through three major techniques: arc discharge, laser ablation, and chemical vapor deposition (CVD) (Vaka et al. 2021; Mathangi and Helen Kalavathy 2020; Soni et al. 2020; Hwang et al. 2020).

In the first method of CNT synthesis, carbon atoms are ascended from solid precursors at high temperatures, while laser ablation heats carbons at 1200 °C or at 4000 K for laser plume (Ismail et al. 2020). By controlled oxidation, arc discharge and laser ablation methods produce pure carbon nanotubes.

In CVD, at high temperatures (550–700 °C), carbon monoxide is passed by heat treatment using a furnace. In addition, the same techniques to produce CNTs are used to synthesize carbon dots (CDs) (Ismail et al. 2020). On the other hand, other techniques have been developed to produce CDs. Among them, hydrothermal carbonization is considered the most robust and the most used method due to its inexpensive costs, ecofriendly nature (Zhao et al. 2020; Kareev et al. 2020; Smirnyagina et al. 2020), electron beam ablation (Shankar et al. 2020), and sputtering (Pukha et al. 2021). Carbon black (CB) is also an important material obtained by combustion (Ismael 2020; Kesavan and Chen 2020).

2.4 3D-Printing Strategies of Carbon Materials

The three-dimensional printing method has been extremely applied in recent years and largely been used for manufacturing devices of multiple applications, for instance, medical diagnostics, environmental sensing, and research as well (Beg et al. 2020; Horst and McDonald 2020; Cardoso et al. 2020; Javaid et al. 2020). 3D printing or additive manufacturing (AM) is applied to fabricate tools; the operation is based on using software connected to printers (Li and Pumera 2021; Guo et al. 2021; Moraes et al. 2020).

Several types of 3D printing techniques have been mentioned earlier to print carbon materials. Based on their respective mechanism and their operational conditions, there are three major techniques for 3D printing: extrusion-based methods, powder-based methods, and photopolymerization (light-induced) methods (Dores et al. 2020; Chin et al. 2020; Zhu et al. 2020; Duty et al. 2018).

2.4.1 Extrusion Technique

This method is based on spreading the carbon material by means of a nozzle, which uses thermoplastic polymeric materials extruded to print objects layer-by-layer from a heated nozzle onto sensor active surface area where it is cooled to below its thermoplastic temperature. Single-, double-, and triple-print-head machines are available for fused deposition modeling, making it a good choice for simultaneous multimaterial 3D printing (Kishore and Sinha 2021). Fused filament formation is a technique applied for three-dimensional printing in the extrusion process. By the

facts, when thinking about 3D printing, the first thing that comes to one's mind is FFF printers (Roach et al. 2020).

2.4.2 Powder Technique

This technique uses laser or electronic beams to fuse polymers and alloys into 3D printing materials with various forms (Chin et al. 2020; Sun et al. 2021; Shi et al. 2021).

2.4.3 Photopolymerization Technique

This method uses light irradiation to cure monomers to polymerize and then forms polymeric composite; this method is fast, easy, and applicable at room low temperature (Zhu et al. 2020; Malinowski et al. 2020).

2.5 Miniaturized Sensor Application

Sensors are tools largely applied in environmental monitoring and food safety, and they are simple and portable and can provide a selective determination of pollutants. Miniaturized sensors are more suitable with large applications in various fields (Pranzo et al. 2018; Vishnu et al. 2020). The miniaturized sensing tools are now applied for monitoring and diagnosing any trace amounts in different fields, including environmental monitoring (Mamun and Yuce 2020), medicine (Brainina and Kazakov 2020; Zhou et al. 2020), food safety (Krishnan and Saraswathyamma 2021).

2.5.1 Miniaturized Sensors for Monitoring Organic Pollutants

Miniaturized electrochemical sensors are successfully applied to determine hazardous compounds (Liao et al. 2016), metal ions (Katseli et al. 2020), and biomolecules (Nagaraj et al. 2010) by variation in the current signal of the modified electrode surface where an electrochemical reaction occurs.

Commonly, electrochemical sensors should have good stability with high selectivity and sensitivity of the miniaturized tools; this can be reached by using biorecognition elements like enzymes and aptamers. Caballero and coauthors studied catechol detection by developing a sensor that offers a good chronoamperometric response with a wide linear response and fast response time (Caballero et al. 2018). Similar works focused on developing a portable sensor for phenol compound detection based on the disposal of cellulosic strips (Khattab et al. 2020).

2.5.2 Miniaturized Sensors for Cancer Detection

Miniaturized sensor systems combining alternating current electrohydrodynamics exhibited good analytical performance for biomolecular detection including cancer (Khondakar et al. 2019). Furthermore, the integration of fluorescence and surface-enhanced Raman spectroscopy extremely raised its applicability for monitoring multiple biomarkers (Kamil Reza et al. 2017). Omar Shaikh et al. developed a portable pen to detect tissue palpation in oral cancer screening (Shaikh et al. 2020). Alizadeh and his group worked on a novel method to develop an electrochemical miniaturized sensor with good performance (Alizadeh et al. 2019).

2.6 Analytical Performance of Miniaturized Electrochemical Biosensors

The linear range is a parameter that indicates the proportional variation between target concentration and the current signal delivered by the biosensor. Carbon-based sensors with a wide linear range improve the equilibrium between two processes, diffusion and reaction, occurred at the interface bulk solution where the target is and the solid surface of the modified carbon electrode. Each sensor has a limit of detection that describes the concentration of the target under which the sensor is unable to detect.

The determination of hydrogen peroxide (H_2O_2) in blood using an indium tin oxide electrode modified by three-dimensional gold material displays a low detection limit of 9.8×10^{-13} M (Purohit et al. 2019). Recently, Kuldeep and coauthors successfully prepared a nanostructure of gold nanoparticles (AuNPs) and graphene oxide (GO) for sensing alkaline phosphatase that provides a low detection limit of 9.10 ($\pm$0.12) U/L (Mahato et al. 2020). Similarly, the team of Ashutosh Kumar has prepared a nanosensor based on iron–gold nanocomposite using glucose as a reducing agent for the detection of biomarker related to liver disease. The sensor displays a low detection limit of 0.14 nM (Kumar et al. 2019b). The analytical performance is entirely depending on the type of biomaterials used for constructing biosensors. The biocompatible catalysts were used according to the biosensing application, and progress on various synthesis methods with new design of immunosensing platforms allows successful application to biomedical application (Kumar et al. 2019a).

As shown in Table 2.1, miniaturizing electrochemical biosensors was carried out with a variety of nanomaterials and conducting polymers to perform their analytical performance, which allows the commercialization of these devices. In our recent review paper, we have investigated different methods to prepare sensing biointerfaces for the determination of phenols (Bensana and Achi 2020). As shown in Table 2.1, the analytical performance of miniaturized electrochemical biosensors is high when using carbon material-based inks (Shi et al. 2020). Similarly, the use of polylactic acid (PLA) as a printing biopolymer presents a good

Table 2.1 Analytical performance of miniaturized electrochemical biosensors for monitoring organic pollutants and for cancer biomarkers

Sensors	L.O.D.	L.R.	Sensitivity	Stability	Target	Ref.
GP-GV/PET	0.08μmol	0.1–8μmol	–	–	Estriol	Pradela-Filho et al. (2020)
GP-GV/PET	9μmol	10–1000μmol	0.021μA/μmol		Catechol	
CB-PLA	0.097μg L^{-1}	10–300μg L^{-1}	0.0523μA/μg L^{-1}	–	Cu^{2+}	João et al. (2020b)
eCAD	0.0048μM	0.005–3μM	248μAμM^{-1} cm^{-2}	–	Pb^{2+}	Shi et al. (2020)
BMIM·BF4/MCN–TB	3.97 pg mL^{-1}	0.01–15 ng mL^{-1}	–	100% two weeks	Breast cancer (BRCAl)	Zhao et al. (2013)
CB-SPE	3μM	5–50μM	0.87 $A.M^{-1}.cm^{-2}$	RSD = 9.0%	Catechol	Talarico et al. (2015)
CB/PLA	1μg L^{-1}	up to 200μg L^{-1}	–	>2.8%	Pb^{2+}	João et al. (2020a)

additive combined with carbon black as high-performance conductive filaments for constructing biodegradable sensors for biofuel analysis (João et al. 2020b).

2.7 Miniaturized Sensors

2.7.1 Smart Sensors

These on-body sensors are prepared using stretchable and flexible platforms and are integrated into communication modules or connected with smartphone systems (Zhang et al. 2020). They are in contact with human skin for monitoring blood oxygen, human body temperature, heart and respiration rates, and blood pressure. Zhang et al. (2020) have prepared directly printed on-body sensors with the integration of smart modules (Fig. 2.1a).

Interestingly, Draz et al. (2020) have constructed nanoparticles and a deep neural network for virus detection by using smartphone systems (Fig. 2.1b). Their miniaturized device can capture virus with Pt-nanoprobes by loading samples into modified microchips with incubation for 20 min to capture the virus.

2.7.2 Papers

Paper-based sensors are the cheapest analytical tools (<1$); they can be mass-produced by 3D printing of a three-electrode system, thus allowing their availability and then commercialization. For instance, these papers are recently applied for monitoring trace levels of heavy metals such as Cd(II) and Zn(II) and also act as excellent adsorbers of biorecognition elements like glucose oxidase for blood glucose determination, as shown in Fig. 2.2a. As presented by Kokkinos et al. (2018), a microfabricated paper was constructed by spray coating thin films of Sn,

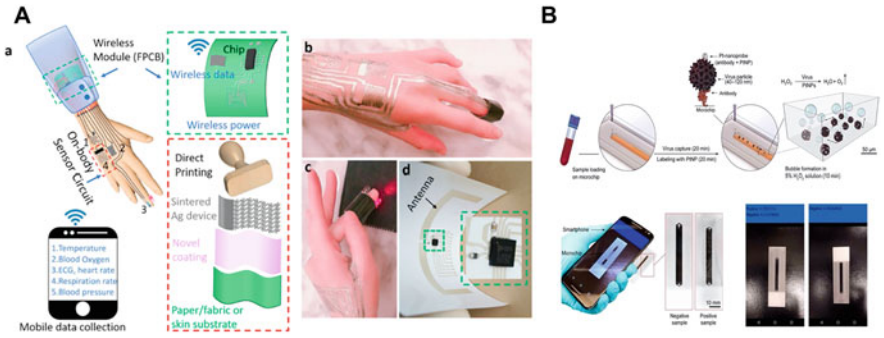

Fig. 2.1 (**a**) Schematic of a wearable sensor based on flexible printed material. Reprinted with permission from Zhang et al. (2020). (**b**) Three-dimensional schematic of the convolutional neural network CNN–nanoparticle-enabled smartphone for virus detection. Reprinted with permission from Draz et al. (2020)

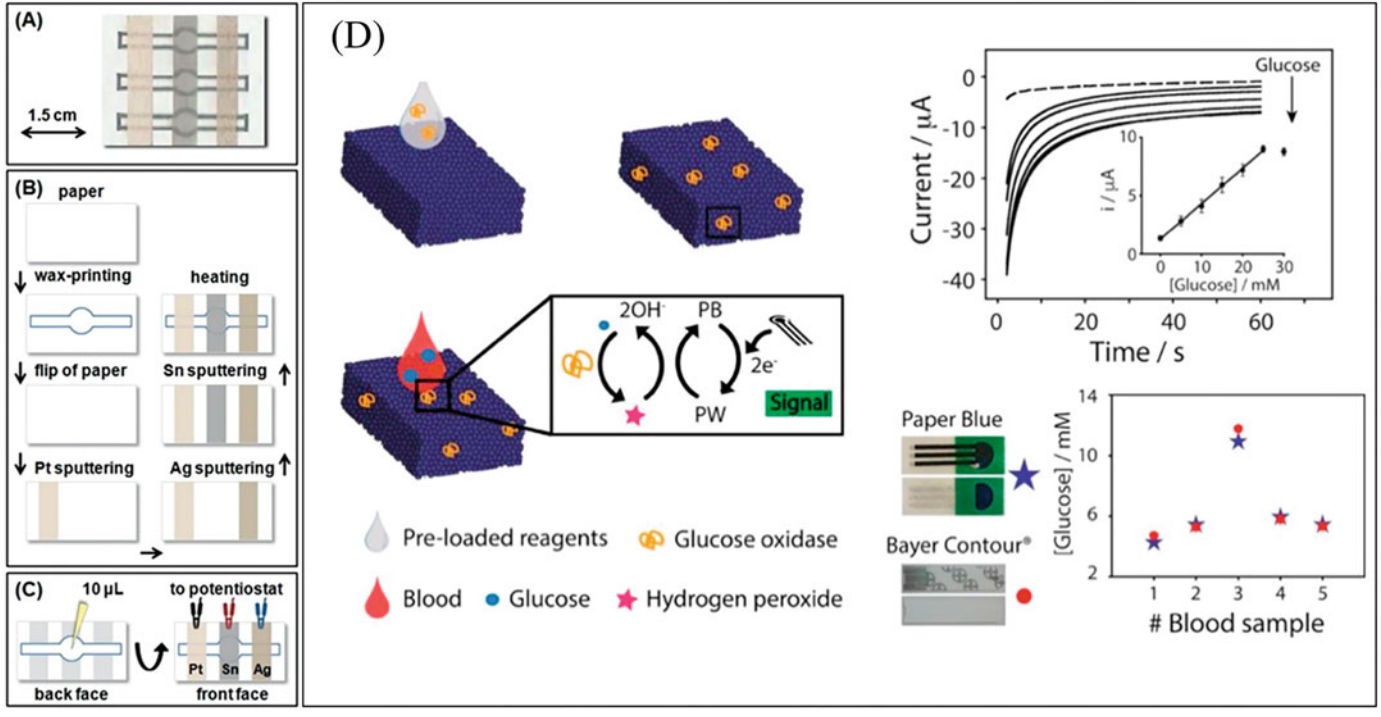

Fig. 2.2 (**a**–**c**) Images of the Sn WE of the ePAD at two different scales. Reprinted with permission from Kokkinos et al. (2018). (**d**) Scheme of the blood paper-based glucose biosensor. Reprinted with permission from Cinti et al. (2018)

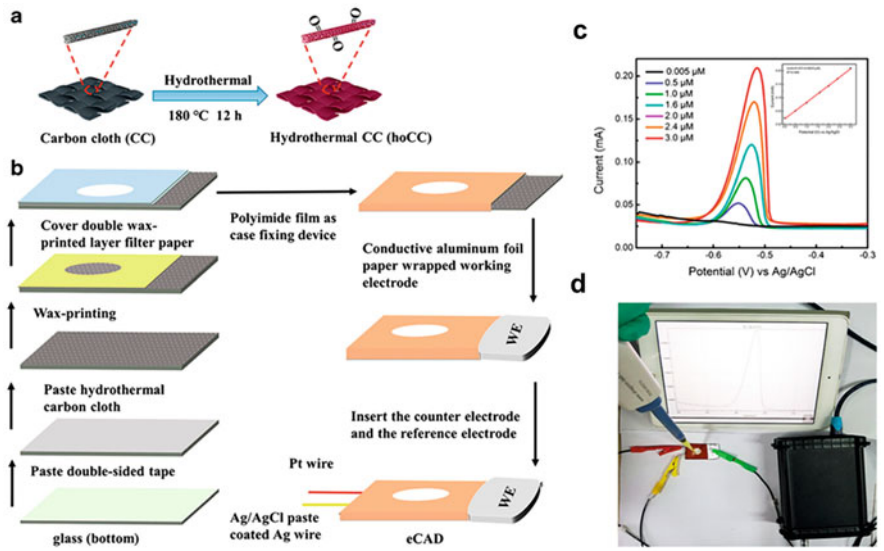

Fig. 2.3 Photograph of the electrochemical sensor based on carbon cloth. Reprinted with permission from Shi et al. (2020)

Pt, and Ag and also contained a wax-printed microfluidic channel as an electrolyte (Fig. 2.2b–d). The paper costs only 0.11$ and provides on-site analysis as an electrochemical sensor for detecting chemical compounds.

Similarly, Huilan Shi et al. (2020) have prepared an electrochemical carbon cloth (CC) by hydrothermal treatment (180 °C for 4 h) to remove unstable oxygen-containing groups, which enhances the catalytic activity of carbon cloth (Fig. 2.3a). These enhancements are due to the availability of carbons to be oxidized (Fig. 2.3b). The paper was used as a sensing platform for the determination of lead ions in water samples with a limit of detection of 4.8 nM (Fig. 2.3c).

2.7.3 Screen-Printed, Stretchable, and Flexible Electrodes

Screen-printed electrodes are mostly used as miniaturized sensing platforms for monitoring chemical compounds. This type of sensor contains three printed electrodes, a counter electrode, a working electrode, and a pseudo-reference electrode. This electrode is printed by two steps, deposing Ag ink as a layer at conducting support followed by coating AgCl film. As illustrated in **scheme 1**, inks based on pure or mixed carbon materials can be used to print counter and working electrodes. Screen-printed electrodes present more advantages; they can offer the possibility of printing on flexible or stretchable conducting supports. They can also specifically be designed to immobilize biorecognition elements like enzymes for monitoring bioelements with more activity (Mădăraş and Buck 1996). Lauro A. Pradela-Filho et al. (2020) have prepared a low-cost electrochemical paper

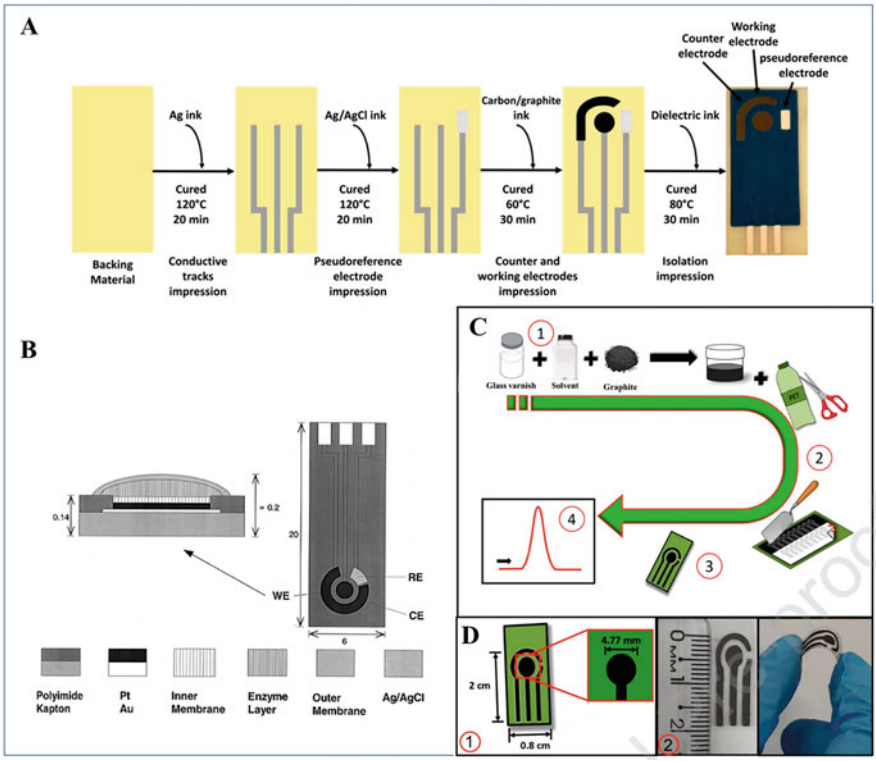

Fig. 2.4 (**a**) Scheme of the screen-printed process step by step. Reprinted with permission from Mădăraş and Buck (1996). (**b**) Scheme of the three-electrode printed system. Reprinted with permission from Molinero-Abad et al. (2018). (**c**) Scheme of the preparation steps of screen-printed electrode. Reprinted with permission from Pradela-Filho et al. (2020)

for the determination of phenolic compounds and estriol hormone with a limit of detection (Fig. 2.4). This technique can produce disposable electrochemical sensors and flexible and easy-to-make miniaturized devices (Pradela-Filho et al. 2020).

2.8 Conclusion

Nowadays, there is an urgent need to construct disposable analytical devices that are ready to use for real-time monitoring with low-cost preparation. This needs knowledge in both 3D printing methods and signal processing technology (DSP)-integrated circuits (ICs). As long as each printing process has advantages and disadvantages, it is highly recommended to choose the suitable printing process taking into consideration the specific application of the printed sensor, the character and the nature of the carbon materials, and the resolution needed for the application.

In addition, there are other extra criteria of selection such as flexibility, complexity, and thermal and chemical stability.

This chapter explores the innovations in the miniaturization of analytical devices like electrochemical biosensors. Different types of sensing platforms are detailed here, including papers, screen-printed sensors, and smart sensors. Thanks to 3D printing technology that makes these analytical tools more disposable with low cost, they were successfully applied for monitoring compounds at trace levels, including even cancer biomarkers. As printing inks on human skin is now possible, these micro-printed tools can henceforth be applied for real-time monitoring of chronic disease.

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Ion Track-Based Nanofluidic Biosensors

3

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Abstract

During the past decade, solid-state nanopores and nanochannels (SSNs) have emerged as a new class of devices for the creation of nanofluidic platforms with diverse applications. In particular, the precise control of ion transport achieved by SSNs paved the way to the development of specific and efficient biological and chemical iontronic sensors with promising technological potential. As biological ion channels play crucial roles in the regulation of vital processes for human cells, they have been a huge source of inspiration toward the design and construction of more sophisticated SSN devices. Today, the academic research on the topic has evolved to many concrete and practical usages, reflecting the potential commercial value of SSNs. Among the different methods available for the nanofabrication of single SSNs, high-energy ion beam (~MeV–GeV) techniques coupled to etching chemical processes are one of the most used due to their control on the size and geometry of the pore. The combination of this advanced nanofabrication technology and different surface functionalization strategies to confer specific target moiety responsiveness to the SSNs were the key point for the extraordinary advances in the area. This chapter aims to provide a closer look

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_3

at the fabrication of SSNs by the ion-track-etching technology and the functionalization strategies in order to build SSNs for biosensing purposes.

Keywords

Nanofluidic · Sensors · Bioanalysis · Analytical performance

3.1 Introduction

Living systems have always been a limitless source of inspiration for scientists both in basic and applied investigations. In particular, ion channels are ubiquitous in nature and play vital roles in many important life processes, such as signal propagation and processing (Pérez-Mitta et al. 2017a; Zhang et al. 2018). Inspired by the key functions of biological ion channels, the development of fully-abiotic solid-state nanochannels (SSNs) has been at the forefront of materials science in recent years (Gleich et al. 2010). Thus, tailorable nanochannels having dimensions comparable to the size of biological molecules and mimicking the function of biological ion channels have attracted attention based on their use as exceptionally sensitive molecule biosensors (Ali et al. 2008). As the biosensing capabilities of the SSNs considerably depend on the surface characteristics of their inner walls, the finely tuning over the surface properties at the nanoscale level plays a crucial role in the biosensing performance.

Among all nanofabrication techniques, ion-track-etching technology is a well-established technique for the development of SSNs in polymer membranes (Spohr 1990). Track-etched foils, as biological protein nanopores, have interesting features such as their selective and asymmetric transport but with enhanced chemical stability, which make them excellent substrates for the design of complex (bio)sensing devices (Tagliazucchi and Szleifer 2016). The surface chemical groups of track-etched nanochannels serve as strategic sites for the proper anchoring of biological building blocks with the capability of binding to or reacting with specific analytes. The integration of these recognition elements into SSNs allows for sensing of biological analytes based on the ionic transport across the nanochannels.

In this chapter, we will first introduce the basic concepts of the ion track-etching technology for the nanofabrication of abiotic nanochannels and the principles that govern the transduction of ionic signals. Then, the strategies for the integration of bioreceptors and some concrete examples of biorecognition mechanisms are addressed.

3.2 Nanofabrication: Ion-Track-Etching Technology

Ion-track-etching technology has been widely used for the creation of nanochannels in both polymeric materials such as polyethylene terephthalate (PET), polyimide (PI), and polycarbonate (PC), as well as other inorganic materials such as silica and

mica (Toimil-Molares 2012; Trautmann 2009). It involves two main steps: (a) the generation of an ion-track via the irradiation of the material with swift heavy ions and (b) the selective ion-track dissolution by performing an appropriate chemical etching.

3.2.1 Swift Heavy-Ion Irradiation

In the first step, a polymeric foil is irradiated with swift heavy ions of MeV–GeV energy (Fig. 3.1(a)). Each swift heavy ion generates along its trajectory in the material a damaged region known as ion-track. For example, at the MAT facilities available at the UNILAC linear accelerator of the GSI Helmholtz Center for Heavy Ion Research (Darmstadt, Germany), materials can be irradiated with heavy ions (^{197}Au, ^{206}Pb, and ^{238}U) with energies of up to 11.4 MeV per nucleon (~15% velocity of the light). Moreover, the number of ions per area unity (ion fluence) that impacts the sample can be adjusted between a single ion and ~10^{11} ions/cm^2 (Fig. 3.1(a)) (Toimil-Molares 2012).

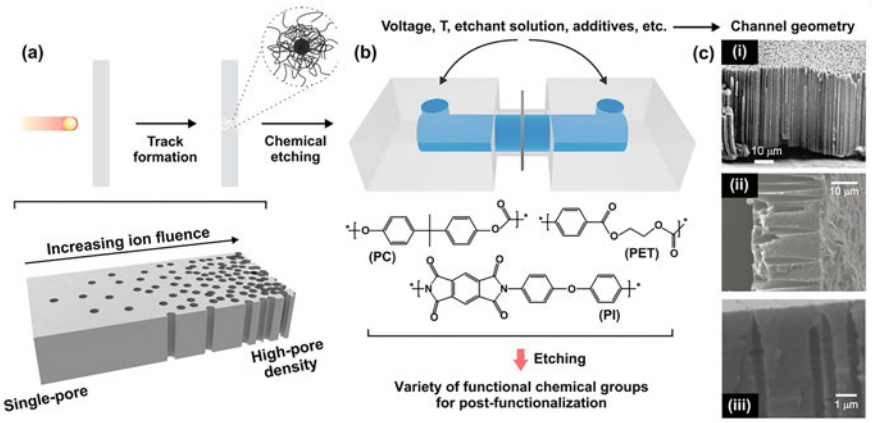

Fig. 3.1 Schematic illustration of ion-track-etching technology. (**a**) Foil irradiation with swift heavy ions (top), which allows the modulation of the channel density by tuning the ion fluence (bottom). (**b**) Chemical etching of the ion tracks by placing the irradiated polymer foil in between two compartments (top), which creates the nanochannels and generates reactive surface groups for post-functionalization depending on the identity of the polymer foil (PC, PET, or PI) (bottom). (**c**) SEM cross-section images of polymer membranes with (1) cylindrical pores (PI) adapted with permissions from (Trautmann et al. 1996). Copyright © 1996 Published by Elsevier B.V.; (2) conical channels (PC) adapted with permissions from (Pérez-Mitta et al. 2018a). Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.; (3) bullet-shaped channels (PET) adapted with permissions from (Laucirica et al. 2020c). Copyright © 2020 Published by Elsevier B.V

3.2.2 Chemical Etching

Taking advantage of the physicochemical differences of the track region compared to the bulk material, it is possible to transform every ion-track into a nanochannel by exposing the irradiated membrane to an adequate chemical (etchant agent) that reacts and selectively dissolves the cylindrical track region (Fig. 3.1(b)). This anisotropic dissolution rate along the damaged ion-track and the isotropic dissolution rate of the bulk undamaged polymer material are denoted track etching rate (v_t) and bulk etching rate (v_b), respectively (Apel and Fink 2004). By modulating the relation between v_t and v_b (track-to-bulk etch ratio $v = v_t/v_b$), it is possible to generate channels with different geometries (Fig. 3.1(c)). On the one hand, with high v values, channels with cylindrical geometries (symmetrical channels) are obtained. For example, in the case of materials such as PC and PET, cylindrical channels can be attained by exposing the irradiated membrane to highly concentrated NaOH solutions (typically 2–6 M), whereas in the case of PI, the etchant agent is usually a NaOCl solution with 13% of active Cl at pH ~9. (Apel and Fink 2004; Pérez-Mitta et al. 2018a) On the other hand, a low v value with asymmetrical etching conditions leads to asymmetrical channels, such as conical or bullet-shaped channels. For example, conical channels can be created by exposing one side of the membrane to the etchant solution and the opposite side to a stopping solution that neutralizes the etchant. In the case of PI, this is achieved by employing a NaOCl solution with active Cl at pH ~12 and a reducing agent solution as an etchant and stopping solution, respectively. In the case of PC and PET, this is achieved by employing a highly concentrated NaOH solution and an acidic solution as an etchant and stopping solution, respectively (Pérez-Mitta et al. 2016, 2018a; Siwy et al. 2003). Bullet-shaped channels can be obtained in PET by adding a small portion of surfactant to the NaOH solution only on one side (Laucirica et al. 2019a, 2020a). All these etching protocols can be performed using different etching time, etchant concentration, and temperature, also in combination with, e.g., applying a transmembrane voltage, pre-exposing the foils with UV light, varying additive concentrations, etc. (Apel and Fink 2004; Apel et al. 2007) The appropriate selection of the specific etching conditions is crucial since it determines the final structural parameters such as the channel aperture sizes and geometry.

In summary, ion-track etching is a versatile and attractive technology to create nanochannels, especially in polymeric materials. The possibility to finely tune the channel density and the relatively straightforward experimental protocol required to adjust the geometrical channel parameters via the appropriate selection of etching conditions are the main advantages. Furthermore, the surface of the track-etched channels synthesized in polymeric materials can be functionalized, exposing a variety of functional chemical groups, such as carboxylic acids, alcohols, phenoxy groups, and amines, which can be easily derivatized for the subsequent integration of stimuli-responsive building blocks or recognition elements (Pérez-Mitta et al. 2015a, 2017b; Laucirica et al. 2020b).

3.3 Transduction by Iontronic Signals

Sensing with nanofluidic devices has been possible by two different approaches: resistive-pulse sensing and steady-state measurements. The former is usually performed by employing nanopores (low aspect ratio, i.e., length ~ radius) and recording the current as a function of time. In contrast, steady-state measurements are more often carried out in solid-state nanochannels (SSNs) (high aspect ratio or, i.e., length >> radius) such as polymer track-etched or anodic aluminum oxide membranes. In this chapter, we will be focusing only on sensing by steady-state measurements (Pérez-Mitta et al. 2017a).

Biosensing with SSNs by steady-state measurements generally involves the recording of current–voltage (*I–V*) curves in the absence and presence of the target analyte. For this, the polymeric membrane containing nanochannels is placed between two half-compartments of a conductivity cell, and a two or four-electrode arrangement is connected to a potentiostat or sourcemeter (with voltage source) to control the transmembrane voltage and to simultaneously record the current. Taking into account that transmembrane current is given by the ion flux, ions are considered the signal carriers and the signal response is often referred to as iontronic output.

To detect and quantify the presence of a target analyte, a measurable change in the iontronic output must be generated. For this, the analyte should trigger a change in the physicochemical properties of the nanochannel surface that affects the readout. Given this, one of the main approaches for constructing sensors and biosensors based on SSNs involves the integration of recognition elements on the nanochannel surface that selectively interact with the analyte in such a way that the channel undergoes a physicochemical change in the presence of the target molecule. There are three established mechanisms to control the iontronic readout via changes on the nanochannel surface: (a) modulation of the surface charge, (b) steric effects or volume exclusion, and (c) wettability changes (Pérez-Mitta et al. 2019). In the case of bio- and chemical sensors, usually, the transduction mechanisms are focused on the phenomena (a) and (b), which will be examined in this chapter. Additional details about ion transport in nanochannels and the different transduction mechanisms are available in Ref. (Tagliazucchi and Szleifer 2016).

3.3.1 Iontronic Response Modulated by Surface Charges

The immersion of a charged surface into an electrolyte solution promotes a redistribution of ions in the interface region of the solution. This region at the vicinity of the surface is designated as the electrical double layer (EDL) in honor of the first models proposed to describe its structure and behavior (Bard and Faulkner 2001; Brett and Oliveira Brett 1993). Within the EDL, the electrostatic potential differs from the value of the bulk solution and, as a result of electrostatic interaction between the ions and the surface charged groups, this region is characterized by an enrichment of counterions (ions with opposite charge polarity than the surface groups) and a depletion of co-ions (ions with the same charge polarity than the surface groups).

The characteristic thickness of the EDL is called the Debye length (λ_D), and its value only depends on parameters related to the electrolyte solution. Typically, λ_D decreases as the electrolyte concentration increases, for example, λ_D acquires values of 9.61 nm and 0.96 nm (at 298 K) for 1:1 aqueous electrolyte solutions with concentrations of 0.001 M and 0.1 M, respectively. In the case of nanochannels, when the channel radius (at least at its smallest aperture) is comparable to the λ_D, (Pérez-Mitta et al. 2017c) the ion concentration inside the channel is controlled by the surface charges, and, therefore, the ion transport across these nanostructures is selective to counterions. The result is the polarization of the ionic concentration (ICP) inside of the channel.

The symmetry of the channel plays a key role in the determination of the iontronic response. Symmetrical systems such as cylindrical channels act as ohmic resistors (Fig. 3.2(a)). Therefore, the response of these devices is characterized by a linear relationship between the current and the transmembrane voltage. Moreover, under surface charge-governed transport conditions, the conductance G (slope of I–V curve) of the channel is directly related to the surface charge density. This behavior was comprehensively explained in a seminal work reported by Schoch and Renaud (Schoch and Renaud 2005). These authors found that the conductance of negatively charged silica nanoslits obeyed the following relation:

$$G = 10^3 \cdot \left(\mu_{K+} + \mu_{Cl-}\right) \cdot C \cdot N_A \cdot e \cdot \frac{w \cdot h}{d} + 2 \cdot \mu_{K+} \cdot \sigma_s^* \cdot \frac{w}{d} \qquad (3.1)$$

where μ_i corresponds to the i-ion mobility, C is the electrolyte concentration, N_A is the Avogadro number, e is the electron charge, σ_s^* is the effective surface charge, and w, h, and d are the weight, height, and length of nanoslits, respectively. Under surface charge-governed transport, i.e., low salt concentration enabling to obtain a λ_D comparable to the slit size, G is dominated by the second term of the right, and therefore, the conductance is dependent on the effective surface charge. Even more, if there is a change in the surface charge, it would be evidenced by a variation in the conductance G. Therefore, one of the most used strategies facing up the development of (bio)sensing devices based on symmetrical track-etched nanochannels involves the modification of the nanostructures with recognition elements that in the presence of certain analytes promote changes in the surface charge density. Thus, the analyte concentration can be related to the changes of the channel conductance and, therefore, quantified in different samples (Fig. 3.2(a)).

When the channel symmetry is disrupted, the ion response is quite different. SSNs with broken symmetry present non-ohmic ion transport properties (Siwy 2006). The iontronic response in asymmetrically modified channels and geometrically asymmetric channels such as conical or bullet-shaped channels is characterized by an ionic current rectification regime (ICR) (Fig. 3.2(b)) (Cervera et al. 2006). In a rectifying regime, the conductance of the system depends on the transmembrane voltage polarity. For one polarization, the ionic current is high (high-conductance branch), but the ionic current is low for the opposite transmembrane polarization (low-conductance branch). This asymmetric behavior is sometimes referred to as

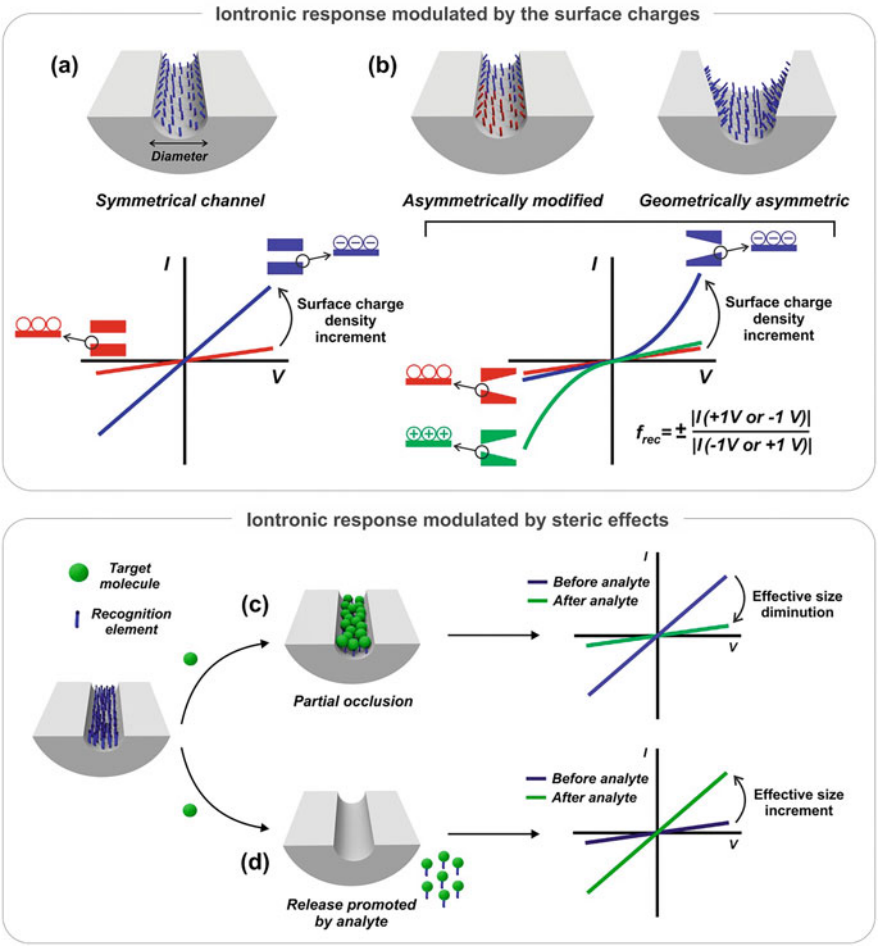

Fig. 3.2 Transduction mechanisms in SSNs. Surface charge effect: correlation between the iontronic response and the surface charge in (**a**) cylindrical (symmetrical) nanochannels and (**b**) asymmetrical nanochannels. Steric effect: changes in the iontronic output due to the partial occlusion (**c**) or aperture (**d**) promoted by the analyte

current rectification or diode-like behavior because of the analogy with electronic diodes. The type of surface charge (positive or negative) determines the direction of the rectification. Thus, the ICR is referred to as anion-driven (anion-selective) rectification or cation-driven (cation-selective) rectification for positively and negatively charged channels, respectively. Then, the diode-like features of the *I–V* curve allow inferring both the surface charge density and its sign (Fig. 3.2(b)). Moreover, the efficiency of the rectification can be quantified by using the rectification factor (f_{rec}) defined as the module of the ratio between the ion currents at the high- and low-conductance branches. Also, the sign of f_{rec} is determined by the sign of the

surface charge. Thus, f_{rec} is a parameter regulated by the surface charge magnitude (Pérez-Mitta et al. 2017b; Yameen et al. 2010). Hence, similarly to the symmetrical case, if the analyte promotes changes in the surface charge density, it could be evidenced by the variation in either the high-conductance branch current values or the rectification efficiency (f_{rec} values) (Fig. 3.2(b)).

3.3.2 Iontronic Response Modulated by Steric Effects

As shown in Eq. (3.1), the channel dimension is a determining magnitude of the channel conductance. Larger inner space enables a higher flux of ions yielding higher conductance values. In contrast, partial or complete blockage of the channel leads to a decrease in conductance (Yameen et al. 2009). These concepts provide the basis for (bio)sensing via steric effects, and at least two common strategies are known. On the one hand, it is possible to decorate the channel surface with a recognition element that interacts with a bulky analyte causing a partial channel blockage, which decreases the conductance (Fig. 3.2(c)) (Guo et al. 2019). On the other hand, if the recognition element is released away from the channel by the interaction with the target analyte, the inner space increases and, consequently, the conductance rises (Fig. 3.2(d)) (Chen et al. 2016; Liu et al. 2016). In both cases, the analyte concentration can be correlated with the changes in the channel conductance and, therefore, quantified in different samples.

3.4 Ion-Track -Based Nanofluidic Biosensors

A biosensor integrates two basic components in the same device: the bioreceptor—a biological molecule that recognizes the target analyte—and the transducer, which is capable of converting the recognition event into a measurable signal (Lee and Mutharasan 2005). The appropriate selection of the recognition elements is central to developing a sensor with the desired selectivity, sensibility, and robustness. Thus, surface functionalization with biological compounds such as DNA, RNA, enzymes, proteins, aptamers, and antibodies, among others, has become popular because these bio(macro)molecules provide (1) a specific recognition of target analytes, (2) high efficiency for specific coupled reactions, as it is the case of enzymes, and (3) the possibility of promoting a signal amplification by fusing biomolecular systems and nanotechnology (Ding et al. 2019). In this context, by combining the inherent signal amplification properties of track-etched nanochannels and the capability of biological macromolecules to bind/react with specific analytes, a variety of biosensors for a vast number of analytes (ions, nucleic acids, proteins, cells, drugs, amino acids, sugars, neurotransmitters, pollutants, and gases) has been developed (Pérez-Mitta et al. 2019). These devices exploit not only the different functionalization strategies of SSNs but also the different mechanisms of recognition between the bioreceptor and the target analytes. In the following sections, we will focus the attention on the general strategies for the integration of biorecognition

elements into track-etched SSNs, subsequently revising some paradigmatic examples of responsive systems based on interactions of the bioreceptor–target analyte for the different families of bioreceptors.

3.4.1 Strategies for the Integration of Bioreceptors

The modification strategy plays a key role in conferring specific properties to the inner surface of the nanochannels, such as wettability, surface charge, and functionality (responsiveness, catalytic activity, biological affinity), or in reducing undesired interactions. Chemical functionalization of nanochannels consists of one or more steps where, generally, the first step is a chemical reaction that allows the introduction of chemical groups onto the inner surface of the channels that serve to attach the molecules of interest in the following step. Here, some of the most employed functionalization strategies are reviewed and commented.

3.4.1.1 Covalent Functionalization: EDC/NHS Coupling Reaction

Carboxylate groups on the etched PET surface can be derivatized by producing amide linking groups from the reaction with primary amine groups in a variety of building blocks. The EDC/NHS approach is a very extended strategy for promoting this coupling reaction. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) combined with N-hydroxysulfosuccinimide (NHS) or sulfo-NHS is employed for activating carboxylate groups for its subsequent reaction with nucleophiles (primary amine groups, for example) (Fig. 3.3(a)) (Apel et al. 2011). This method has proved to be successful, and it is commonly used in the construction of nanopore-based biosensors (Ali et al. 2010a; Vlassiouk et al. 2009).

3.4.1.2 Electrostatic Self-Assembly

Electrostatic adsorption of polyelectrolytes is an alternative technique to covalent chemistry that allows creating a thin polymer film on nanochannel surfaces (Fig. 3.3(b)). In a typical experiment, the nanochannel membrane is immersed into a solution of a polyelectrolyte with the opposite charge to that of the channel walls (Pérez-Mitta et al. 2017b; Laucirica et al. 2019b, 2020d). Although polyelectrolyte deposition on flat surfaces is a well-understood process (Netz and Andelman 2003), nanoconfinement effects have to be taken into account for adsorption in nanochannels. Therefore, a successful modification of its inner surfaces via electrostatic adsorption requires considering variables such as the channel diameter, the molecular weight of the polyelectrolyte, and the ionic strength and pH of the solution (Gilles et al. 2018).

After the first layer of electrolyte is absorbed on the surface, the net charge of the surface inverts and becomes the same as that of the adsorbed polyelectrolyte and is possible to sequentially adsorb another polyelectrolyte of opposite charge in order to assemble a polyelectrolyte multilayer. This process is known as layer-by-layer (LbL) self-assembly (Decher et al. 1992). Several groups have used the LbL self-assembly method to asymmetrically functionalize SSNs, observing that the rectifying

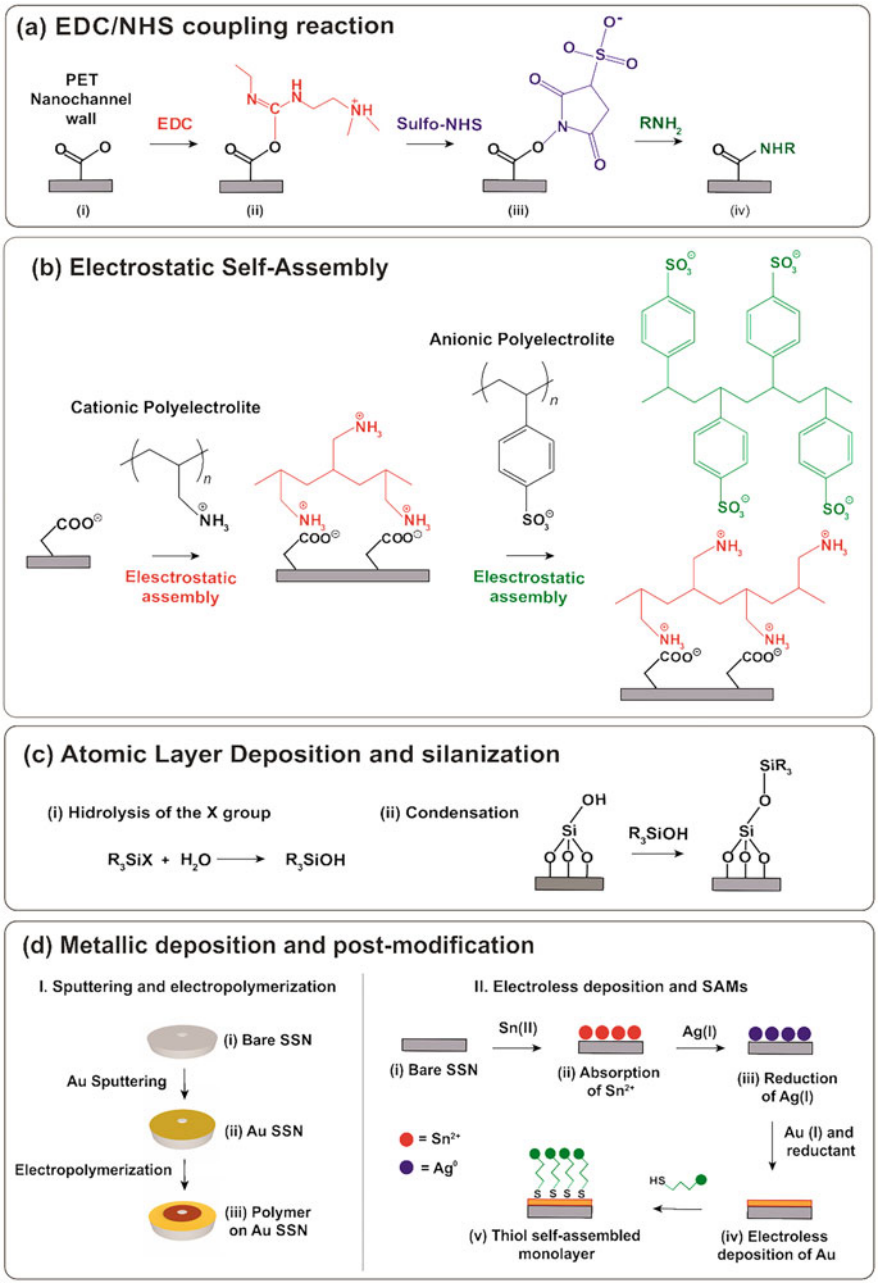

Fig. 3.3 Illustrative scheme of the different types of functionalization strategies: (**a**) EDC/NHS coupling reaction, (**b**) electrostatic self-assembly, (**c**) atomic layer deposition and silanization, and (**d**) metallic deposition and its possible postmodifications. (**b**) was adapted with permission (Ali et al. 2010c). Copyright © 2010 American Chemical Society

properties are strongly affected by the result of the conditions and the nature of the most external polyelectrolyte capping layer (Ali et al. 2010b; Ma et al. 2018).

3.4.1.3 Atomic Layer Deposition and Silanization

Another type of covalent method of surface functionalization of SSNs is the atomic layer deposition (ALD) process, with which thin films of a variety of materials, such as SiO_2, TiO_2, and Al_2O_3 oxides (Sander et al. 2004; George 2010; Spende et al. 2015; Sobel et al. 2015; Ruff et al. 2018; Kumari et al. 2018), can be produced. Silica surfaces present a combination of siloxane groups, which can behave as weak acids or weak bases depending on the pH conditions.

Moreover, organosilanes are commonly employed for silica functionalization. Silanes have general formulas $RSiX_3$, R_2SiX_2, or R_3SiX, where R is an organic group that confers functionality to the surface and X is a hydrolyzable group, which is lost during coupling to the silica surface. A representative scheme of this functionalization mechanism is presented in Fig. 3.3(c). This modification has proven to be successful in some systems, such as glass nanopores (Zhao et al. 2013) and alumina (Tan et al. 2011), although exploration in single-pore polymer nanochannels coated with SiO_2 continues to be a challenge.

3.4.1.4 Metallic Deposition and Postmodification

Nanochannel surfaces can also be metalized by thermal evaporation or *sputtering*, which allows the preparation of asymmetric gold coatings, i.e., in one side of the membrane (Pérez-Mitta et al. 2015b). The good conductivity of the Au film enables further functionalization steps of the metal surface by electropolymerization (Fig. 3.3 (d)) (Laucirica et al. 2020a; Pérez-Mitta et al. 2015c; Tian et al. 2010). The combination of both Au-sputtering and electropolymerization in nanochannels not only allows using the metalized membrane as an electrode (controlling its membrane potential and ionic transport) but also controlling the pore diameter.

Another efficient method to create metallic films is the electroless plating method (Ohno 1991). This technique is especially recommended for polymeric substrates that require mild modification conditions and can be described as an autocatalytic redox reaction in which metal cations in solution (Au(I), for example) are reduced onto a substrate to form a metal layer. Commonly, this process is carried out in the presence of surface-immobilized metal nanoparticles that act as catalysts of the reaction. As an example, the Au-electroless protocol employs a mixture of an Au (I) complex and a strong chemical reductant (e.g., formaldehyde) in the presence of Ag nanoparticles (Muench et al. 2011). The success of this methodology has been demonstrated by several authors on PET and PC track-etched membranes (Gao et al. 2014; De Leo et al. 2007).

On the other hand, self-assembled monolayers (SAMs) of thiols on gold (and other coinage metals) constitute a versatile pathway to introduce surface chemical functionalities (Love et al. 2005). Depending on the nature of the terminal group, the one-step self-assembly process can lead to the formation of a monolayer of predefined functional groups on the surface. This method has been commonly used for the functionalization of gold surfaces due to its ease of preparation and

the good stability of the gold–thiolate bond at ambient conditions (Fig. 3.3(d)). For the application of this surface chemistry to SSNs, the membranes must be previously metalized. In this sense, it is possible to coat the surfaces of nanochannels with a thin gold layer and then form a SAM of thiols with specific chemical functionalities (Tian et al. 2011; Martin et al. 2001; Nasir et al. 2014).

3.4.2 Biorecognition Mechanisms

The different biorecognition mechanisms are presented below according to the nature of the recognition element.

3.4.2.1 Nucleic Acids

In a seminal work, Harrel et al. presented in 2004 the integration of DNA into single conically shaped PC-based nanochannels coated with a thin layer of gold to build artificial ion channels (Harrell et al. 2004). The authors proved that the extent of rectification could be precisely controlled by either a simple chemical method (varying the DNA chain length) or a simple physical method (changing the nanochannel mouth diameter). After that, many efforts have been dedicated to the integration of DNA architectures into SSNs in order to achieve the detection of a wide variety of analytes including ions and nucleic acid molecules.

Mechanical Transduction Modulated by DNA Complex Structures

Buchsbaum et al. presented a new concept for building synthetic DNA-modified nanochannels that could simultaneously respond to pH and transmembrane potential changes (Buchsbaum et al. 2014). By attaching DNA oligomers containing protonable adenine (A) and cytosine (C) at the narrow opening of an asymmetric SSN, they showed that a pH-dependent reversible closing mechanism was responsible for the iontronic output. Specific DNA secondary structures known as G-quadruplexes (G4) were also employed for the construction of proton-gated nanofluidic nanochannels (Wang et al. 2009). After grafting the walls of a conical-shaped single track-etched nanochannel with G-rich human telomere strand (a type of G4 DNA), the DNA underwent a potassium-responsive conformational change between a random single-stranded structure (no K^+) and a four-stranded G4 structure (presence of K^+) (Fig. 3.4(a)), which induced a change in the effective pore size and consequently in the ion transport properties of the device (Fig. 3.4(b)). Furthermore, immobilizing C-quadruplex and G-quadruplex DNA molecules onto the top and bottom tip side of a cigar-shaped nanochannel, Liu et al. presented a bioinspired pH and K^+ double-gated nanosystem (Fig. 3.4(c)) (Liu et al. 2015). By adjusting K^+ and H^+ (chemical effectors) concentrations, the two gates of the nanochannel can be opened and closed alternately or simultaneously.

The Hybridization Strategy as Recognition and Binding Mechanism

Hybridization is the process by which a synthetic DNA probe or primer binds via Watson–Crick base pairing to a biological DNA target sequence (Khodakov et al.

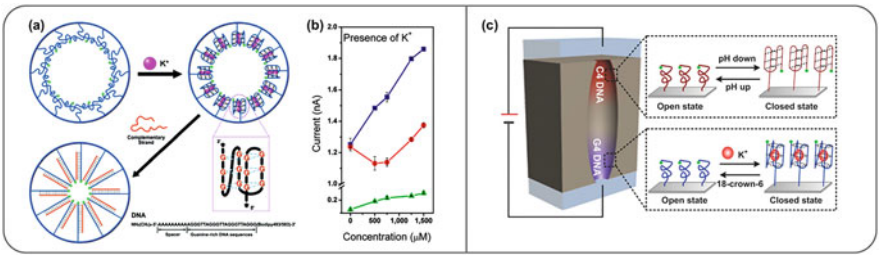

Fig. 3.4 (**a**) K^+-responsive G4 DNA. In the presence of K^+, the packed rigid quadruplex structures partially decrease the effective pore size. After adding complementary DNA strands, G4 DNA forms a closely packed arrangement of double-stranded DNA. (**b**) Current vs. K^+ concentration curve before G4 DNA modification (blue); after G4 DNA modification (red); after the addition of the complementary DNA strands (green). Reproduced with permission (Wang et al. 2009). Copyright © 2009 American Chemical Society. (**c**) Schematic illustration of the bio-inspired potassium and pH cooperative double-gated nanochannel. Reproduced with permission (Liu et al. 2015). Copyright © 2014 WILEY-VCH Verlag GmbH & Co

2016). Because of its simplicity and robustness, hybridization is the basis of many DNA analysis and diagnostic methods, in many cases in combination with amplification techniques to increase its sensitivity (Khodakov et al. 2016). In 2005, Vlassiouk et al. reported a pioneering work that used hybridization as a strategy to detect target DNA oligomers with AAO membranes (Vlassiouk et al. 2005). From that moment on, many attempts have been made to build nucleic acid sensors based on a hybridization mechanism in different types of nanochannels.

DNA Oligonucleotides

An illustrative example of a track-etched membrane-based DNA biosensor was developed by Sun et al. in 2016 (Sun et al. 2016). The authors reported the construction of nanofluidic channels for label-free, ultrasensitive, and highly sequence-specific detection of DNA (Fig. 3.5). The DNA probe oligonucleotides (bioreceptors) were immobilized by self-assembly on PEI-modified conical track-etched PET multi-SSNs. After that, BSA was adsorbed in order to avoid further nonspecific interactions with other negatively charged analytes (Fig. 3.5(a) and (b)). The as-prepared SSNs presented a near ohmic behavior due to their almost zero surface charge density. DNA detection was evaluated by monitoring the rectification efficiency of the diode-like behavior upon hybridization. A strong increment in the rectification efficiency was found when increasing the target complementary DNA sequence concentration, which could be explained by the increment in the net surface charge of the SSN that yields a binding-type dependency of the rectification factor on the target concentration (Fig. 3.5(c)). This device was able to discriminate complementary DNA (c-DNA) from noncomplementary DNA (nc-DNA) and one-base mismatched DNA (1bm-DNA) samples with high specificity (Fig. 3.5 (d)), even in real serum samples.

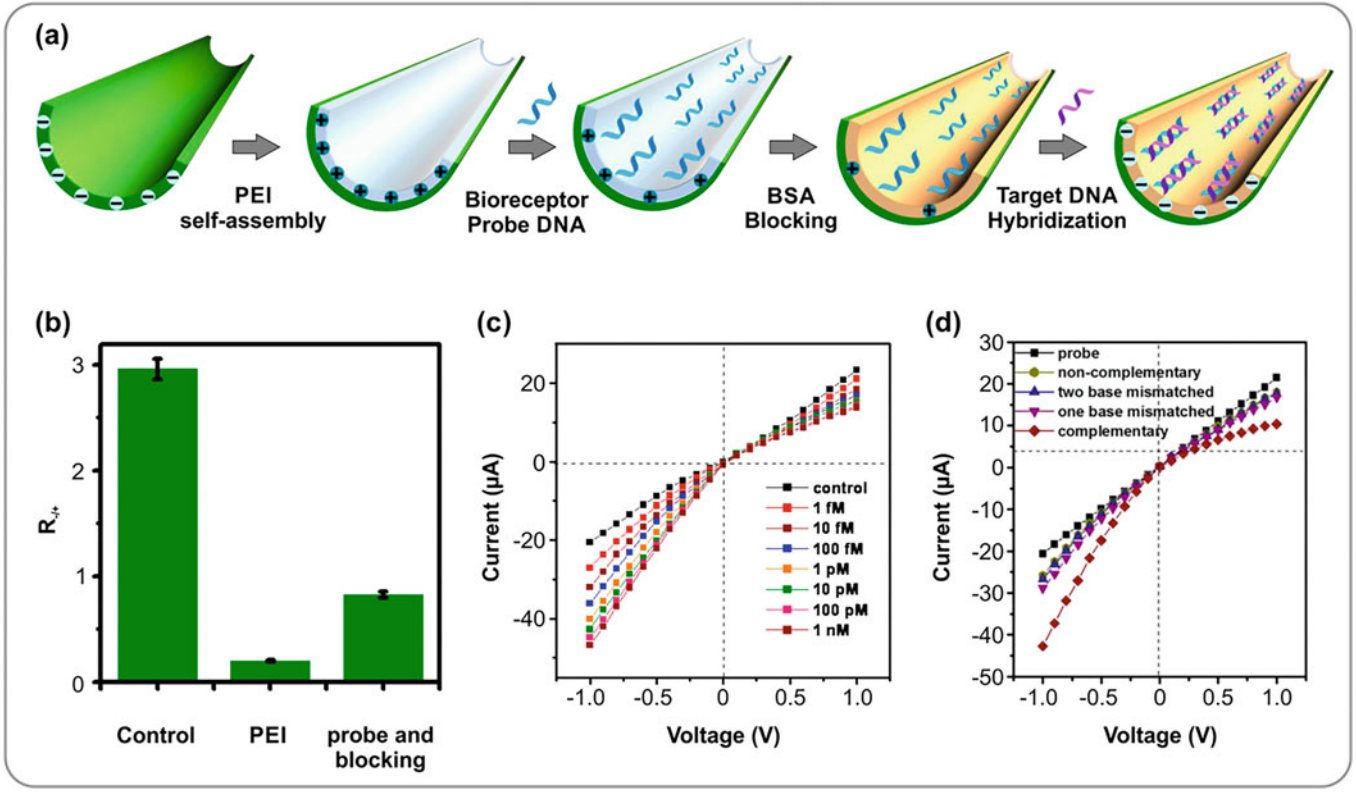

Fig. 3.5 (**a**) Schematic illustration of the probe DNA-functionalized PET nanochannel biosensor construction for DNA detection. (**b**) Rectifying characteristics of the layer-by-layer assembly process on the conical nanochannel ($R_{-/+}$ is defined as the absolute value of the current at −1 V divided by the current at +1 V). (**c**) *I–V* curves for different c-DNA concentrations. (**d**) *I–V* curves of the nanochannels after treated with 1 nM nc-DNA, 2bm-DNA, 1bm-DNA, and c-DNA. Adapted with permission (Sun et al. 2016). Copyright © 2016, Elsevier

DNA Superstructures

Further than double-strand hybridization, DNA superstructures have been utilized to create more efficient gating mechanisms in nanochannels (Pérez-Mitta et al. 2019). An instructive example was reported by Jiang and co-workers, who achieved the sub-nanomolar sequence-specific DNA detection (Liu et al. 2013). Track-etched PET nanochannel membranes were modified with predesigned capture DNA probes, and, in the presence of the target DNA, alternating units of signal probes (denoted as S1 and S2) were successively hybridized, bringing long concatamers (Fig. 3.6(a)). These DNA superstructures efficiently block ionic transport across the membrane, leading to a decrease in the transmembrane ionic current (Fig. 3.6(b)). Moreover, the DNA probe was modified with an aptamer sequence so that the presence of small target molecules caused the disassembly of the preformed supersandwich structures (Fig. 3.6(a) and (b)). With this strategy, the detection of oligonucleotides and ATP was successfully achieved.

Aptamers

Aptamers are nucleic acid molecules that can be rationally designed and tailored for a specific target (Dunn et al. 2017). Its use is promising because these molecules can be engineered into sensors, actuators, and other devices with relatively simple methods, which is central to emerging technologies. In this context, Li et al. reported the creation of a bioinspired adenosine artificial regulatory receptor (ARR) based on the self-assembly of designed adenosine (AD) aptamers onto gold-sputtered PET multi-nanochannels (Li et al. 2016). The selected recognition element of the ARR was a sequence-specific aptamer (SSA) that binds adenosine with a high affinity. In terms of the iontronic output, SSA self-assembly produced very low ionic currents (OFF state) due to the randomly stretched single-stranded structure of SSA that partially blocked the tip of the nanochannel. The exposition to AD and its binding to the SSA induced a conformational change in the aptamer that opened the pore (ON state). More specifically, AD and SSA formed beanpod structures that, in terms of ion transport, showed an increase in the ionic current (ON state).

DNA Hydrogels by the Hybridization Chain Reaction (HCR)

The concept of hybridization chain reaction (HCR) was introduced by Dirks and Pierce in 2004 (Dirks and Pierce 2004). They reported the mechanism by which stable DNA monomers (hairpins H1 and H2) assembled only upon exposition to a target single-stranded DNA fragment. Hairpins play the role of monomer DNA building blocks that hybridize only when an initiator strand (target DNA) triggers a polymerization reaction into a nicked double helix. The amplification of the initiator recognition event continues until H1 or H2 is completely consumed. Using HCR strategy, smart DNA hydrogel-functionalized potassium-responsive nanochannels were developed (Wu et al. 2018). For this aim, a PET membrane with a single conical channel was coated on the tip side with an Au film and then functionalized with a thiolated nucleic acid (initiator) (Fig. 3.7(a)). Then, DNA hydrogels were self-assembled via HCR using a series of hairpins (Fig. 3.7(a) and (b)). The DNA hydrogel-modified channel displayed a high rectification factor due

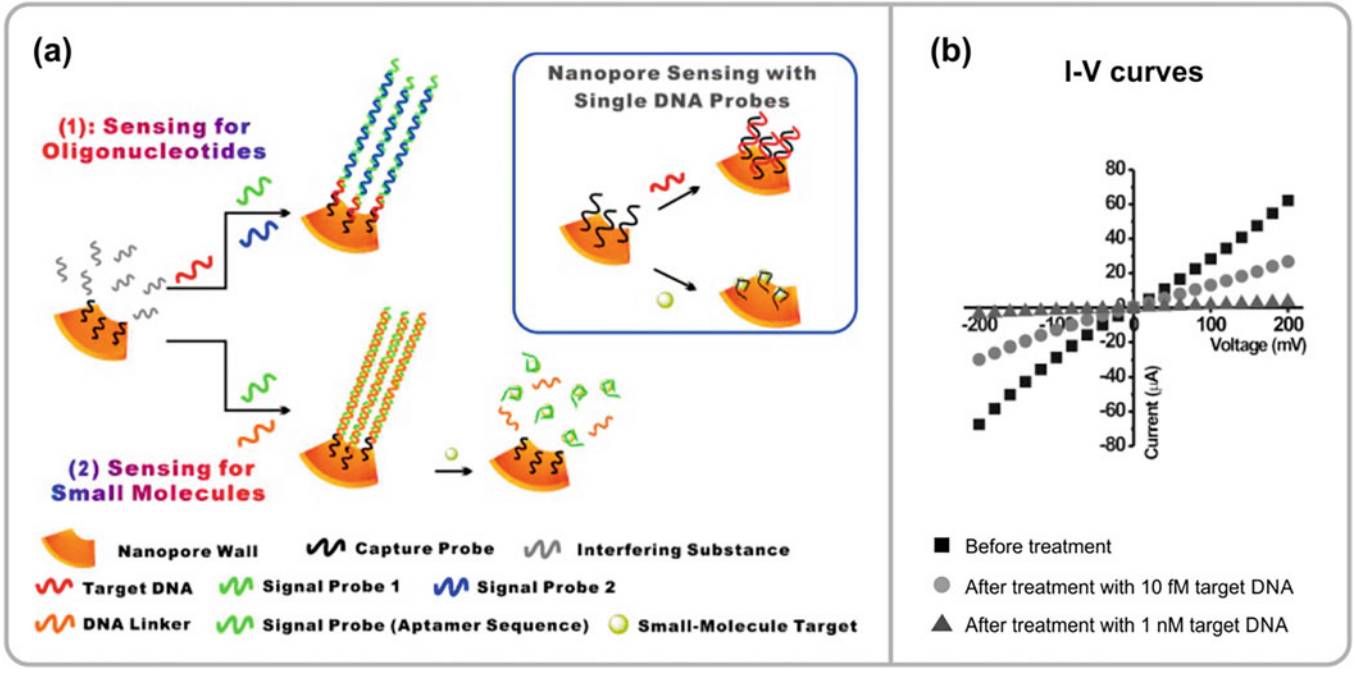

Fig. 3.6 (**a**) Improved biosensing strategy for oligonucleotides and small molecules. (**b**) Detection of DNA as monitored by nanopore conductance: *I–V* curves before (black small square) and after treatment with 10 fM (black small circle) or 1 nM (black up-pointing small triangle) of target DNA. Adapted with permission (Liu et al. 2013). Copyright © 2013, John Wiley & Sons, Inc.

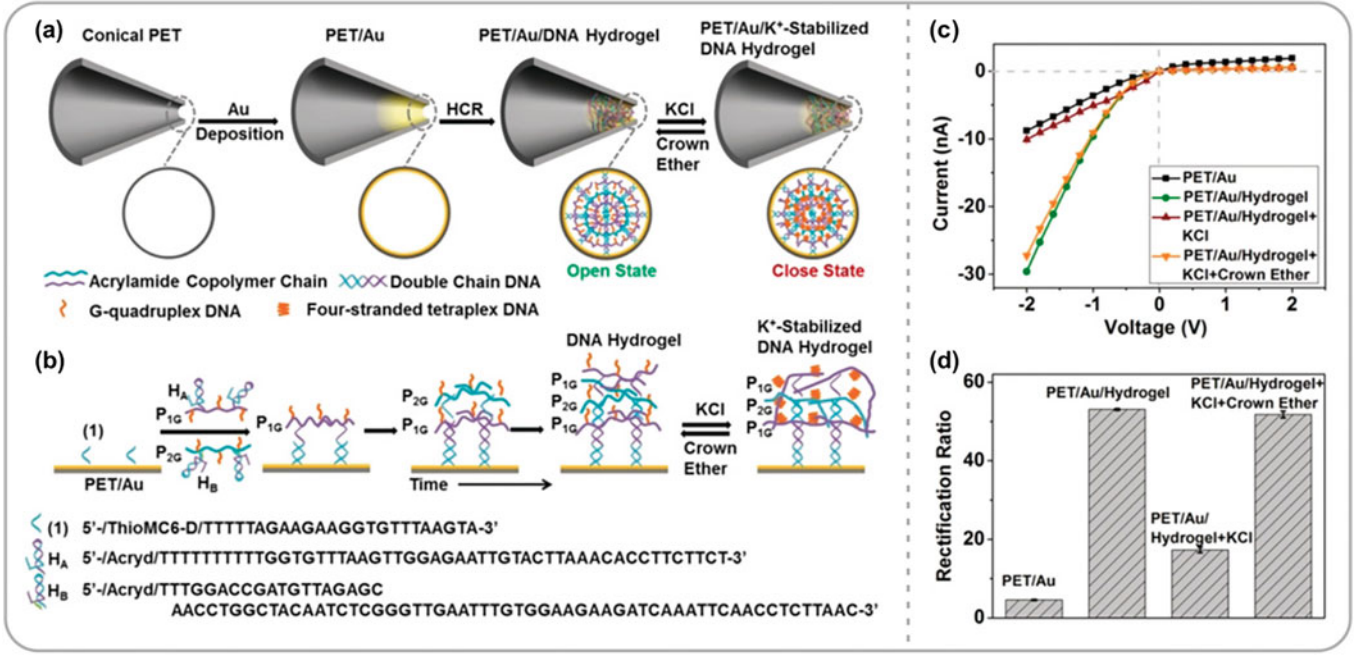

Fig. 3.7 (**a**) Scheme depicting the preparation process of the DNA hydrogels-based ion channel. (**b**) Detailed formation and transition of DNA hydrogel networks. (**c**) I–V curves and (**d**) rectification ratios of the conical nanochannel under different conditions (0.1 M LiCl, 10 mM Tris-HCl buffer, pH 7). Reproduced with permission (Wu et al. 2018). Copyright © 2018 John Wiley & Sons, Inc.

to the hydrophilicity and negative charge provided by the hydrogel (OPEN state) (Fig. 3.7(c) and (d)). When exposing the system to K^+ ions (0.05–1 M), a decrease in the rectification factor was observed (CLOSE state). Besides, by treating with 18-crown-6-ether solutions, K^+ ions could be released, leading to a softening of the DNA hydrogel structure.

Integration of DNAzymes

DNAzymes, also identified as deoxyribozymes or DNA enzymes, denote single-stranded DNA molecules with catalytic capabilities. In the last years, many efforts have been assumed to assess a variety of DNAzymes for innovation-driven applications such as gene regulation (Achenbach et al. 2004). Also, it is known that ions play an important role in maintaining normal physiological conditions and regulating several biological processes in living organisms (Hille 2001). Here, we present some relevant advances in the development of ion sensors based on DNAzyme-biofunctionalized track-etched nanochannels (Guo et al. 2019; Chen et al. 2016; Tu et al. 2021).

Chen et al. reported the construction of a nanofluidic device with Cu^{2+}-modulated ion transport properties by integrating a specific Cu^{2+}-induced self-cleaving DNAzyme into a polymer multi-nanochannel membrane (Chen et al. 2016). The creation included the nanochannel surface grafting with a short DNA sequence (DNA(2)) partially complementary to a DNAzyme (DNA(1)). Thus, DNA(2) partially hybridizes DNA(1) to form a double-stranded DNA (dsDNA). The DNAzyme-modified channel showed a diode-like behavior characterized by a negative surface as a consequence of the high negative charge provided by dsDNA. When exposing the membrane to Cu^{2+}, a decrease in the rectification factor was observed, in line with a Cu^{2+}-promoted break of DNA(1) into three fragments and its subsequent dissolution, which produced a decrease of the negative charge density.

Another example of a Zn^{2+} detection system based on cylindrical PET multichannel modified with both DNA supersandwich structure and Zn^{2+}-requiring DNAzyme was developed by Liu et al. (Liu et al. 2016) A capture probe, a sessile probe, a DNAzyme, and auxiliary DNA probes were immobilized at the nanochannel surface. The as-prepared DNA-modified channel showed an ohmic behavior with low conductance ascribed to the partial occlusion of the channel. On the contrary, in the presence of Zn^{2+}, a cleavage of the sessile probe and the stripping of the DNA supersandwich from the DNAzyme structure occur, leading to an increase in the conductance.

3.4.2.2 Proteins and Enzymes

In 2016, Ali et al. immobilized bovine serum albumin (BSA) on the surface of a single asymmetric nanochannel to develop a tryptophan (L-Trp) sensor (Ali et al. 2016). BSA molecules serve as chiral receptors since they enantioselectively recognize Trp over D-Trp through specific BSA–L-tryptophan interactions. In order to provide the channel with functionalization sites for BSA, dopamine (DA) was self-polymerized, allowing the deposit of a polydopamine (PDA) thin film onto the channel surface (Fig. 3.8(a)). Then, BSA amine groups reacted with PDA through

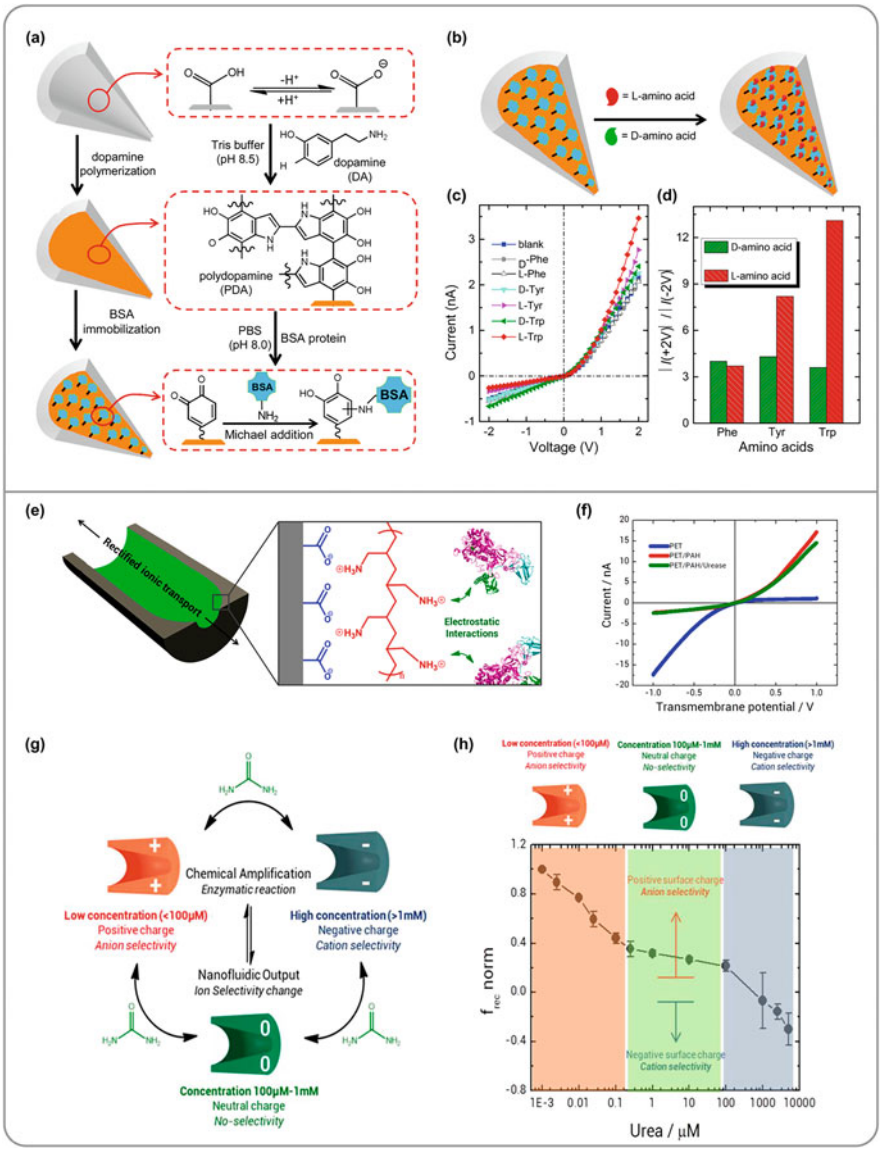

Fig. 3.8 (**a**) Functionalization of the PET nanochannel for L-tryptophan biosensing. (**b**) Specific binding of amino acid enantiomer onto BSA-modified nanochannel surface. (**c**) I–V curves of the BSA-modified SSN before (blank) and after exposure to 1.5 mM solution of D-/L-Phe, D-/L-Tyr, and D-/L-Trp (100 mM KCl, pH 5.8). (**d**) Changes in the rectification factor (f_{rec}) at 2 V. Reproduced with permission (Ali et al. 2016). Copyright © 2016 Elsevier B.V. (**e**) Urease-modified single PET nanochannel. (**f**) *I–V* curves before and after each modification step at pH 6. (**g**) Scheme of the biochemical degradation of urea and the nanofluidic output of the nanochannel through the amplification of the enzymatic reaction. (**h**) Calibration curve in terms of normalized rectification factor *vs* urea concentration. Reproduced with permission (Pérez-Mitta et al. 2018b). Copyright © 2018, American Chemical Society

the Michael addition reaction. In terms of ion transport, the specific BSA–L-tryptophan interactions led to changes in the surface charge density that modulated the ionic transport through the nanochannel (Fig. 3.8(b), (c) and (d)).

Even more, the integration of enzymatic processes into SSNs confers them outstanding specificity on target analytes as a result of biological recognition. An illustrative example was provided in a pioneering work by Pérez-Mitta et al. (Pérez-Mitta et al. 2018b) The authors provided evidence on the mechanism for sensing urea by using a urease-modified single PET nanochannel. First, the electrostatic assembly of poly(allylamine) (PAH) on the etched bullet-shaped PET nanochannels was performed, and afterward urease was electrostatically adsorbed on the PAH-modified channel (Fig. 3.8(e) and (f)). Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, causing a local pH increase and, thus, a decrease in the protonation degree of the PAH, reducing the positive surface charge density in the channel. This biosensing device was able to reach a limit of detection of 1 nM of urea (Fig. 3.8(g) and (h)).

3.4.2.3 Other Biomolecules

Amino Acids

Recently, Lou et al. reported a new L-tyrosine functionalized SSN-based strategy to detect H_2O_2 released from living cells without insertion procedures (Lou et al. 2020). First, cervical cancer living cells (HeLa) were incubated on a PET membrane with cylindrical nanochannels. After that, the PET surface carboxyl groups were functionalized by coupling chemistry with L-tyrosine-generating phenolic hydroxyl groups on the nanochannel surface. Afterward, the addition of an aggregation-induced emission luminogen (TT) in the presence of horseradish peroxidase (HRP) and H_2O_2 released from living cells derived in the formation of di-tyrosine linkages and TT oligomerization. This fact provoked a partial blocking of the ion transport pathway, which was detected by ion transport measurements. A high sensitivity response to H_2O_2 was obtained as, for 1 nM H_2O_2, a resistance of more than 100 kΩ was measured (+0.2 V).

3.5 Conclusions and Outlook

Solid-state nanochannels have become a versatile platform for the design and development of biosensing devices based on nanofluidics. Due to the influence of the ionic surface charge and the effective channel size on the ionic current through the nanochannels, the functional coupling of biorecognition entities on the nanochannel surface and their specific interaction with target molecules affect the recorded ionic current due to the caused changes in ionic surface charge and/or effective channel size, simultaneously enabling specific responsiveness and sensing. Some biological molecules, such as nucleic acids and enzymes, are able to exhibit interactions with some specific targets with high efficiency, but they need to be properly integrated into the confined environment of the inner surface of the

nanochannels. In this chapter, the basis of the application of SSNs for biosensing purposes, including the nanochannel fabrication and the transduction of iontronic signals, was presented together with some illustrative examples of how the proper integration of biorecognition elements within the inner surface of the channels allows sensing and quantifying a variety of analytes in solution from the changes in the iontronic response.

Due to the nanometer size of the active sensor components, i.e., the nanochannels, the miniaturization of the SSN-based technology should become straightforward, naturally heading toward the use of SSNs for ultrasensitive detection and quantification of biomarkers and drugs for clinical, pharmaceutical, and forensics applications in miniaturized devices.

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Clinical Biosensors: Considerations and Development Process

4

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Abstract

The desire for efficient, timely, and cost-effective medical diagnostics has impelled biosensor technology and research progression. There are significant challenges in the development process of biosensors to meet the continually increasing demands. The fabrication and development process should produce high performance and yield without sacrificing cost-effectiveness. This need necessitates continuous and lean development methodologies to fabric, characterize, and miniaturize biosensors and biosensing systems to render more effective outcomes. Clinical biosensors combine detection and transduction units to detect chemical or biological substances or responses and transform them into electrical, optical, thermal, piezoelectric, or electrochemical signals. Most biosensors aim to detect a biological signal from a specific analyte to monitor the biological functions and environment. Another lesser-known class of biosensors, wearable biosensors for clinical applications, is used to measure biopotential, pathophysiological, or biological signals noninvasively. This chapter focuses on the lesser-known class of biosensors deployed using wearable form factors, emphasizing noninvasiveness, cost-effectiveness, and portability. This chapter also summarizes the technological challenges that have deterred the

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_4

development and execution of biosensors., with a brief description of the opportunities and future outlook for biosensors in clinical and healthcare applications.

Keywords

Biosensors · Bioelectronics · Healthcare · Wearables · Nanobiotechnology · Vital signs

4.1 Introduction

Research on wireless wearable technologies and the healthcare industry have been closely intertwined in the modern era as noninvasive and ubiquitous monitoring solutions are gaining significant grip among patients and healthcare practitioners. Healthcare expenditure is on an increasing trend and a continuous upward spiral (California Health Care Foundation 2012; Health, United States 2017). Healthcare expenditures increased by 143%, from $1370 billion in 2000 to $3337 billion in 2016. The predicted cost will approximately be $4500 billion in 2020. Accordingly, ever-increasing costs influence health insurance and other healthcare-related tribulations. The potential means to control the healthcare costs include improving preventive medicine and early detection and identifying the underlying causes of disorders and diseases. Improvement in preventive medicine can be initiated by educating people about healthy lifestyles, origin and identification of symptoms of various disorders, and prevention of various chronic diseases.

However, the quality of the effort is only effective with a smaller group of people because of the abundant variables that climb with the population's growth. Early detection and identification of the causes of chronic disorders and diseases lead to a considerable decrease in the cost of treatments. Recurrent and uninterrupted monitoring is one of the critical factors in the early detection of chronic diseases. However, repetitive hospitalization or hospital visits may result in a substantial increase in cost. One of the solutions to address these issues could be remote point-of-care (POC). The remote POC systems facilitate monitoring the patient and utilizing the patient data to diagnose the diseases and disorders at the comfort of home instead of a patient requiring visiting a hospital or a physician. Therefore, the cost of hospitalization can be reduced by implementing POC systems. Identification of causes leading to chronic diseases can also reduce the cost of treatment. Hospital visits and readmissions are one of the major causes of this increase in healthcare costs. The ever-increasing healthcare costs are one of the primary motivating factors to reduce unnecessary hospital visits and readmissions by utilizing technology advancement. According to the Centers for Disease Control and Prevention, cardiovascular disorders were significant factors leading to death (Health, United States 2017; CHS Data Brief 2018). In the United States, cardiovascular disorders-related deaths resulted in 23.8% of all diseases resulting in mortality.

There is a need for compact, low-cost, reliable, and ubiquitous patient monitoring systems that can be used for health monitoring (Yancy et al. 2013; Bergmann et al. 2012). While multiple approaches (Farshchi et al. 2006, 2007; Mundt et al. 2005; Yao et al. 2005; Rasid and Woodward 2005; Fulford-Jones et al. 2004; Obeid et al. 2004; Mohseni et al. 2005; Irazoqui-Pastor et al. 2002; Modarreszadeh and Schmidt 1997) are used to build an efficient wireless system, robust and real-time monitoring systems that enable continuous diagnostic quality measurements are highly desirable. The measured parameters may include vital signs, electrocardiogram (ECG), electroencephalography (EEG), electrooculography (EOG), electromyography (EMG), skin conductance, or Galvanic skin response (GSR), blood pressure, rate, body and skin temperature, oxygen saturation, and the like. Generally, a biosensor consists of a sensing component and a transducer. The sensing component functions as a receptor that receives the signals from the source. Similarly, the transducer converts the acquired signals to electrical, optical, thermal, piezoelectric, or electrochemical signals. The biosensor typically works in conjunction with an electronics unit to amplify, filter, and process the signals. The processed signals are used to derive meaningful outcomes. The combination of the biosensor and the electronics unit is called the biosensor system. This chapter focuses on the fundamental aspects, considerations, development and fabrication processes, challenges, and future perspectives of biosensors and their healthcare and clinical perspective applications.

Scientific developments have sparked the need for refinement in the healthcare industry. Research groups and companies are looking forward to developing efficient and accurate systems by harnessing technology evolution and advancement. The use of technology, especially wireless communication in healthcare diagnostics, facilitates the acceleration of the diagnostics process, thereby harmonizing the primary goal of diagnostics to identify a medical abnormality in a shorter period. Currently available noninvasive monitoring systems, where electrical recording is the primary monitoring modality, use electrodes to establish contact with the patient's skin using a conductive gel. The electrodes are connected using leads or cables to the recording equipment. The patient must be near the equipment due to the wired electrical connections between the body and the equipment. The gel-based adhesive electrodes and numerous cables make it uncomfortable for the patient and cause poor patient compliance resulting in improper and low-quality signal recordings because of motion artifacts. Some of the commonly monitored biopotential signals are electrocardiogram (ECG), electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG). The gel-based Ag/AgCl electrodes used for monitoring these signals also require skin preparation by cleansing and shaving to remove the dead skin and hair.

Moreover, the wet conventional Ag/AgCl electrode has some detriments such as skin irritation and difficulty in long-term monitoring because the gel dries out after a few hours resulting in an increase in the skin-electrode impedance. Generally, physiological parameters are monitored over an extended period during the patient's everyday life to obtain diagnostic quality signals. As the drying of the gel increases skin-electrode impedance, it results in low signal fidelity and quality. Additionally, noises, such as motion artifact and powerline interference, are introduced to the

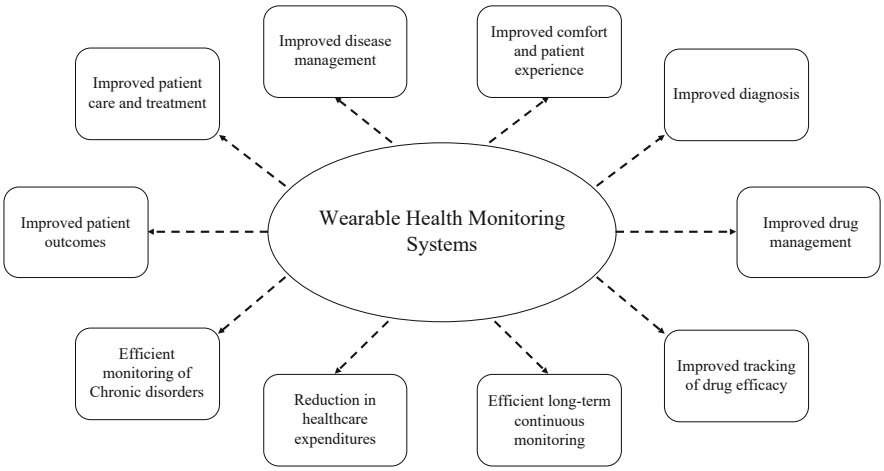

Fig. 4.1 Wearable health monitoring systems and outcomes

biopotential signals as the electrode floats over the electrolytic gel during monitoring. To overcome these drawbacks, dry electrodes can be used. These dry electrodes are held against the skin surface to establish contact with the skin without the need for electrolytic fluids or gels (Fig. 4.1).

4.2 Wearable Biosensors for Clinical Applications

Biopotentials are electrical signals generated by physiological processes. These signals are generated by the excitable cells and their electrochemical activity. The excitable cells produce an action potential, which acts as the primary source of biopotentials. Some of the commonly monitored biopotential signals are electrocardiogram (ECG), electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG). Biopotentials are electrical signals generated by the pathophysiological processes in the human body. Biopotentials are the information transfer between the living cells by assisting all the biochemical processes. Some of the biopotential signals of interest are listed in Table 4.1 (Neuman 1998; Malmivuo and Plonsey 1995; CThompson02, CC BY-SA 4.0 n.d.; Rai et al. 2013; Shyamkumar et al. 2014; Ramasamy and Varadan 2018).

4.3 Applications of Clinical Biosensors

Hung et al. have designed a wearable system to address the growing need for long-term patient-centered health monitoring for aging and elderly patients. The system consists of integrated textile electrocardiogram (ECG) electrodes, intelligent finger-ring photoplethysmogram (PPG) sensor, miniaturized optical fiber-based

Table 4.1 List of parameters and measurement modalities

Parameter	Measurement modality	Description
Electrocardiogram (ECG)	Surface electrodes	Electrical activity of the heart by measuring the potential difference between the given lead positions
Electromyogram (EMG)	Surface electrodes	Electrical activity of the skeletal muscles
Electrooculogram (EOG)	Surface electrodes	Electrical activity of the resting potential of the retina
Electroencephalogram (EEG)	Surface electrodes	The neurophysiological activity of the neurons in the brain and recording the electrical activity of the brain
Blood pressure (BP)	Arm cuff-based monitor	Force exerted by the flow of blood
Temperature (T)	Temperature probe (body) or skin temperature sensors (skin)	Body or skin temperature
Respiration/respiration rate (R)	Piezoelectric/ piezoresistive sensor, surface electrodes	Movements indicative of inspiration and expiration with respect to time
Oxygen saturation (SpO_2/SaO_2)	Pulse oximeter	The amount of oxygen in the blood
Skin condition and metabolites (SC/M)	Galvanic skin response sensors, surface electrodes	The electrical conductance of the skin is associated with the activity of the sweat glands
Heart sounds (HS)	Accelerometer, microphone, condenser, acoustic sensors	A record of heart sounds and murmurs
Activity (A)	Accelerometer, gyroscope, magnetometer, GPS	Measurement of acceleration, orientation, and location

temperature sensor, eye dynamics monitor, global positioning system (GPS) module, and wireless capability (Hung et al. 2012) (Fig. 4.2).

4.3.1 Neurological Disorder Monitoring

4.3.1.1 Parkinson's Disease (PD)

Many movement disorders can be caused by abnormal functioning of the central and peripheral nervous systems. Long-term monitoring of the gait has been cited as a valuable tool in assessing patients suffering from Parkinson's disease. Moore et al. (Moore et al. 2007) have developed such a system. They used an ankle-mounted wireless sensor to capture every stride over 10-h epochs. They have shared results from five patients suffering from PD and demonstrated the efficacy of long-term gait monitoring in the home environment.

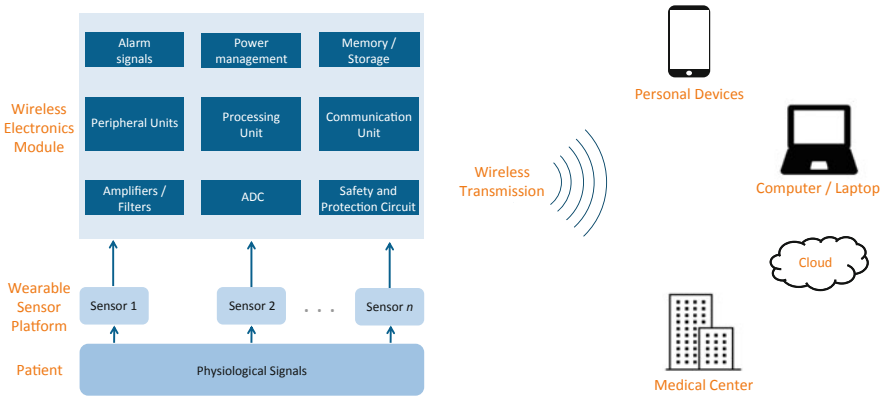

Fig. 4.2 Data flow in a typical biosensor system

Based on the hypothesis that acceleration measurements are reliable predictors of motor activity and can also be used as a classifier for normal and abnormal gait activity in patients suffering from PD, in 2004, Bonato et al. (Bonato et al. 2004) proposed nonlinear data mining approaches to detect the motor fluctuations associated with the manifestation of PD. They explored parameters like fractal estimates, approximate entropy values, and correlation dimension estimates and showed their association to tremors. For the collection of data for their study, accelerometer (ACC) and surface electromyographic (EMG) signals were recorded while patients were asked to perform a set of activities (sitting, finger-to-nose, tapping, sit-to-stand, walking, and stand-to-sit). They placed ACCs in the right and left upper arm, forearm, thigh, sternum, and right shin. In addition, they placed EMG sensors on the left and right biceps brachii, right erector spinae, right vastuslateralis, and right tibialis anterior. The EMG and ACC data were acquired with an ambulatory setup. Thus, they showed that data mining techniques coupled with wearable sensor deployments could be powerful tools to classify motor patterns of primary and secondary movement disorders in PD, such as tremor, rigidity, dyskinesia, akinesia, and dystonia in a manner that is both objective and automatic.

4.3.1.2 Tourette Syndrome

Tourette syndrome is a neurological disorder that exhibits as tics in motor and phonic function. The intensity of these tics may vary over time. Diagnosis and evaluation of this disorder require the practitioner to observe several signs, both visual and auditory. These challenges exist despite the definition of clear scoring procedures in the literature like the TS scale. In 2009, Bernabei et al. (Bernabei et al. 2010) analyzed a sample of 12 subjects diagnosed with TS. They used a 3D accelerometer, a wearable actigraph, and a Bluetooth© transmission module with a rechargeable Li-ion battery.

They post-processed the signal using nonlinear median filtering and an adaptive thresholding technique to differentiate abnormal tics from normal movements. They

demonstrated that with specific frequency and threshold criteria, a wearable device could differentiate between normal and abnormal tics with an overall sensitivity, specificity, and accuracy of (mean ± SD) 75 ± 10%, 70 ± 15%, and 74 ± 14%, respectively.

4.3.2 Neonatal Care

In neonatal care, wearable monitoring systems based on sensorized textiles are desired due to two reasons: (1) traditional sensors use adhesives that are usually harsh on the skin of newborn children and (2) traditional monitoring systems have several wires that impede parent's access to the newborn, which is known to be important for the development of newborn children. The wearable system for newborn children can be in the form of a pajama, as described by Andreoni et al. (Andreoni et al. 2011). They have conducted a comprehensive survey on the choice of yarn applied to sensitive pajamas. The yarn consisted of different proportions of silver. The quantity of silver in the yarn was optimized for conductivity. The pajamas were connected to a wireless transmission device. The system composed of these two components was compared against standard Ag-AgCl adhesive electrodes and was shown to give excellent and reliable electrocardiographic measurements.

4.3.3 Blood Flow Monitoring

Kuwabara et al. have designed and implemented a new appcessory for monitoring peripheral blood flow in daily life. An appcessory is a combination of a smart device app and an accessory.

Their motivation comes from the fact that blood flow in the peripheral arteries can give us a great deal of understanding into an individual's physical and mental state. Moreover, blood flow is traditionally measured using laser Doppler equipment that is of a desktop form factor at this time. Their design is small, lightweight, and wearable. The smart device app that they have designed offers real-time data visualization and supports cloud connectivity to provide advanced data processing capabilities. The accessory is connected to the intelligent device wirelessly through low-energy Bluetooth. Through their use of Bluetooth low-energy and intermittent signal processing algorithms, they were able to have appcessory operation times of more than 24 h (Kuwabara et al. 2014).

4.3.4 Sports Monitoring

Both the general population and athletics teams have greater access to wearable devices and sensors that provide performance and body state metrics. For example, functional movement sequences, workloads, and biometric markers can all be used to gather information during training and provide valuable feedback to athletes that

can reduce the risk of injury and improve their performance. Wearable devices used to observe movement can include pedometers, goniometers, accelerometers, gyroscopes, and global positioning satellite (GPS) devices. In addition to the movement measuring systems, physiological sensors measure heart rate, sleep quality, temperature sensors, and integrated sensors.

Li et al. have performed a comprehensive review of all sensor systems in research and development to monitor human performance. The ability to monitor high-acceleration movements is a significant advancement that gives us valuable information in training for competitive contact sports (Li et al. 2016).

4.3.5 Pregnancy Monitoring

In both developing and developed countries, maternal and infant health is a vital public health concern. Complication during pregnancy can lead to maternal and infant death and can also be associated with miscarriage, stillbirth, and preterm birth outcomes. Wearable technology has been shown to help in behavioral modifications and appropriate lifestyle choices that help in ensuring a healthy pregnancy and normal delivery. The challenges faced in research deal with the inference of physiological adaptations, lifestyle adaptations, and enabling longitudinal monitoring throughout pregnancy based on the sensor data collected by the wearable devices (Penders et al. 2015). The focus on using wearables to track lifestyles during pregnancy is on five attributes—physical activity, sleep, stress levels, diet, and weight management. Physical activity has mostly been captured using energy expenditure (EE) measurements, pedometer measurements, or through questionnaires. Studies have also combined these measures with physiological signal measurements but have not revealed conclusive predictors of activity (Berntsen et al. 2011). The findings conflict each other in overestimation and underestimation (Smith et al. 2012). Stress assessments are usually performed with wearables using extracted parameters like heart rate, heart rate variability (Shea et al. 2008), galvanic skin resistance, and respiration rate. The validation of these tools pertaining to their use for pregnant women is minimal at this time, but promising advancements have been made in studies involving the general population. Several factors during and immediately after pregnancy influence the sleep patterns of women, and some of these changes are detrimental. General discomfort, increased night-time urinary frequency, fetal movement, and fatigue during pregnancy will affect sleep patterns. Immediately after giving birth, the hormonal levels are restored to pre-pregnancy levels, and it may take up to 12 weeks to return to normal. Traditionally, sleep monitoring is performed using polysomnography (PSG), and these techniques are not suitable for at-home monitoring. The wearable solutions to this requirement have been in the form of actigraphy (Lee and Gay 2004), which gives information about the time of sleep but not the quality of sleep. Several activity trackers and wearables make assessments of sleep quality through measurement of ECG, EEG, and GSR.

4.4 Sensors and Materials

This report has thus far described the various implementations and deployments of wearable systems for health monitoring in different contexts, target individuals, and lifestyles. One of the critical components of a wearable system is the sensing element that performs the operation of transduction. Especially in the case of wearables, these sensing elements need to be lightweight, flexible, and consume as little power as possible, if not completely passive. This section describes the various advances in sensing technology categorized by the type of sensor and the measurand that it senses or measures.

4.4.1 Flexible Pressure Sensors

The design of flexible pressure sensors needs careful consideration for the functional material of the sensor. The choice of material with superior electronic properties and specificity in the transduction of external stimulus is key to designing an optimal sensor. Therefore, it is critical to developing appropriate functional materials for pressure sensing purposes. Especially for flexible pressure sensors, the combination of mechanical compliance, appropriate electrical performance, low temperature, and large-area processing is critical for creating flexible pressure sensors with desired qualities. The functional materials are most often chosen to transduce the pressure signals into changes in capacitance, piezoresistivity, or piezoelectricity. The most often used active materials are poly (dimethyl siloxane) (PDMS), rubrene, micro- and nanostructured polymers. Capacitance, piezoresistivity, and piezoelectricity are the most frequently used methods for signal transduction, while PDMS, rubrene, micro- and nanostructured polymers, polyvinylidene fluoride (PVDF) and poly [(vinylidenefluoride-co-trifluoroethylene] copolymers. A comprehensive survey of flexible pressure sensor structures and materials is presented in (Zang et al. 2015).

4.4.2 All Elastomeric Flexible Temperature Sensors for Body-Attachable Wearable Sensors

Motivation for the development of stretchable physical sensing devices comes from its potential applications in several deployments like human–machine interface, electronic skin, and personal health monitoring. For example, Trung et al. have reported a transparent and stretchable (TS) temperature sensor with a gated structure based on intrinsically transparent and stretchable materials to understand electrical properties and sensing mechanisms of sensing materials under applied stimuli. In their device, all of the layers of the device were intrinsically transparent and stretchable materials, which can be easily coated directly onto a transparent, stretchable substrate by using a simple spin-coating method and lamination techniques.

Composite materials formed by inserting conductive and temperature-responsive reduced graphene oxide (R-GO) nanosheets into an elastomeric polyurethane

(PU) matrix were served simultaneously as the temperature sensing layer. The temperature sensor exhibited stretching up to the strain of 70% and the impressively high sensitivity of ≈1.34% resistance change per °C. The responsivity of the device to temperature was nearly unchanged after 10,000 cycles of stretching at 30% strain. The TS-resistive and TS-gated devices were able to detect minute temperature changes as small as 0.2 °C and were highly responsive to the temperature of human skin. They further demonstrated that their sensor structure could detect temperatures on hot objects and also on human skin (Trung et al. 2016).

4.4.3 Potentiometric Sensor for Monitoring Wound pH

Guinovart et al. have demonstrated a pH potentiometric sensor incorporated in a bandage to measure the pH levels in a wound area. The potentiometric sensor used electro-polymerized polyaniline (PANi) for both the reference and the working electrode. The range of pH measured by the sensor was 5.5–8. They did not observe a significant carry-over effect. They were able to test the performance of the sensors in vitro using buffer solutions to emulate the composition of a wound (Guinovart et al. 2014).

Fiber-based sensor structures are highly desirable for wearable electronics because they are inherently light, flexible, and conformable. Textile-processing technologies that were effective at ambient conditions have been used extensively to fabricate many types of fibrous structures.

Recent advances in nanotechnology have facilitated the building of electronic devices directly on the surface of fibers or inside them. However, there are significant gaps in knowledge when it comes to the process of imparting electronic functions to porous, highly deformable, and three-dimensional fiber assemblies. Additionally, to incorporate wearable technologies, these structures need to have adequate resistance to wear from regular and daily usage (Zeng et al. 2014).

4.4.4 In Situ Perspiration Analysis

Human sweat is a rich source of physiological information. Since sweat can be sampled at the level of the skin, perspiration analysis is noninvasive. Analysis of perspiration requires the detection and estimation of several different analytes. Gao et al. have demonstrated a mechanically flexible and fully integrated sensor array for multiplexed in situ perspiration analysis. Their sensor has the ability to simultaneously measure several different types of analytes like glucose, lactate, sodium ions, and potassium ions. Furthermore, they have incorporated a temperature sensor to measure the skin temperature to calibrate the analyte sensors. They have presented a comprehensively integrated sensor system incorporating flexible sensing elements, signal processing elements on flexible substrates, and wireless transmission capabilities (Gao et al. 2016).

4.4.5 Electrochemical Sensors

Electrochemical, piezoelectric, and optical transducers have been used to detect various analytes in samples of body fluids. The high performance, portability, and sensitivity have made electrochemical sensors the predominant choice. However, most sensor systems require a blood sample from the patient, which makes them intrusive and causes discomfort and inconvenience. In addition, there are several application scenarios where continuous monitoring of analytes is required, for example, diabetes management, athletes requiring fitness level assessments, monitoring of drug treatments, and real-time monitoring of pathogens. In all of these scenarios, the use of an invasive sampling method of body fluids is not practical.

4.4.6 Saliva-Based

The area that has seen the most advancement in incorporating a saliva-based sensor is the partial dentures. There are several analytes of interest in the mouth, like pH and fluoride concentrations. There are several existing implementations of electrochemical sensors in partial dentures. However, they have practical limitations like temperature variations affecting the reading, replacing several teeth with the actual sensing devices, and possible leakage of internal solutions. Minamitani et al. (Minamitani et al. 2002) designed a temperature sensor-integrated liquid-free-iridium pH sensor to overcome these limitations. This sort of device can be miniaturized and worn by an individual. Ideally, a saliva-based sensor should follow the contour of the individual's mouth without causing any inconveniences. Recently, Mannoor et al. demonstrated a tattoo that can be placed on the enamel for continuous wireless monitoring of bacteria by bio-functionalizing antimicrobial peptides on graphene-modified silk tattoo substrates (Mannoor et al. 2012).

4.4.7 Tear-Based

Tears contain several biomolecules from various glands like lacrimal glands, surface epithelial cells of the eye, meibomian glands, blood, and goblet cells. Tears can even be used to measure glucose levels because of their high correlation with the levels in the blood. Strip-based substrates were used to fabricate the earliest form of ocular sensors. These devices are developed by first fabricating bare electrodes onto flexible or stretchable substrates using standard lithographic techniques. Low-cost thick film printing technology has also been used to develop high-fidelity ocular sensors. Then, fabrication methodologies, such as drop casting and polymer entrapment and direct mixing of biomolecules within inks, have been utilized to functionalize the electrode transducers with receptors. Strip-based ocular sensors have been developed for monitoring keratoconjunctivitis sicca (Ogasawara et al. 1996), transcutaneous oxygen (Iguchi et al. 2005), and glucose (Kagie et al. 2008). However, these sensors were made on semi-flexible substrates that could cause irritation of the eyes, which

in turn could cause the analytes to be diluted. These limitations are overcome with sensor designs that are accommodated in soft lenses.

A polyethylene terephthalate (PET) contact lens with amperometric glucose sensors, which were bio-functionalized activated and deactivated glucose oxidase and built-in wireless communication and charging, was demonstrated by Yao et al. (Yao et al. 2012).

Real-life implementations are yet to be demonstrated. Power sources for such sensors are still a research challenge. Several of the analytes present in tears are potential candidates for energy sources.

4.4.8 Fabric/Flexible Plastic-Based Sensors

Fabrics are an excellent substrate for the fabrication of wearable sensors as they are worn close to the body, and materials such as wool, cotton, and nylon have highly conducive chemical properties for the incorporation of chemical sensors. For example, a wearable potentiometric sodium sensor for cystic fibrosis monitoring was demonstrated by Schazmann et al. (Schazmann et al. 2010). A conductometric sensor that is fabric-based for measuring the level of hydration was demonstrated by Coyle et al. (Coyle et al. 2009). Wearable potentiometric sensors for several other analytes like pH, NH^{4+}, and K^{+}, and Cl^{-} have been demonstrated by other research groups, which have fabricated them on carbon nanotube-modified fibers (Guinovart et al. 2013a) and by screen printing technology (Gonzalo-Ruiz et al. 2009). Other sensors have been reported to measure transcutaneous oxygen (Mitsubayashi et al. 2003) and humidity (Zampetti et al. 2009).

4.4.9 Epidermal-Based Sensors

Wearable sensor deployments often need conformal contact between sensing surface and the skin. Clothing and fabric are not always suitable for this requirement as they are often in contact with all parts of the skin. Moreover, chemical sensing is most efficient if sensors are placed directly on the skin surface and not on fabric that loosely contacts the skin. Thus, the fabrication protocols for temporary tattoo-based electrochemical sensors were formulated (Windmiller et al. 2012). Incorporating commercially available temporary tattoo papers with functionalized receptor and reagent layers induces selectivity in sensing-specific analytes like acidity, ammonia (Guinovart et al. 2013b), sweat lactate (Khodagholy et al. 2012), alcohol, and sodium levels in perspiration.

4.4.10 Skin Interstitial Fluid-Based Sensors

Vital information can be acquired from interstitial skin fluids (ISFs). One of the key areas of focus has been glucose monitoring (Vashist 2012). Iontophoresis-based

electrochemical glucose sensing is the most widely recognized technique (Tierney et al. 2001). Several challenges have impeded the commercial success of products that utilize these techniques, like complaints of skin irritation. These types of challenges will need to be overcome with research in flexible sensing materials (Bandodkar and Wang 2014).

4.5 Conclusion

Wearable textile-based nano-biosensor systems with the mobile platform are a unique class of unobtrusive, continuous health monitoring with significant benefits for neurological and cardiovascular patients or high-risk patients. The bioelectromagnetic principles of origination and propagation of bioelectric signals (EEG, EOG, EMG, ECG) show that measured electric potential represents the cumulative electrical activity of the sources of these signals, i.e., neurons of the brain tissue and myocytes of heart tissue. The dry electrodes can be used for long-term monitoring because they do not face the problem of drying of gel, and they are reusable in the form of a wearable garment. The textile-based wearable nano-biosensor systems discussed in this article can measure neurological signals and identify anomalies to diagnose targeted neurological and cardiovascular disorders. These disorders range from a chronic condition to safety to rehabilitation and improved quality of life. The bioelectromagnetism principles of neural and cardiac bioelectric signals and the performance of textile-based nano–bio sensors provides a unique perspective to the potential for the development of novel wearable systems that harness the potential of textile-based nano–bio sensors and wireless platform for understanding the neural and cardiac function in and out of hospital setting in unprecedented detail. The sensor systems can be used to diagnose and treat neurological disorders such as autistic spectrum disorder, traumatic brain injury (TBI), and neuroprosthesis. In addition, they can be used for highly specialized cardiac monitoring, such as vectorcardiography (VCG), impedance cardiography (ICG) or tomography, tumor detection, and prevention from sudden cardiac death by detection of T-wave alternans. For the patients and medical healthcare professionals to adapt to wearable medical device technology, the devices and systems must meet challenges in form factor, device longevity, and wireless communication requirements specific to each application. Patterns of design for wearable systems architecture is an expanding body of knowledge and is becoming a viable alternative to conventional health monitoring technology.

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5 Development and Implementation of Portable Biosensors in Microfluidic Point-of-Care Devices for Pathogen Detection

Natish Kumar, Monika Kumari, and Ravi Kumar Arun

Abstract

Massive pathological outbreaks have frequently impacted global risk, emphasizing the necessity for on-site sample analysis techniques that are rapid, reliable, and responsive to accelerate diagnoses and enable early action. The point-of-care examination makes it easier to quickly identify analytes close to patients, enabling improved identification, control, and treatment of the pathologic infection. It also provides fast medical decisions as the diseases can be diagnosed early, leading to better clinical outcomes for patients. The material used to manufacture the device is essential in microfluidic technology. Inorganic, polymeric, hydrogels, and paper are the four broad categories of materials utilized in microfluidic chips. Soft lithography, photolithography, conventional machining, and laser ablation are some of the technologies used to fabricate microfluidic devices. Electrochemical, electro-chemiluminescent, colorimetric, and enzyme-linked immunosorbent assay is the most common sensing technology integrated with microfluidics to detect microbes and biological analytes. This chapter will investigate how microfluidic technology has been utilized to develop portable biosensors and the current trends of these nanosensors for point-of-care diagnosis of various pathogenic diseases.

Keywords

Microfluidic · Biosensor · Point-of-care detection · Lab-on-a-chip

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_5

5.1 Introduction

5.1.1 Microfluidics: Basic Concept

The definition of microfluidics depends upon two major characteristics: one of the characteristics is channel size, which has to be small in micrometer scale, and the other characteristic is the handling of volumes in nano-liter of liquids by microchannels for (10–100 μm) liquid flow, integrated with the other components such as microscale inlet valves, a specific system for pressure controller micro-pumps, and miniaturized outlet sinks along with various instruments for the study of liquids (Whitesides 2006; Park et al. 2011; Sin et al. 2013). The central parameter for describing the efficiency of fluids is the Reynolds number (Re), which is mainly influenced by the inert forces of viscous ratio. $Re > 10^3$ usually denotes systems for a macroscopic platform, whereas $Re < 10^2$ represents systems for a microfluidic platform. In microfluidic devices, the low Re number denotes laminar flow or nonturbulent flow, which is a key and common function of miniaturization of devices. In a nonturbulent or laminar flow system, diffusion at the interface between fluids is responsible for mixing of fluids. The diffusion time is reduced in microfluidics due to the small sizes, and the mixing process, which is diffusion-based, has now become an effective proposition to the mechanical parts. Based on the parameters mentioned above, the next generation of detection methods are being developed that reduce the individual steps such as sample preparation, reagent handling, bioreaction, and detection of biological analyte to a single-step procedure in a single platform based upon microfluidics (Nasseri et al. 2018; Demello 2006).

5.1.2 History

Researchers have been interested in the behavior of fluids confined to a tiny diameter since Hippocrates (400 BC), Galen (200 AD), and Theophilus (700 AD). The goal of this work was to decipher the functioning of the human body. The origin of microfluidic device fabrication with micromechanics is usually linked to Stanford University's position on gas chromatography and IBM's development of inkjet printer nozzles in the late 1960s (Castillo-León 2015) (Fig. 5.1).

The first commercially accessible continuous types of laser printers were developed by IBM researchers in 1976. Professor George Whitesides, one of the founding members of the microfluidics and lab-on-a-chip communities, wrote an essay in 1998 on the fast prototyping of microfluidic devices using polydimethylsiloxane (PDMS) (Verma et al. 2015). Due to its variable device shape, simple instrumentation, and ease of integration with other technologies, this novel technique greatly influences chemical and enzymatic processes, DNA-based applications, immunoassays, clinical diagnostics, and cell-based application proteomics (Choi et al. 2012). Studies involving cells and organs on chips provide an excellent example of the influence of 3D imprinting technology (Castillo-León 2015). Through the sophisticated sensor, microfluidic technology has taken the lead in

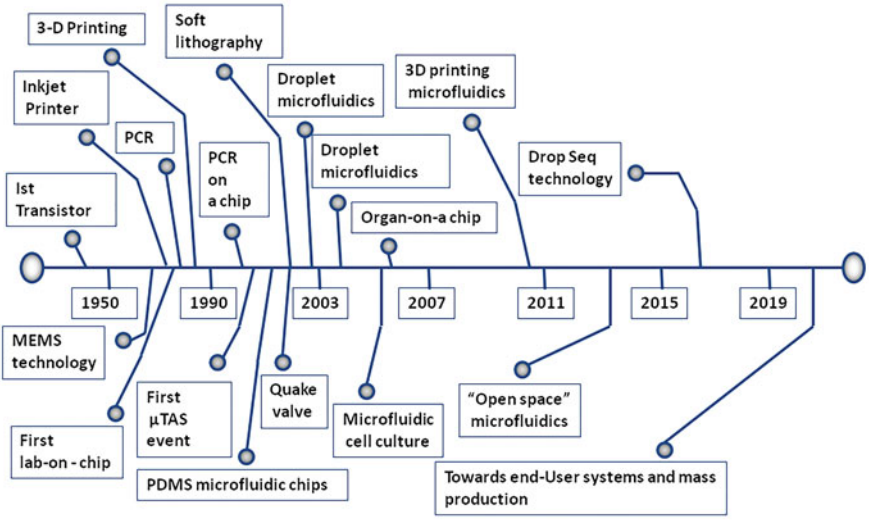

Fig. 5.1 Historical timeline of development in microfluidics technology

creating point-of-care diagnostics. In medicine, microfluidic chips shorten the time between diagnosis and therapeutic therapy, which is critical for patient safety (Chiu et al. 2017).

5.1.3 Role of Microfluidics in Biological Applications

The early diagnosis and surveillance of pathological infectious diseases are important for optimizing precise treatment, reducing mortality rate, and improving overall cost-effectiveness in the health sector. More precise and rapid diagnostic technologies are critical in the clinical diagnosis of pathogenic microorganisms such as viruses, prokaryotic bacteria, parasites, eukaryotic fungi, protozoa, and prions. Infectious diseases, for example, an acquired immunodeficiency syndrome (AIDS), Zika virus, malaria, tuberculosis (TB), and Ebola, kill millions of people per year (Na et al. 2018; Govindaraju et al. 2019). Microfluidics-based sensors have gained a lot of traction in the production of miniaturized biosensor devices for medical health purposes to address the existing issues. Advanced infrastructure, time-consuming methods, and expensive reagents are incompatible with resource-constrained settings in current diagnostic systems. While scalable material-based and paper platform technologies provide new ways to create point-of-care diagnostic assays for several medical applications, they face technological challenges when it comes to integrating with different detection systems. Microfluidic diagnostic portable devices have grown in popularity due to no need for expert operators and their rapid detection, high accuracy, responsive on-site clinical sample detection technique, ease of use, and disposable nature (Shafiee et al. 2015).

5.1.4 Point-of-Care (POC) Devices

Over the past two decades, modern generation biosensors have been gradually deployed for tracking and detecting biological analytes. Microfluidic systems' small size, high performance, and portability, combined with the ability to optically track and calculate the chips, have allowed the diagnostic approach that is integrated with significant advantages over traditional approaches**.** The use of miniaturized microfluidic platforms has facilitated developments in biochemistry and biomedical, medicine (biomedical devices), and chemistry (analytical), and biology. Microfluidic devices are used in many areas, such as pathogen and disease optical detection and surface chemistry science. Rapid detection of various diseases using convenient and robust "lab-on-a-chip" (LOC) devices have now become increasingly significant, especially in resource-poor areas (Reyes et al. 2002). Since they can offer critical input on health-related conditions to healthcare professionals and outpatients in remote environments and in real-time fashion, LOC diagnostic platforms are also considered a substitute to a centralized laboratory. LOC techniques may be used in combination with traditional diagnostic methods, specifically in the case of infectious diseases (Dittrich et al. 2006). LOC systems are mainly based on DNA, proteins, and cells that can assist physicians in diagnosing a broad range of pathogenic diseases and therefore selecting the appropriate treatment because of their quick, rapid, and highly precise detection. Microfluidic diagnostic technologies are evolving all the time in order to improve healthcare systems. In biological and therapeutic methods, these techniques utilize different ways of optical detection to classify and measure complex biomolecules. Microfluidic-based biosensors are thoroughly studied along with various detection techniques, depending on specimens (analytes), in the pursuit of fabricating on-demand (POC) devices. For manufacturing POC for clinical diagnostics, the WHO has set up certain standards that these devices should be inexpensive, specific, sensitive, rapid and robust, accurate, user-friendly, apparatus-free, and deliverable to end-users (ASSURED). Instant results and quick recording of disease status are necessary to build up diagnostic devices (Souf 2016; Nguyen et al. 2017). Nanoparticles such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), magnetic nanoparticles, carbon nanoparticles, liposomes, and QDs have now been used combinedly with enzymes and other molecules to enhance the sensitivity of paper-based analytic devices (López-Marzo and Merkoçi 2016). As a detection test for viral pathogens such as H1N1 virus infection, researchers build up an RNA extraction technique, which is paper-based and would allow an in-situ amplification reaction from RNA. This amplification reaction can be done exactly after the RNA extraction that takes place on the paper extraction matrix and also eliminates the off-chip step for the elution process. Reverse transcription loop-mediated amplification assay (RT-LAMP) is fast and isothermal and does not involve a thermal cycler. Furthermore, the RT-LAMP was intended to include probes that facilitate direct downstream visual analysis on an immune chromatographic or lateral flow detection (LFD) test strip, similar to those platforms that are used in common pregnancy tests, obviating the need for detection equipment (Rodriguez et al. 2015).

5.1.5 Importance of Microfluidics-Based Sensors

Through microfluidics application, a large number of bio-chemical analytes, biomarkers, small molecules (DNA, RNA, and proteins), cells, and pathogens can be detected using different sensing analytic technologies, including chemiluminescent, electrochemical, and colorimetric methods, which have been developed for quantitative detection of the virus (Xing et al. 2020). In comparison to lab setups, the development, ease of production, and marketing of microfluidics-based devices are much simpler than those of other approaches. Furthermore, when introduced to the market, it requires less space and investment; thus, it is useful not only in the scientific or technical area but also in everyday life (Zhao and van den Berg 2008). Several low-resource settings lack the necessary equipment and infrastructure to conduct these diagnostic tests and analyses, forcing innovative workarounds to address this mostly unresolved issue in densely populated countries. Many diagnostic tests with built-in analytic capabilities are being developed using microfluidic technologies appropriate for low economical regions. Engineers and researchers developed innovative microchip manufacturing technologies that would be difficult or impossible to achieve using macroscale approaches. These microchips have found therapeutic applications since they need minimal biofluids for sample processing and can generally be performed quickly and efficiently (Sackmann et al. 2014). On a microchip, a new area of microfluidic devices has recently evolved to mimic in vivo organ function. This innovative "organ-on-a-chip" device integrates many well-known microfluidic components into a single in vitro device, helping developers to more accurately replicate in vivo operations. Scientists utilize microfluidics to solve this problem by developing potentially transformative ways to bring down the cost of prescribed drug development (Sackmann et al. 2014; Low et al. 2021).

5.2 Materials for Microfluidic Device Fabrication

In microfluidic technologies, the material used to fabricate the device is extremely important. A microfluidic chip is a molded or etched series of microchannels. Output holes are drilled into the chip to link the microchannel network to the macro-environment. Microfluidic chips, which also have valves for active flow control, can effectively accommodate fluids in a variety of applications. The materials used in microfluidic chips must be sufficient and possess the required properties**.** Chip materials have traditionally grown to reflect two significant microfluidic technology trends: effective microscale research platforms and low cost manageable (portable) analyses. The four broad categories of materials used in microfluidic chips are inorganic, polymeric, hydrogels, and paper.

5.2.1 Inorganic Material

5.2.1.1 Silicon

The first substance used in microfluidics was silicon, but it was soon replaced by glasses and then polymers. Silicon was initially chosen because of its high thermo-conductivity, ease of metal deposition, resistance to organic solvents, and stable electro-osmotic mobility. However, due to its stiffness, this material is difficult to treat, making it difficult to produce active microfluidic components such as valves and pumps. Dangerous materials used in the welding process necessitate the use of safety facilities as well. Both of these drawbacks, along with the high cost of silicon, make it an unappealing substrate for constructing microfluidic chips (Ren et al. 2013; Nge et al. 2013).

5.2.1.2 Glass

Following the preliminary reliance on silicon, the glass was chosen as a substrate for the construction of microfluidic chips. Prior to its incorporation into the microfluidic sector, quartz or glass capillaries for capillary electrophoresis (CE) and gas chromatography (GC) microchannels were used. For detection of glutathione from cellular supernatant, researchers used electrophoresis and chemiluminescence in every glass system (Zhao et al. 2009). Glass is an amorphous medium that is both optically translucent and electrically insulating. Standard photolithography or wet/dry etching methods are commonly used to process this material. The thermostability and solvent compatibility of glass/silicon systems contribute to additional essential applications (Mu et al. 2009). Glass is compliant with biological samples; it is also a gas-tight material with low nonspecific adsorption (Mellors et al. 2008).

5.2.1.3 Ceramic

Low-temperature cofired ceramic is usually used in ceramic microfluidic applications. Ceramic cofired at a low temperature (LTCC). This ceramic is made of aluminum oxide and prepared by laminate sheets that are assembled, patterned, and then heated at high temperatures. Because of its laminar structure, LTCC can be shaped into complex three-dimensional devices, and Fakunle and Fritsch demonstrated low nonspecific adsorption in an LTCC system using an enzyme-linked immunosorbent assay (ELISA) (Fakunle and Fritsch 2010). The electrical and mechanical properties of LTCC are outstanding, and it has high efficiency. LTCC technology is used to create a sophisticated micro-electromechanical system and micro-opto electromechanical system packages that incorporate electronic measuring, power, and signal conditioning circuits. Furthermore, electrical, optical, gas, and fluidic networks are implemented in a single box (Ren et al. 2013; Nge et al. 2013).

5.2.2 Polymers

Chips that are polymer-based were launched after several years of silicon/glass chips. A broad range of polymers are available, and this allows for considerable

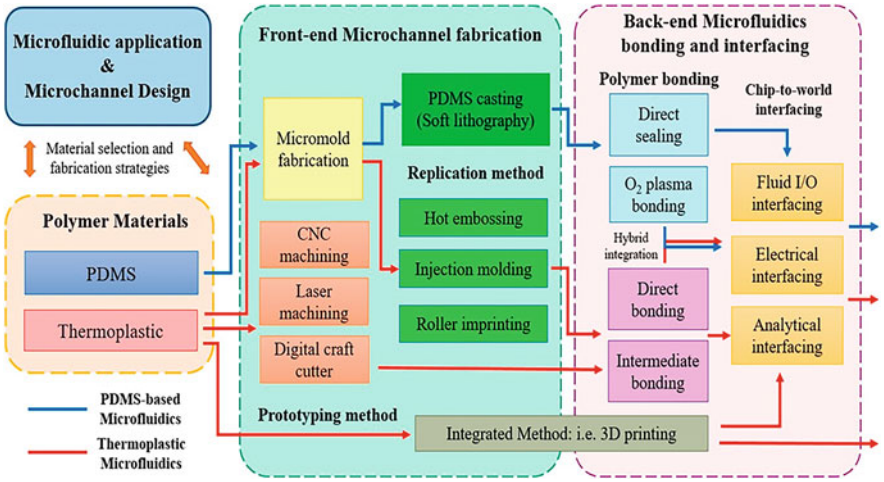

Fig. 5.2 Diagram of the fabrication process for polymer microfluidics. The PDMS-based microfluidics fabrication process is shown in blue, while the thermoplastic microfluidics fabrication procedure is shown in red. Figure source, reprinted with permission: ref. (Tsao 2016)

versatility in selecting a suitable material with unique properties. Polymers, which are less expensive and easier to produce than inorganic materials, have now become the most commonly used microchip materials. Polymers are divided into three categories depending on their physical properties: elastomers, thermosets, and thermoplastics (Fig. 5.2).

5.2.2.1 Elastomers

Elastomers are made up of cross-linked chains of polymers that are usually inter-twist; they can expand or constrict when subjected to extrinsic force and revert to their native form when the extrinsic force is removed. Polydimethylsiloxane (PDMS) is a broadly used elastomer in microfluidics (PDMS). Elastomers allow cost-effective and rapid prototyping as well as high-density valve integration on a microchip, enabling dynamic and parallel fluid processing as well as in-channel cell culture. Plastics are both simple and cheap to microfabricate, making them a suitable replacement for elastomers (McDonald and Whitesides 2002; Stroock and Whitesides 2003).

5.2.2.2 Thermoplastics

Thermoplastics are built up by densely cross-linked polymers, which are easily moldable when heated to their glass transition temperature but preserve their native form when they are cooled. These types of materials are usually more amenable and robust to micro-machining techniques, optically transparent, resistant toward small molecules, and more rigid than elastomers' permeation. Polycarbonate (PC), poly (ethylene glycol) diacrylate (PEGDA), poly(methyl methacrylate) (PMMA),

polyethylene terephthalate (PET), and polystyrene (PS) are among the thermoplastics that can be used to manufacture microfluidics-based products on a large scale (Nge et al. 2013).

5.2.3 Hydrogels

Hydrogels are formed by hydrophilic polymer chains that are organized in 3D networks, which span in an aqueous medium, and can contain up to 99% water. Hydrogels are extremely porous and have manageable pore sizes, which allow small molecules along with bioparticles to pass through. Hydrogels are useful for encapsulating the cells for 3D culture due to their aqueous nature and high permeability. Chemists may make use of hydrogels as an extremely porous structural medium, which facilitates molecules to diffuse without producing bulk fluid flows. Microfluidics has become particularly concerned in biological or medical research and bio-mimicking because of its rapid development (Ghaemmaghami et al. 2012).

5.2.4 Paper

For paper-based chip fabrication, a biosensor is an extremely cheap and user-friendly material. Clinical diagnosis, environmental monitoring, and food safety surveillance are among promising applications of microfluidic-based biosensors discussed. Paper-based sensors have gradually increased because of their broad availability, nature of hydrophilicity, and affordability. Paper-based biosensors are generally favored due to their high surface-to-volume ratio and lesser amount of volume requirement; these are the major reasons particularly observed in the development of the paper-based sensor (Wang et al. 2012). Paper is a highly porous cellulose matrix that is good at wicking liquids. As some areas of a paper are hydrophobically modified, the capillary effect specifically guides the aqueous solution added to the paper through the hydrophilic zone (Martinez et al. 2010). The fact that paper has a high surface-to-volume ratio, is structurally porous, and has a low-volume constraint, these are the primary explanation for the privilege in the construction of paper-based sensors (Wang et al. 2012). In the sensor production, the paper is chosen on the basis of its fabrication steps involved in designing the device as well as its application in specific areas. In the field research, different paper-based devices such as filter papers are primarily used in microfluidic platforms to make a device and sensor creation. A hydrophobic nitrocellulose membrane, on the other hand, is appropriate for nonbinding biomolecules, DNA, proteins, and other molecules (Liana et al. 2012).

Commonly utilized technologies for fabricating paper-based biosensing components include photolithography, cutting, laser, paper origami, and wax printing, all based on 1D, 2D, and 3D spatial and surface changes. Sealing the pores, cutting, flexographic printing, wax patterning, shaping, inkjet printing, and alkyl

ketene dimer printing for integrating the 2D features are the essential development procedures (Mahato et al. 2020).

5.3 Fabrication Technique for Microfluidics Devices

Microfluidics is rapidly growing in a research field that primarily emphasizes manipulation of small volume of fluid on the microscale level, and it is recognized most commonly by devices with critical dimensions of less than 1 mm. As the field is continuously growing, there are several different methods that have emerged for channel fabrications with the required dimensions. Different methods for microfluidic device fabrication include photolithography, soft lithography, conventional machining, and laser ablation (Fiorini and Chiu 2005) (Table 5.1).

5.3.1 Soft Lithography

In microfluidics field, there is remarkable work performed using soft lithography, which was introduced by Whitesides in 1998. Particularly, polydimethylsiloxane (PDMS) has been well documented in the soft lithography method. Soft lithography has facilitated a low-expertise way toward micro/nanofabrication, and it also plays an essential role in microfluidics, ranging from simple channel fabrication to the design of micropatterns onto a surface or within a microfluidic channel (Xia and Whitesides 1998; Kim et al. 2008).

The soft lithography protocol offers an overview of the methods, which primarily include printing, molding, and design using the stamp as an elastomeric. Soft lithography also gives a chance to make a structure with well-defined curves in a three-dimensional structure and tolerance of materials with a wide variety and generates controllable surface chemistries in a well-defined manner, which is usually companionable with medical/biological applications. Soft lithography is also experimentally convenient with lesser cost and has come up as a technology that gives access for a number of useful applications, including microfluidics, lab-on-a-chip for pathogen detection, cell biology, micro-electromechanical systems, and flexible electronics/photonics (Qin et al. 2010). Soft lithography is the collective name that consists of recently developed fabrication techniques for micro- and nano-structures. They have several applications, especially in biological sciences (Nur and Willander 2019). In the detection of pathogens, there are several applications of microfluidic biochips. There are several novel types of microfluidic systems, and new techniques that can be used for pathogen detection like viruses (e.g., HVB, HIV, ZIKA) have also been covered. Moreover, next-generation techniques relying on high sensitivity, specificity, and lower consumption of expensive reagents suggest that fast result generation can be achieved via optical-based bacterial cell detection. The introduction of smartphones in observation has replaced microscope-based observation, resulting in significantly improved detection of cell and other analytes and also

Table 5.1 Recent studies on microfluidics-based sensing technologies in pathogen detection

Substrate	Name of pathogen	Sensing technique integrated with microfludics	Analytical performances	References
PDMS	*Plasmodium vivax*	Electrochemical detection	The limit of detection of ~40 *vivax* infected RBCs (*Pv*-iRBCs)/10 μL blood sample in 5 min	Singh et al. (2021)
Silicon base and Pyer glass cover based channel	Detection of viruses	ELISA based	22 ng mL^{-1}; the limit of detection time was shortened from >3.25 to <30 min	Liu et al. (2005)
Paper, lateral flow test strip	Influenza (H1N1)	RT-LAMP	Detection limit of viral load of 106 copies/mL, contributing a tenfold enhancement over current prompt immune-assays	Rodriguez et al. (2015)
Cellulose paper	Human immunodeficiency virus-1	Electric and optical sensing	Can detect multiple biotargets selectively, with sensitivity and repeatability	Shafiee et al. (2015)
Lateral flow test strip	Human immunodeficiency virus-1	Nucleic acid lateral flow assay	LOD is 0.1 nM	Hu et al. (2013)
Silicon chip	Zika, Chikungunya, and dengue viruses	RT-LAMP	Clinically relevant sensitivity; detection of Zika virus as low as 1.56×10^5 PFU/mL from whole blood, low reagent consumption	Ganguli et al. (2017)

Transparent indium–tin–oxide (ITO) coated glass	Rubella virus	Electrochemical immunoassay	High sensitivity	Rackus et al. (2015)
Polycarbonate (PC) plate	HIV-1	Sandwich immunoassay	Low-cost, simple, and efficient operation, limits of detection (LODs) of 0.17 and 0.11 ng/mL for p24 antigen	Li et al. (2019)
Silicon, PDMS	*S. typhimurium*, *E. coli*	*Fluorescence* immunoassays	The detection limit of the sensor was 103 CFU/mL *Salmonella*	Kim et al. (2015)
Silicon wafer, a spin coater, UV aligner, PDMS, a photoresist (SU-82050)	Airborne pathogenic	ATP-bioluminescence	System can determine the existence of airborne microbes within 10 min	Lee et al. (2008)
Gold nanoparticles (AuNPs) attached on 1,6-hexanedithiol (HDT)	*Aspergillus fumigatus*	Electrochemical biosensor	In standard buffer and real sample, the biosensor detects glip-T with an extraordinary detection limit of $0.32 \pm 0.01 \times 10^{-14}$ M and $0.81 \pm 0.01 \times 10^{-14}$ M, respectively	Bhatnagar et al. (2018)

facilitating simplistic data processing, which helps in easy transfer of data for presentation purposes (Nasseri et al. 2018).

5.3.2 Photolithography

Microfabrication in microfluidics domain photolithography is one of the easiest and most essential methods used to design precise patterns in the material. In the photolithography system, a prototype or shape can be etched with critical exposure of a light-sensitive polymer to ultra-violet light (Ma et al. 2010). Photolithography has been used as a major method in the fabrication of microfluidic devices. It consists of exposing a substrate that is coated with photoresist to light in a manner that it inclusively developed regions that can be protected from (or subjected to) following fabrication processes such as deposition or etching (Lin et al. 2002) as the most obtainable light source in nature is sunlight, which consists of various types of light across a spectrum ranging from infrared, through visible light, to UV. At the ground level, UV-radiation light represents around 5% of solar energy, and the spectrum of radiation lies around 290 and 400 nm. Eventually, radiation is mostly used as a light source for the fabrication (Etzel and Balk 1999). One of the research articles explains FLASH (fast lithographic activation of sheets), which describes an instant method for laboratory prototyping of microfluidic devices in paper. Nowadays, paper-based microfluidic devices are growing as a new technology for various applications in diagnostics for the rising world, as simplicity and low cost are vital in the application in the biological field, specifically the diagnosis of pathogens (Martinez et al. 2008).

5.3.3 Wax Screen Printing

In the microfluidics segment, the fabrication is well established by using screen printing for the fabrication of chemical sensors and biosensors because there are various benefits such as versatility, miniaturization, low cost, and the opportunity of mass production (Renedo et al. 2007). There are numerous different forms of printing surfaces that can be used, such as ceramic, glass, cotton, and paper or similar fabrics. The type of ink used in the screen printing process is also determined by the printing surface and the intended usage. Characteristically, liquid inks and dyes are the printing materials. In one of the research, the printing material was used as a solid wax for screen-printing hydrophobic barriers on paper (wax screen printing method) (Tudorache and Bala 2007). Wax is environmentally friendly; moreover, it is extremely easy and cheap to attain than photoresist or PDMS. The fabrication method is well organized such that the process nowadays is accomplished without the use of a UV lamp, clean room, organic solvents, or sophisticated instrumentation. From previous reports, wax printing needs a wax printer ($2500 US), but recent progress in printing screen methods has made them inexpensive and broadly available around the world (Carrilho et al. 2009).

5.3.4 Laser Ablation

In the fabrication method, the laser ablation for machining the microflow channel has various advantages, mainly including trouble-free, rapid, and one-time ablation to complete the fabrication process. This technique is widely used, the method to machine main polymer materials is used, and the glasses can use the microflow channel on the surface. The glass-based materials are generally used because of their excellent surface stability, solvent compatibility, and optical properties due to the straightforward and well-understood fabrication system (Giridhar et al. 2004; Cheng et al. 2005; Li et al. 2011). Characteristically, in previous research, laser ablation is particularly referred to as the technique of ablating and a microflow channel machining on the surface of a polymer material by using a carbon dioxide laser with a wavelength of 10.6 μm (Nieto et al. 2010). There are various micro-machining processes for constructing microfluidic devices utilizing glass as a material that have been extensively established; the choice is based on how the materials will be handled, as well as the form and size of the key features. Hot embossing, injection molding, and further thermo-forming method provide high throughput and cost, but they are ineffective in the case of glass (Becker et al. 2000). Fabrication method for microchannels on glass by laser-ablation method has been explored and reported using carbon dioxide, UV, and ultra-short pulse lasers (Nieto et al. 2010; Stjernström and Roeraade 1998; Sohn et al. 2005; Flores-Arias et al. 2009). In the case of transparent materials, in the visible spectral range, laser ablation should preferably be executed with ultraviolet radiation since the linear optical absorption is in this wavelength range. Altogether, the laser ablation has provided a benchmark for machining channels of microfluidics devices.

5.4 Microfluidics-Based Sensing Technologies in Pathogen Detection

Pathogenic microorganisms include any microorganisms that have the capability of causing human or animal diseases, including viruses, bacteria, fungi, protozoa, helminths, etc. They can be easily transferrable from one host to another by air, body fluids, food, water, etc., causing national and international panic and economic losses (Yu et al. 2017a). There is a need for faster, portable diagnostic methods that have a more accurate result. The conventional identification method of pathogenic microorganisms includes large cell numbers of a pure cell culture, with time and labor consuming enrichment and pre-selection steps. For example, the developed world standards for target pathogen diagnosis, including culture, enzyme immunoassay, and polymerase chain reaction (PCR), often take between 2 to 4 days. Moreover, since most centralized laboratories are limited to large cities, nowadays near-patient testing using point-of-care (POC) devices has become increasingly important. Therefore, robust and portable diagnostic devices are capable of providing quick information on pathogens that is mainly helpful in reducing rates of mortality, hospitalization, and timely isolation if the pathogens are infectious.

Although, in the past two decades, many different biosensors have been developed, there is still a need for miniaturized, low-cost, disposable biosensors with the capability of rapid detection and precise identification of an extensive range of pathogens (Lazcka et al. 2007). For pathogen identification, microfluidic systems have a medium for RT-PCR, RT-LAMP, nested-PCR, nucleic acid hybridization, ELISA, fluorescence-based assays, sample preparation multiplexer (SPM), and CRISPR. Microfluidic devices coated with specific antibodies for capturing pathogens can be used to detect pathogens present in a solution. Using H1N1 virus as a model, researchers have developed a microfluidic chip that detects RNA-based viruses from throat swab samples (Ferguson et al. 2011). Microfluidics-based biochemical analysis enables quick detection of pathogenic microorganisms. With the help of the mass spectrometry technique, which can illuminate the molecular structure and molecular weight of analytes, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been wildly used for bacterial identification since the 1990s (Zhang et al. 2018). Moreover, a simple and real-time paper microfluidic assay coupled with smartphone detection has been developed for detecting ZIKA from complex sample matrices through RT-LAMP (Ferguson et al. 2011). Using normal electrochemical methods, the biosensor was used to detect ZIKA by calculating variations in the electrical signal with changing virus concentrations in buffer and serum (Tancharoen et al. 2018). In a study, affinity-based biosensing mechanisms were developed with Quantum dots (QDs) examined in the domain of the microfluidic approach. For example, the combination of microfluidic technology and QD-based affinity biosensors is presented in order to build a stronger technical platform for COVID-19 virus diagnostics. Microfluidic devices offer a wide range of methods for detection of RNA viruses such as H1N1, H3N2, H9N2, Hendra, and influenza B viruses. These accurate methods of detecting RNA viruses might also have the potential for detecting the novel coronavirus that has caused a global issue of Covid-19 (Park et al. 2019). Microfluidics and lab-on-a-chip technologies have been fascinating for the miniaturization and integration of conventional laborious equipment. Various functions can be realized in small sensors, and microfluidic channels can be used to combine them into a single device. Many attempts have been made in this direction to miniaturize and integrate different functions for the identification of food-borne pathogens using PCR (Tancharoen et al. 2018) (Fig. 5.3).

5.4.1 Chemiluminescent Assay

Chemiluminescence immunoassay is an innovative approach focused on radiation immunity analysis and enzyme-linked immunoassay that is nonradioactive, free of carcinogenic compounds and does not pollute the atmosphere or humans. It is very useful in the early detection of many diseases because of its quick action, high sensitivity, and consistent outcomes (Nasseri et al. 2018). Chemiluminescent assay, combined with microfluidic-based apparatus, has been made for quick and easy-to-use recognition of antibodies and proteins against various pathogens. Antibody

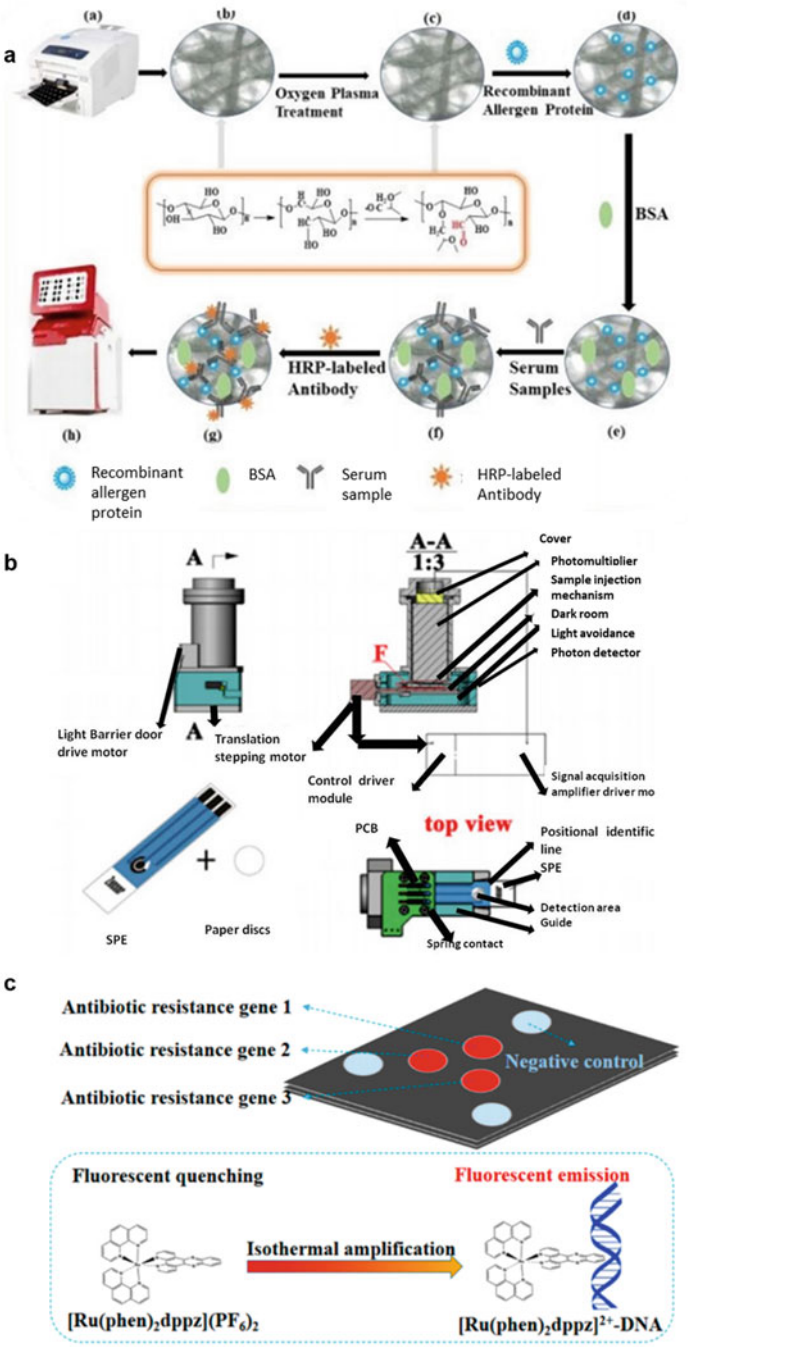

Fig. 5.3 Detection techniques and recent applications of μPADs. (**a**) Chemiluminescent detection of the paper-based immunoassay using horseradish peroxidase (HRP)-labeled antibody. (**b**) Electrochemiluminescent detection of the antigen of hepatitis B virus from clinical serum samples. (**c**) Antibiotic resistance gene detection via fluorescence sensing using a light source (Lim et al. 2019)

sensitivities in these assays are generally proportional to the sample's chemiluminescence. This method has recently been used to identify the nucleocapsid protein of the SARS coronavirus using RNA aptamers (Sayad et al. 2016). A new nanozyme-based chemiluminescence paper assay was developed for highly sensitive and specific recognition of SARS-CoV-2 spike antigen, combining nanozyme and enzymatic chemiluminescence immunoassay with a lateral flow strip (Ng et al. 2018). The focus of this research was to create a stable Co–Fe@hemin-peroxidase nanozyme that catalyzes chemiluminescence like natural peroxidase HRP, therefore amplifying the immunological response signal. The SARS-CoV-2 recombinant spike antigen had an identification limit of 0.1 ng/mL and a linear range of 0.2–100 ng/mL. Furthermore, the test's sensitivity for pseudovirus was equivalent to the ELISA technique, reaching 360 TCID50/mL. A multiplexed sandwich-chemiluminescent enzyme immunoassay for the simultaneous detection of *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Yersinia enterocolitis* was developed in one research. To accomplish multiplexed identification of the four diseases, a contemporary polystyrene 96-well microtiter plate format with each centre well having four subwells in the rim was created. Each subwell was immobilized with monoclonal antibodies appropriate for the microorganisms. After the samples were administered to the main wells, the bacteria that immediately responded to the accompanying monoclonal antibody were collected in one of the four subwells. The peroxidase activity of the bound polyclonal labeled antibodies in each well was ascertained using a low-light charge-coupled imaging system and an enhanced luminol-based chemiluminescent combination, after which a mixture of peroxidase-labeled polyclonal antibodies against the four bacteria was implemented. The test was straightforward and fast, with a limit of quantification of 10^4 to 10^5 CFU/mL for all bacterial species. The method's precision was adequate, with recovery values varying from 90 to 120% as compared to findings from a traditional culturing technique. This approach can be used as a screening tool to assess if these pathogenic bacteria are found in various foods (Basiri et al. 2021).

5.4.2 Electrochemical Assay

A transducer, also known as an electrode, is used in electrochemical biosensors for microbial bacterial identification. When target pathogens bind to an electrode using an electrochemical technique involving the electrode and a pathogen-containing electrolyte solution, the chemical energy associated with biorecognition is converted to electrical energy. In situ detection of pathogens on surfaces, rapid pathogen detection using low-cost platforms, sample preparation-free detection of pathogens in various matrices, multiplexed detection of pathogens in practical matrices, and pathogen detection via wireless actuation and data acquisition formats are all possible with electrochemical biosensors. As a result, pathogen detection electrochemical biosensors are now widely utilized in water safety, food, medical diagnostics, environmental monitoring, and bio-threat detection (Cesewski and Johnson 2020). Previously, a microfluidic RT-PCR device and an electrochemical

DNA sensor were used to achieve fast detection for initial viral infection screening. A paper-based microfluidic interface was also developed for multiplexed electrochemical detection of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) antibody markers in serum samples. It was the first paper-based electrochemical immunosensing device with multiplexing and telemedicine capabilities for diagnosing HIV/HCV co-infection. An electrochemical microfluidic paper-based immunosensor array (E-lPIA) and a portable multichannel potentiostat make up the interface, which can conduct enzyme-linked immunosorbent tests on eight samples concurrently under 20 min (using a prepared E-lPIA). The platform's multiplexing capacity now allows it to create numerous measurement data for HIV and HCV markers from a single sprint, and the findings will be sent to a remote site for telemedicine. This device is small, low-cost, high-throughput, and user-friendly because of the unique convergence of paper-based microfluidics with mobile instrumentation. Researchers that have created electrochemical biosensors for pathogen detection explain the transduction components, electrochemical methods, biorecognition elements, and biosensor output in depth. The material of the electrode and the type factor of transduction elements are discussed (Reyes et al. 2002; Cesewski and Johnson 2020). Researchers who have created electrochemical biosensors for pathogen detection describe the transduction components, biorecognition elements, electrochemical methods, and biosensor output in depth. The electrode material and transduction element type factor are explored. The availability, processing, and immobilization techniques address aptamers, antibodies, and imprinted polymers, among other biorecognition components for pathogen detection (Dittrich et al. 2006; Housecroft and Constable 2010). A continuous-flow polydimethylsiloxane (PDMS) microfluidic RT-PCR chip and disposable electrical printed (DEP) chips were utilized in one of the experiments for fast amplification and sense of novel influenza (AH1pdm) virus of swine origin. There were four zones on the RT-PCR chip: an RT reaction zone, an initial denaturation zone, a heat cycle zone for PCR (two-step PCR), and a pressurizing-channel zone to prevent air bubbles from forming. To assess electrochemical signals, methylene blue was added to the RT-PCR mixture. The RT-PCR took only 15 min to complete, and the DEP chip detected the amplifiable reduction signals right away. The MB reduction current on the DEP chip with the amplicon was significantly lower than on nonamplified controls. The DEP chip for quick electrochemical sensing and this microfluidic technology for rapid RT-PCR are compatible and may create a portable diagnostic test device (Souf 2016; Yamanaka et al. 2011).

5.4.3 Calorimetric Assay

One of the earliest pathogen identification assays is the colorimetric assay. Colorimetry is a technique for utilizing colored compounds to determine the concentration of analyte in a sample. A colorimeter is an instrument that tests the absorbance of a given wavelength of light to determine the concentration of a solution (Wang et al. 2018). Respiratory tract infections are a widespread cause of disease and death

worldwide, and specific viral pathogens may cause them. For diagnosing various respiratory viruses, researchers developed an optimized microsystem focused on real-time colorimetry. This microsystem unit combines an eight-channel microfluidic array chip with a reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) processor for point-of-care screening of viral infection in the respiratory tract. The entire detection procedure may be accomplished (sample aggregation, nucleic acid extraction, sample loading, real-time detection, and signal output). Furthermore, researchers have developed gold nanoparticles that are incredibly efficient in identifying pathogens due to their capability to change color rapidly and efficiently as their environment changes (Kaarj et al. 2018). The point-of-care device, utilizing the RT-LAMP amplification of nucleic acid (Choi et al. 2018), has been developed to tackle the recent outbreak of the Zika virus, leading to creating a point-of-care system for rapid virus identification in the resource-limiting region. Researchers created a wax-printed paper microfluidic chip that uses (Choi et al. 2018) undiluted tap water, human urine, and diluted (10%) human blood plasma to demonstrate the produced simple and responsive ZIKV assay. The paper pore size, shape, and channel dimension of different paper microfluidic chips were examined and adjusted to enable appropriate separation of direct-use biological samples (tap water, urine, and plasma) during capillary action-driven flow. Because of their versatility, practicality, and cost-effectiveness, colorimetric biosensors that detect target analytes with the naked eye have got a lot of coverage. Nanomaterials have recently been used for fast and sensitive identification of pathogenic viruses and bacteria as flexible signal transduction and amplification mechanism. Yu et al. published a paper that explored how nanomaterials and bioreceptors can be further combined to create a rapid and responsive colorimetric detection device for pathogen detection in the future. After the ZIKA virus RNA had flowed to the paper microfluidic chip detecting zone, it was excised and placed on a heated plate at 68 °C for the addition of an RT-LAMP combination, including a pH indicator. In 15 min, visible color variations from adequate amplification were detected and measured using smartphone visualization. The detection limit was as low as one copy per liter. The current system may be used to identify dengue virus (DENV), flaviviruses, and Chikungunya virus (CHIKV), as well as other readily spread microbial pathogens, potentially leading to field-based diagnostics (Yu et al. 2017b) (Fig. 5.4).

5.4.4 ELISA for Virus Detection

The enzyme-linked immunosorbent assay (ELISA) is a technique for detecting and quantifying molecules including proteins, antibodies, hormones, and peptides. It has been frequently employed in microfluidic devices recently, resulting in a quick and low-cost way to diagnose RNA viruses. Typical ELISA and fluorescence-based Luminex tests take three stages and many hours to complete, but combining this approach with the microfluidic technology has resulted in effective and quick diagnosis. Within 60 min, researchers developed an ELISA microfluidic device for

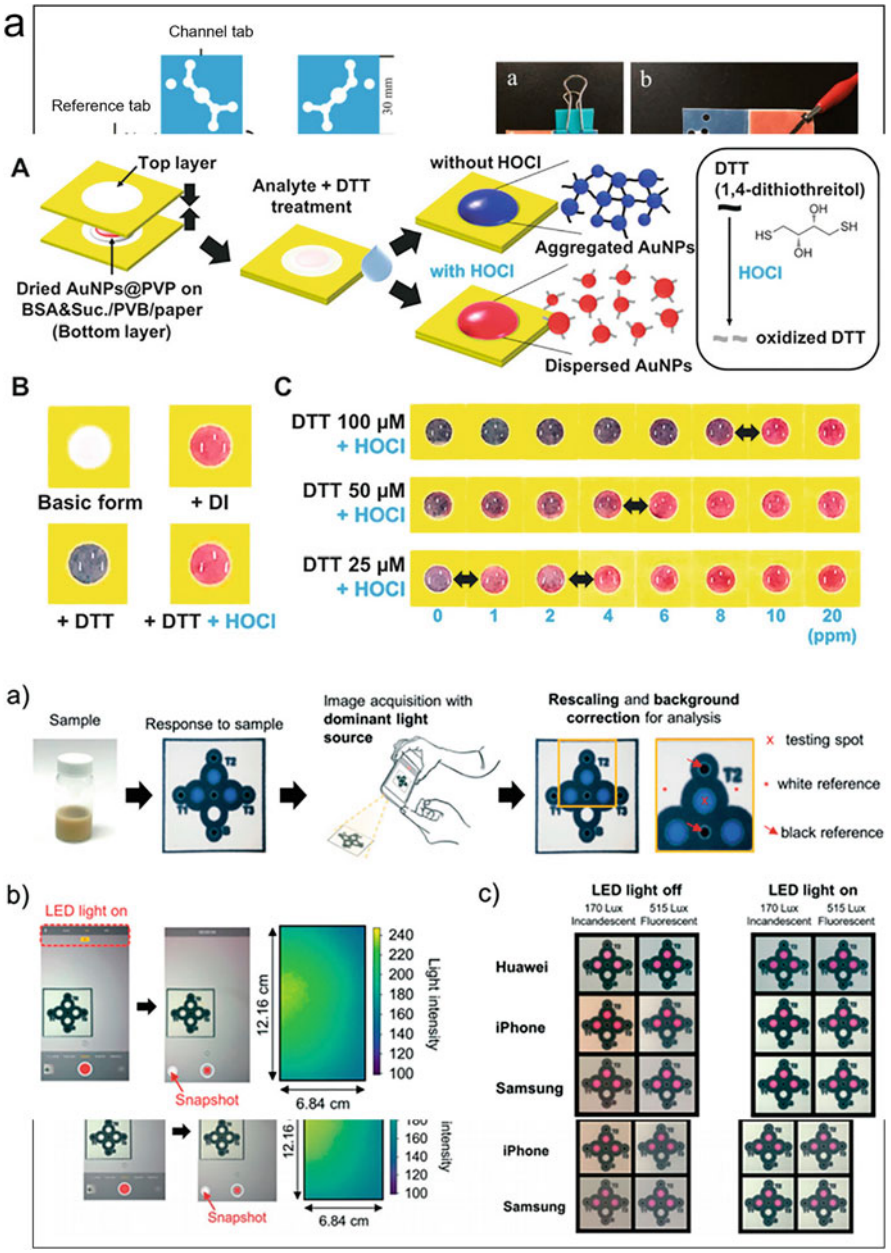

Fig. 5.4 Detection techniques and recent applications of μPADs. (**a**) Colorimetric sensing of HOCl via AuNPs by controlling the concentration of dithiothreitol. (**b**) Colorimetric sensing using a smartphone with an integrated light source. (**c**) Electrochemical detection of microRNA with chromogenic reaction (Lim et al. 2019)

detecting different pathogens as Hendra virus IgG antibody (Liu et al. 2005). One of the research used a bead-based microfluidic technology to produce a quick and responsive enzyme-linked immunosorbent test (ELISA) for pathogen detection with quantum dots as the labeled fluorophore. When compared to a standard ELISA on the same virus, the target virus's lowest detectable concentration was increased from 360 to 22 ng/mL, the detection time was cut in half from 3.25 to 30 min, and the quantity of antibody absorbed was reduced by 14.3 (Nguyen et al. 2020).

5.5 Conclusion

This chapter has covered the technological advancements of microfluidic devices toward the materials for device fabrication, fabrication technique, and sensing technologies in pathogen microorganism detection. We also discuss the primary methods for microorganism detection systems used in microfluidics: electrochemical, colorimetric, electrochemiluminescent, and enzyme-linked immunosorbent assay. POC biosensors are made of inorganic and organic materials such as glass, silicon, PDMS, PC, PMMA, PET, PS, and paper. Due to its wide availability, hydrophilic nature, portability, self-driven fluidic properties, and affordability, the paper has become one of the potential materials for manufacturing bioanalytical sensors. Stability, reproducibility, and mass manufacturing are critical criteria for clinical applications of microchips, necessitating the introduction of new fabrication methods and materials for microfluidic chips. Despite significant improvements in microfluidics, due to the requirements of external devices, there are still apparent issues and drawbacks in system miniaturization and integration, finding it challenging to implement in resource-constrained situations. Multiple parameter identification is necessary for clinical diagnosis, therapy, and prognosis to obtain a proper evaluation. Consequently, integrating several candidates such as proteins, nucleic acids, and other bioanalytes into a single platform fully automated may facilitate a better understanding of the disease and enhance the practical, specific application of inaccurate POC to improve detection credibility. In the future, several microfluidics-based technologies for bacterial extraction from blood, urine, and identification procedures may be combined, enabling fast, untargeted, and accurate detection of a broad spectrum of pathogens from patient clinical blood samples.

Acknowledgments The authors acknowledge the facilities and support provided by the Director of IIT Jammu. The CSIR-UGC Junior Research Fellowship supports NK. DBT-BIRAC assists MK. The position supports by the DBT-BIRAC funding under project No. RP0042 is gratefully acknowledged. The authors are also thankful to Vikas Kumar for collecting research articles.

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Recent Trends in Clinical Diagnosis for Viral Disease Detection Based on Miniaturized Biosensors

6

Naveen Kumar Singh, Himali Horo, and Vikky Rajulapati

Abstract

Rapid detection of a virus through its biomarkers over miniaturized device for disease diagnosis is in critical demand that requires optimal clinical outcomes for public health. Traditional in vitro testing for viral infectious diseases is time-consuming and requires well-equipped laboratories, skilled personnel, and bulky equipment. With the recent advancement in the multiplexed miniaturized diagnostic technologies, biosensor-based machineries can deliver point-of-care devices that match or outshine conventional standards concerning time, precision, and cost. Broadly classified, modern biosensors take advantage of nano and microfabrication technologies with diverse sensing strategies, such as mechanical, optical, and electrical transducers. This chapter reviewed the miniaturized biosensors for their point-of-care and point-of-need analytical performance in various diseases and their ground-level problems. Miniaturized biosensors for virus disease detection are complex analytical tools that combine interdisciplinary understandings based on biological chemistry, electrochemistry, materials science, and enzymology. This chapter discusses different types of biosensors for viruses and their biomarker detection for various diseases, their properties, the methods and techniques used for sensor fabrications, and their applications in different fields with some selected examples. We evaluate the advances of

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_6

biosensors for virus diseases diagnostics and discuss the critical challenges that need to be overcome to miniaturize diagnostic biosensors in real-world settings. The future approach focuses on the advanced strategies to fulfill current unmet clinical needs.

Keywords

Clinical diagnosis · Miniaturized biosensors · Microbial detection · Virus

6.1 Introduction

Viruses are astonishing pathogens associated with severe morbidity and mortality throughout the world since human history. They are highly infectious in nature, and the absence of deep knowledge and an effective prevention system is the main reason behind their devastating health impact. In the present context, immigration, industrialization, and the gap in efficient point-of-care (PoC) detection systems potentially contribute to the possibility of commonness and outbreak of viral infections around the globe. The advancement of current science and availability of pathogen-specific therapy options for viral infection increased the need for point-of-need efficient diagnostic tests. Improved knowledge about surface chemistry and advanced nanomaterials led to the discovery of several novel methods for virus detection over miniaturized systems. But the outbreak of new viruses such as MERS coronavirus (MERS-CoV), novel strains of influenza viruses A and B, and SARS coronavirus 1 or 2 (SARS-CoV-1/2) brings new challenges to better diagnostic systems. Since symptoms may be similar in different viral infections and may range from a minor cold to severe respiratory disease, it requires a fast and accurate diagnosis. A rapid and reliable diagnostic test to identify the pathogenic virus in infected people is vital to control and eradicate the challenge caused by viral infection (Fig. 6.1). The viral testing is hampered by limited testing capacity, cost,

Fig. 6.1 Need of suitable viral diagnostic assay

and logistics of deployment, often leading to prioritized testing for specific high-risk groups. Real-time reverse transcriptase-polymerase chain reaction (RT PCR)-based assays are considered the gold standard for COVID-19 diagnosis. Several other methods such as loop-mediated isothermal amplification (LAMP), clustered regularly interspaced short palindromic repeats (CRISPER), RT-qPCR, duplex RT-qPCR, sequencing-based assay, enzyme-linked immunosorbent assay (ELISA), and lateral flow immune assay are employed for the detection of SARS CoV-2 (CDC 2020; La Marca et al. 2020). However, except lateral flow immunoassay (LFIA), all of these methods are difficult to be employed in point-of-care/point-of-need (PoC/PoN) applications and resource-constrained environments as they require skilled operators and expensive instruments (Tang et al. 2020). Most LFIA is based on the application of antibodies. However, they suffer from limitations such as false-positive results, poor stability, batch variation, and qualitative or semi-qualitative in nature (Ravi et al. 2020; Sidiq et al. 2020). Among the different types of available recognition probes for detecting antigens from a biological specimen, several other advanced bio-probes such as aptamer, affimer, and small fragment antibodies emerge as suitable probes compared to antibodies and possess several advantages. These advanced recognition probes are inexpensive, rapid scale-up, and can integrate with other technologies to strengthen their performance and application during this unprecedented condition (Acquah et al. 2021; Singh et al. 2018). Electrochemical spectroscopy, either in voltammetry or amperometry modes, has recently obtained huge attention in the field of biosensor due to its exceptional sensitive signal transduction ability and ability to integrate over miniaturized electronics systems (Barfidokht et al. 2019; Sun and Hall 2019; Venkatesh et al. 2018). The key rationales for using an electrochemical system as a biosensing platform are its quick reaction time, high sensitivity, and the possibility of developing small and low-cost integrated devices using advanced and existing technologies (Hsu et al. 2018; Saha et al. 2014).

6.1.1 Viruses as Intracellular Parasites

Viruses are nanometer-sized entities that have the potential to cause severe threats to living cells. As they cannot grow, replicate, or produce their energy, they are not considered alive. However, they can infect a host cell by implanting their genetic materials, hijacking the cellular functions, and utilizing the cell's machinery and energy for replicating their genetic materials. In other words, an infected host cell synthesizes viral proteins instead of their standard metabolic products. The new progeny viruses that are generated attack other cells, and the process goes on (Knipe and Howley 2013). All types of viruses share a typical body structure consisting of a protein shell enclosing a nucleic acid genome. The nucleic acid can be a deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA) and, in both cases, single- or double-stranded. The protein shell is called a capsid and is made of repeating units of a single protein or a few different proteins. In some cases, the capsid is surrounded by one or more lipid bilayer membranes studded with virus-coded glycoprotein on

its exterior surface. The capsid and this membrane are together called a viral envelope. These types of viruses are called enveloped viruses. The main goal of the capsid and the viral envelope is to safely carry the viral gene to the suitable host cell through the extra-cellular environment, retaining its infectious properties. After successfully transferring the viral gene, its next tasks are to attach the virus to the host cell, cross the plasma membrane, and then uncover the nucleic acid genome (Lucas 2010; Perlmutter and Hagan 2015). The diseases caused by viruses are vast and have the potential to cause severe risk to humans, animals, and plant life. In humans, some viruses may cause minor infections like the common cold, stomach flu, and measles, while some may cause severe threats like Ebola, rabies, HIV/AIDS, dengue, polio, hepatitis, and smallpox. HIV and hepatitis have killed millions of people over the years, while some evolving viruses have caused severe outbreaks. Not all viral infections are spread from person to person but can also be spread by the bites of infected insects and animals (Stollar 1993). The severity of infection depends upon the types of the virus and the immune system of the host.

6.1.2 Importance of Diagnosis

Viral infections and related diseases have become one of the major threats to mankind. A large number of highly infectious diseases and outbreaks are initiated by viruses, which have caused severe loss to life, society, and the economy (McKee and Stuckler 2020). Their minimal size and simple morphology, which is susceptible to mutation, challenge the development of wide-use and long-term viral detection systems. Their isolation and visualization are challenging compared to the other microorganisms, thus requiring advanced procedures and technologies (Draz and Shafiee 2018). Viral infections show diverse symptoms, including flu-like, gastrointestinal troubles, rashes, immunodeficiency, tissue and organ damage, etc. In several cases, a sign of viral infection might be confused with other infections such as bacterial, leading to an increased risk of improper prognosis and medication. Hence, a precise diagnosis of a viral infection is essential for a clinician to determine the proper clinical prognosis and effective treatment protocol (Qureshi and Niazi 2020). Moreover, with rapid and spontaneous mutation, viruses can infect host cells with different novel mechanisms, and if a new infection arises in the human population, it's highly required to detect and categorize the agent to prevent outbreaks and epidemics (Dangalle 2021; Parrish et al. 2008). Therefore, the development of a dynamic virus diagnostic system for rapid, precise, simple, and long-term detection is always of utmost importance (Draz and Shafiee 2018).

6.1.3 Virus Detection Approach, Past to Current

The nanoscale dimension of the virus enforces a great challenge in the development of a suitable point-of-care/need detection system for wide use. The first virus detection was performed in the early 1950s through an electron microscope. These

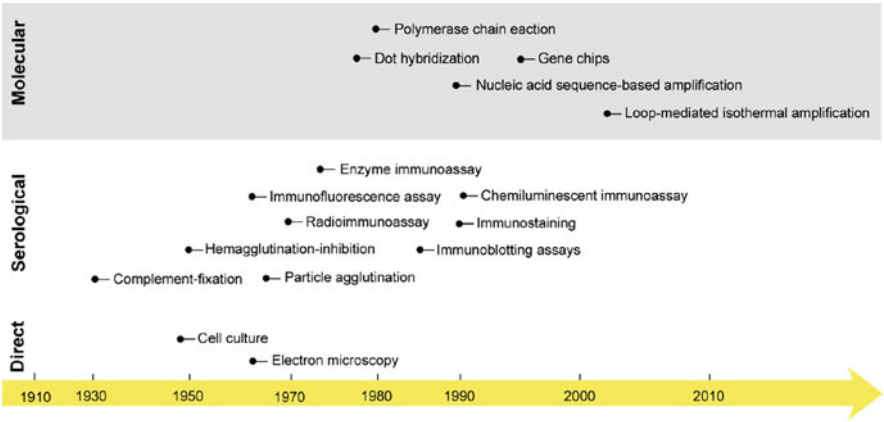

Fig. 6.2 A history and evaluation of virus detection techniques. The image is adapted from Draz and Shafiee (2018)

techniques formed the basis of all known classification and detection systems for a period of decades and remain the main tools for studying and investigating the biochemical and morphological properties of viruses. Although they are suitable for virus detection, their practical application has been restricted due to associated disadvantages such as cost, time, and safety. Two other major developments in the early 1980s bolstered the field of diagnostic virology: (1) the advent of a variety of immunoassays and (2) the introduction of polymerase chain reaction (PCR) (Fig. 6.2). It was accompanied by various successive serological and molecular detection techniques, which rapidly emerges as a mainstream laboratory-bound technique for clinical diagnosis of the virus. The serological immunoassay depends on the antibody and antigen-based detection of viral-specific antigen or corresponding humoral response generated against the specific virus, respectively. Tests that are commonly performed on serological specimens include complement fixation, neutralization, enzyme-linked or radiolabeled immunoassay, immunoprecipitation or immunochromatographic assay, and fluorescent or chemiluminescent immunoassay. Their principle of operation involves conjugating specific antibodies to a variety of signal reporting systems, such as chromo or fluorogenic substrate enzyme materials. The serological assay is a comparatively simple low-cost system and suitable for large-scale rapid testing but suffers from antibody cross-reactivity and high false-positive rate.

On the other hand, molecular techniques are more sensitive and specific (accurate), hence gaining interest in the field of virus detection. The discovery of new polymerases and the magnificent invention of polymerase chain reaction (PCR) have created a breakthrough for virus detection. Furthermore, a high degree of associated specificity in hybridization for DNA and simple modification capability led to many innovations in research and development of virus detection with genotyping and mutation prognostication. The molecular-based approach can be classified into amplification techniques (PCR, loop-mediated isothermal amplification, strand

displacement amplification, and sequencing) and nonamplification techniques (Southern or northern blotting, dot blot, and in situ hybridization). This system has revolutionized diagnostic testing in hospitals and in the community, enhancing the ability to control many viral infections. However, it is instrument dependent, expensive, and time-consuming and requires a highly skilled workforce.

6.1.4 Biosensor

A biosensor is an integrated device that can detect the presence or concentration of a specific chemical or biological analyte in a sample. It consists of a biorecognition element coupled to a transducer and an electronic processor. The biorecognition element can be any biomolecule like enzymes, aptamer, antibodies, cells, nucleic acids, tissues, molecularly imprinted polymers immunosystems, biomimetic, etc., that can specifically interact with the analyte and induce a biochemical activity. This activity is transformed into a decipherable signal by the transducer, followed by the processing of the transduced signal into visualized data by the signal processor (Aliofkhazraei and Ali 2014; Michelmore 2016). Biosensor finds wide application in the biomedical sector for the detection of numerous diseases. Detection of the biomarkers associated with different stages of a disease with high sensitivity and selectivity can help early detection and determine the infection rate and proper prognosis (Etzioni et al. 2003). Biosensors can be broadly classified based on the receptors and the transducing method used for the detection. Based on the receptors, they are classified by the types of biomolecules used. And based on the transducing method, they are classified as electrochemical, thermometric, piezoelectric, optical, and microbalance (QCM) biosensors (Atay et al. 2016). The electrochemical sensors are further classified into amperometric, potentiometric, voltammetric, and impedimetric (Srivastava et al. 2020).

6.1.5 Point-of-Care Biosensors

Point-of-care testing (POCT) or near-patient testing is one of the most effective approaches of a biosensor application. It is a diagnosis system that provides provision for rapid on-site testing and gives lab-quality results within minutes to a few hours. POCT kits are portable and easy to operate. They do not require specimen preparation, laboratory facilities, or trained personnel. POCT can be a very noteworthy approach to address an emergency, mass testing, remote and low resource healthcare sectors, patients requiring frequent health monitoring, and issues of the patients with chronic conditions and aged people. Since POCT ensures rapid diagnosis, it can enable proper disease monitoring and management and quick prognosis and can help a patient to start early treatment. POCT is designed as self-contained miniaturized kits with low detection limits, ultra-sensitivity, accuracy, specificity, and rapid and easy assaying methods. Various prototypes of POCTs have been fabricated, which include lab-on-chip, nanomaterial-based, labeled and label-free,

wearable, and wireless. Lab-on-chip mostly uses microfluidics to integrate multiple functions like sample preparation, reaction, separation, and detection into a few centimeters to millimeter-sized chip. Continuous flow of analyte through microchannels allows the fresh sample to encounter the sensing element embedded in the channels, thus increasing the sensitivity. Moreover, mass transfer is faster in the channels making the diagnosis faster.

Initially, microfluidic channels were fabricated using silica and glass by photolithographic technique. However, various types of polymeric materials such as poly (carbonates) (PC), poly(methyl methacrylate) (PMMA), poly(dimethylsiloxane) (PDMS), poly(ethyleneterephthalate glycol) (PETG), poly(vinyl chloride) (PVC), poly(styrene), poly(imide) (PI), cyclein olefin polymers (COP), and cyclein olefin copolymer (COC) have also been developed. PDMS is widely used in cell-based chips because of its high gas permittivity and optical transmissivity (Rodrigues et al. 2017; Tsao 2016). Paper-based microfluidics is also being in trend because of the large number of advantages it possesses. Paper is inexpensive, abundantly available, highly biocompatible, disposable, environment friendly, and easily functionalized for binding biological samples, it can be patterned by photolithography and easily stored, and most importantly, paper facilitates wicking of liquid, which induces transport without pump (Berthier et al. 2019; Xu et al. 2016).

Nanomaterial-based POCTs explore the exceptional properties of nanoparticles such as small size, high surface-to-volume ratio, electrical and thermal conductivity, magnetic, optical, and fluorescence. They are used as fluorescence quenchers, optical probes, biochemical labels, and biomolecule immobilizing platforms because of surface functional groups. The use of nanomaterials has been shown to enhance the working of sensors with various different mechanisms. For example, in electrochemical, they increase the electron transfer rates; in enzymatic, they reduce the distance between enzyme and electrode (Murphy 2006); in optical, the noble metal nanoparticles increase surface plasmon resonance (SPR) (Choi et al. 2020) and in colorimetric, the color change phenomena due to the state of nanoparticles aggregation can be beneficial (Aldewachi et al. 2018).

6.2 Virus Biomarkers and Associated Challenges with Virus Detection

National Institutes of Health (NIH) has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" (Atkinson et al. 2001). Thus, biomarkers are a part of or are expressed in a biological system that can be analyzed for a normal and abnormal process occurring in the body. Biomarkers of viral infections have been expressed in various biological systems like plasma, sputum, urine, sweat, saliva, etc., and can be analyzed for monitoring the occurrence and severity of viral disease (Hwang et al. 2018).

6.2.1 Types of Virus Biomarkers

The viral biomarkers can be broadly classified into two classes: direct and indirect biomarkers. The former is extracted directly from a virus, which includes viral nucleic acid (DNA/RNA) and antigenic proteins, while the indirect biomarkers are expressed in the host cells in response to a viral infection such as an antibody.

6.2.1.1 Nucleic Acid Biomarkers

Viral nucleic acids (DNA/RNA) have been potential biomarkers for the detection, monitoring, and prognosis of specific viral infections. Advancement in nucleic acid detection techniques enabled wide use of the biomarkers in clinical implementation. PCR, microarray, and LAMP are the most generally practiced techniques for nucleic acid analysis (Shen et al. 2020). However, real-time polymerase chain reactions (qRT-PCR) are now being in trend for early, sensitive, and specific nucleic acids detection (Prabhakar and Lakhanpal 2020). A DNA/RNA biosensor consists of a single-stranded oligonucleotide immobilized on a transducer that can detect its complementary strand (biomarker) by surface hybridization. The transducer converts this hybridization event on the electrode surface to an analytical signal (Ozer et al. 2020).

Based upon the type of nucleic acid they hold, viruses are classified as DNA and RNA viruses. The DNA virus consists of double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA). Replication in DNA viruses occurs by DNA-dependent DNA polymerase. Large DNA viruses (>10 kb) comprise dsDNA, while small DNA viruses comprise circular, ssDNA, or dsDNA (Sanjuán et al. 2016). The pathogens under this type are African swine fever virus (ASFV), varicella-zoster virus (VZV) causing chickenpox and shingles, variola virus (VARV) causing smallpox, herpes simplex virus 1 (HSV-1), and hepatitis B virus (HBV) (Babkin and Babkina 2015; Bauer et al. 2013; Chen et al. 2020; Liang 2009; Vizoso Pinto et al. 2011).

RNA virus generally consists of single-stranded RNA (ssRNA) or sometimes double-stranded RNA (dsRNA). The ssRNA can be categorized into positive sense (ssRNA (+)) and negative sense (ssRNA (−)) RNA. An ssRNA (+) genetic material can execute both as a genome and messenger RNA (mRNA). Thus, they can be directly translated into protein by the host cell ribosomes. During replication, the ssRNA (+) encodes genes for an RNA-dependent RNA polymerase (RdRp), which catalyzes the synthesis of negative-sense antigenome that acts as a template for the formation of new ssRNA (+) (Payne 2020). This virus covers over one-third of all virus genera and includes several pathogens such as human immunodeficiency virus (HIV), coronaviruses (CoV), West Nile virus (WNV), Zika virus (ZIKV), hepatitis A virus (HAV), hepatitis C virus (HCV), hepatitis E virus (HEV), dengue virus (DENV), Japanese encephalitis virus (JEV), human rhinoviruses (HRVs), and chikungunya virus (CHIKV) (Nagy and Pogany 2012). The genetic material in ssRNA (−) acts as a complementary strand for mRNA and needs to be converted to ssRNA (+) before translation using RNA polymerase. The replication in ssRNA (−) occurs by synthesis of positive-sense antigenome as the template by RdRp. All

the virus under this type consists of a lipid envelope enclosing the nucleocapsid. Some of the pathogens that come under this type are influenza A causing Spanish flu, swine flu, bird flu, Asian flu, Hong Kong flu, influenza B and influenza C viruses, measles morbillivirus (MeV), Ebola virus (EV), Marburg virus (MARV), hepatitis delta virus/hepatitis D (HDV), human parainfluenza viruses (HPIVs), Nipah virus (NiV), mumps virus (MuV), and human respiratory syncytial virus (RSV).

The nucleic acid detection approaches possess several advantages, such as excellent sensitivity, specificity, and beneficial for virus that cannot be cell-cultured. However, some major challenges that are associated along with the detection procedures of nucleic acid biomarkers (Leland and Ginocchio 2007; Zhong et al. 2007) can be listed as follows:

(a) DNA can get modified or degraded by nucleases and other substances.
(b) RNA isolation requires high precautions as it is very unstable and susceptible to chemical and enzymatic hydrolysis. The presence of 2′-hydroxyl group on the pentose ring and the ubiquitous presence of RNase enzyme makes it liable to chemical and enzymatic degradation.
(c) RNase inhibitors like diethyl pyrocarbonate (DEPC) used to protect RNA from degradation are highly toxic and carcinogenic, which has to be handled with high precautionary measures.
(d) Any mutations in the DNA/RNA sequence may be missed by the particular primers and probes employed in the detection procedures.
(e) Detection of RNA in diagnosing some viruses like Zika is possible only after a few days of onset of the symptoms.
(f) Technical expertise and expensive instrumentations are required mainly for low-volume analytes.

6.2.1.2 Protein Biomarkers

A virus contains a large number of proteins in its body structure, comprising of structural and nonstructural proteins. For example, the SARS-CoV-2 virus is made of four structural and 29 nonstructural proteins. Detection of these viral proteins can deliver information about the existence of a particular viral infection in a body. Protein biomarkers have always been favored over nucleic acid biomarkers as the nucleic acid biomarkers encompass tedious steps of isolation, purification, and processing stage, which is time-consuming and expensive, while protein biomarkers are easy to isolate and require simple sample preparation steps. Moreover, an extensive range of analytical instrumentation is available that can identify and quantify proteins (Kaur et al. 2020).

The antigenic viral surface glycoproteins are a significant component of an enveloped human pathogenic virus. They play a pivotal role in viral infectivity and immune evasion. Glycoproteins are formed by glycosylation (covalent attachment of carbohydrate to protein backbone), which is a post-translational modification process. Direct detection of these glycoproteins or indirect detection of the developed antibodies in the host cell is an evolving discipline in virus diagnosis. Lectins or monoclonal antibodies mostly do the glycoprotein recognition. However,

various other biochemical processes have been established to investigate glycoproteins (Banerjee and Mukhopadhyay 2016).

A viral envelope is composed of three types of glycoproteins: membrane protein (M), envelope protein (E), and spike protein (S). The S protein is a type of large class I fusion protein that plays a key role in binding and penetrating the host cell. They bind to the angiotensin-converting enzyme 2 (ACE2) receptor present on the host cells and facilitate virion transfer. The M and E proteins are primarily responsible for forming the virus assembly (Shajahan et al. 2020). Some of the glycoproteins associated with the particular types of viruses are spike (S) glycoprotein in SARS-CoV-2, hemagglutinin and neuraminidase in influenza virus, gp120, gp160, and gp41 in HIV, spike Gp1-Gp2 in EV, nonstructural glycoprotein NS1 in DENV, and G-1 and G-2 glycoprotein of HSV-1. Some of the techniques used for the detection of the viral protein are ELISA, chromatographic techniques, radiolabeling, and fluorescence-based assays (García-Cañas et al. 2007). However, some of the major challenges are associated with the detection of viral proteins (Feng et al. 2020; Leland and Ginocchio 2007). Some have been listed below:

(a) Detection of trace amounts of viral protein is a challenge, as they cannot be amplified like nucleic acids.
(b) The nonexistence of antibodies against each protein of a virus limits the development of the protein detection process.
(c) Because of the complex structure and high molecular weight of the glycoproteins, their separation and purification are difficult.

6.2.1.3 Serological (Antibody) Biomarkers

Antibodies, also known as immunoglobulins, are the Y-shaped proteins formed as a body's defense mechanism against infection by specialized white blood cells called B lymphocytes. An antibody binds to a specific antigen protein and inhibits its action in various ways, such as neutralization, opsonization, and complement activation. Antibodies produced for a particular virus differ from the antibody produced for another. Thus, the detection of antibodies produced in response to a specific viral antigen can be an effective approach in diagnosing the occurrence of a particular viral infection.

An antibody test analyzes the level of a specific antibody in the sample. Serum samples are generally used for the detection of developed antibodies. However, saliva, sputum, nasal swab, and dried blood spots have also been used depending upon the type of infection.

Five major classes of antibodies are produced in a body in response to viral infections. They are as follows:

(a) Immunoglobulin A (IgA), found in high concentration in the mucosal secretions, serum, salivary glands, lacrimal glands, intestinal fluids, and colostrum.
(b) Immunoglobulin G (IgG), the most common antibody found in blood and tissue fluids and the only antibody that can cross the placenta.

(c) Immunoglobulin M (IgM), the first antibody produced in response to an infection, found in blood and lymph fluid.
(d) Immunoglobulin E (IgE), produced in the very low level in the serum; however, the level increases in the presence of allergens.
(e) Immunoglobulin D (IgD), mainly found on the B cell surface, acting as a receptor for antigen.

The IgG, IgM, and IgA antibodies are primarily responsible for neutralizing the infectivity of a viral infection. IgG antibodies are very important and show high specificity to their respective antigens. Therefore, IgG detection is in common practice for virus diagnosis. Some of the conventional methods used for the detection are ELISA, immunofluorescence assay, hemagglutination inhibition assay, neutralization assay, and western blot (Corrales-Aguilar et al. 2016). IgG-based diagnostics have been used to detect some glycoproteins such as spike (S) glycoprotein of SARS-CoV-2 and G-1 and G-2 glycoprotein of herpes simplex virus and have shown high sensitivity and specificity.

Some of the major challenges associated with the antibody biomarkers are listed below:

(a) Occurrence of cross-reactivity between antigens if the antibody against a specific antigen has a competing high affinity toward another antigen. This might occur when two antigens have similar epitopes.
(b) False-negative results can occur if the amount of antibodies in the test specimen is below the detection limit of the assay or insufficient antibodies have been produced at that stage of infection.

6.3 Sensor against Viral Diseases

6.3.1 COVID-19 or Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2)

There are several biomarkers that can be significant for the detection of SARS-CoV2. These are ssRNA, antigen, antibody, blood, urine, infection, blood gas index, hemagglutination level, and cytokine levels. In the clinically used approaches, several biosensors have been established for the recognition of COVID-19 (Jalandra et al., 2020). This biosensing system can be used as a body wearable, smart band, plasmonic photothermal sensor, optical sensor, and nano- and cell-based sensors. Seo et al. (2020) reported a biosensor identifying SARS-CoV2 from clinical samples based on the field-effect transistor (FET) approach. The sensor was fabricated with graphene sheets on a coated transistor, followed by an antibody precisely against the SARS-CoV2. The manufactured sensor performs current clinical samples from COVID-19 patients with better results as antigen protein. The samples are collected from COVID-19 patients' nasal swab specimens. This biosensor has been detecting the SARS-CoV2 protein from clinical samples up to the level of 1 fg/mL

concentration using buffer and 100 fg/mL from the biological sample fluid. Furthermore, the sensor detected the virus-related culture in clinical samples with approximately 2.42×10^2 copies/mL, as Seo et al. (2020) reported. A recently reported biosensor was manufactured with the help of gold nanoparticles and SnO_2/F electrodes (fluorine doped with tin oxide), immobilized with monoclonal antibodies of COVID-19. The sensor showed high sensitivity between 1 fM and 1 μM for recognition of COVID-19Ag (antigen). The fabricated sensor effectively senses COVID-19Ag in buffer up to 10 fM, 90 fM with eCovSens, and 120 fM for spiked samples from saliva (Mahari et al., 2020). This biosensor device detected COVID-19Ag within 10–30 s from patient saliva samples. Djaileb et al. (2020) reported a surface plasmon resonance sensor identifying antibodies of nucleocapsid against SARS-CoV2 from human serum. The sensor could respond with 10 μg/mL anti-r nucleocapsid and yield of 221 RU. Qiu et al. (2021) designed a biosensor with the coupled characteristics of plasmonic, such as merging the photothermal plasmonic effect and surface resonance plasmonic. The detection of changes in clinical samples is an additional and encouraging approach for COVID-19 diagnosis. The dual-functional biosensor showed a high response toward SARS-CoV2 samples with the lowest recognition of 0.22 pM and detected a precise target from a gene pool. A recent study of SARS-CoV2 RapidPlex has been reported, which targets the multiplex of biomarkers such as C-reactive protein of saliva samples and serum, anti-spike protein of IgM and IgG, and nucleocapsid protein (NP) (Torrente-Rodríguez et al., 2020). RapidPlex showed the samples' S/B relation range between 10.5 and 12.4 in serum and 2.81 NP, 3.24 S1-IgG, 1.62 S1-IgM, and 1.76 CRP in saliva; the NP concentration range of 0.1 to 0.8 μg/mL for serum and 0.5 to 2.0 ng/mL for saliva in COVID-19 clinical patient samples; IgG in the range of 20–40 μg/mL in serum and 0.2–0.5 μg/mL in saliva and IgM in the range of 20–50 and 0.6–5.0 μg/mL in COVID-19 patient serum and saliva, respectively; and a CRP range of 10–20 and 0.1–0.5 μg/mL in COVID-19 patient serum and saliva, respectively. The positive samples showed higher signals than negative samples, which significantly proved the accurate evaluation of the COVID-19 biomarkers in biofluids using biosensors. Recently, a biosensor label-free miniaturized smartphone-supported signal detection for COVID-19 diagnosis has been reported (Chandra, 2020). This development of an electrochemical immunosensor-based approach will be a potential for the point-of-care device for the detection of SARS-CoV2. The commercially viable and clinical practice nano-bioengineered approaches for COVID-19 diagnosis are minute details on RT-PCR immunodiagnostic assays. Tripathi and Agrawal (2020) reported the electrochemical label-free detection of DNA hybridization, a possible method for identifying COVID-19. The high contagious (rate of infectivty) nature of SARS CoV-2 virus imposes serious challenges and restrictions on the healthcare workers at diagnostic centers. It brings up the need for a suitable user-friendly and pragmatic sensor for detection of COVID-19 at home with minimal settings. With a particular focus, researchers have developed a method to employ a glucometer for detection of SARS CoV-2 virus from human saliva at very low cost, i.e., ~ 3 USD. The assay was based on antisense (complementary DNA) displacement assay from aptamer on binding of target antigen (SARS CoV-2 spike or nucleocapsid protein). To translate

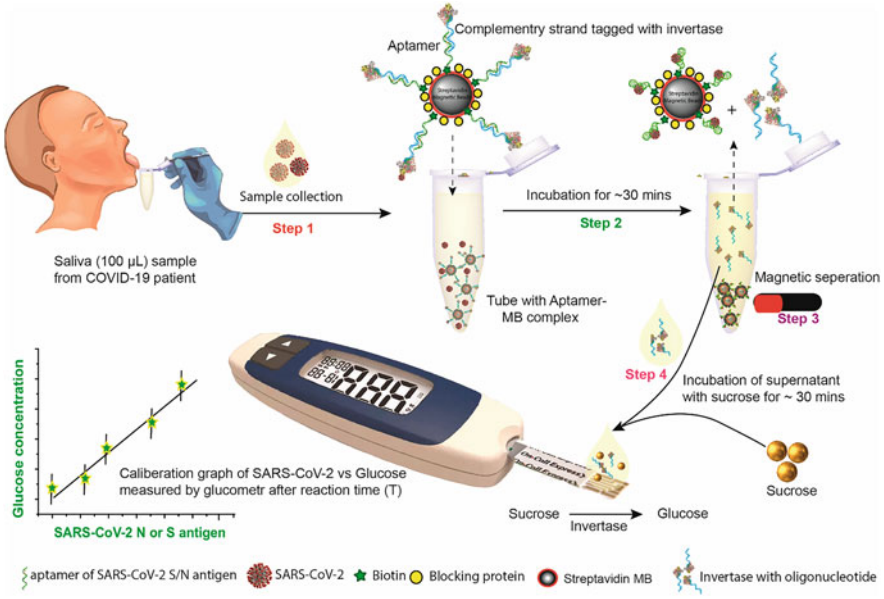

Fig. 6.3 Assay scheme. Biotin-aptamers (anti-S or anti-N protein) are annealed to the complementary invertase–oligonucleotide and pre-assembled on streptavidin-coated magnetic beads (MBs). Next, samples containing SARS-CoV2 virus, or viral (S/N) protein, are incubated with this pre-assembled complex (Steps 1 and 2). The binding of the virus or viral protein to the aptamer triggers a conformational switch releasing the invertase–oligonucleotide into solution (Step 3). The virus-bound aptamer–MB complex is separated using a magnet, and the supernatant containing invertase–oligonucleotide is collected (Step 4). The invertase–oligonucleotide solution is then incubated with sucrose, which is converted to glucose and measured by a commercially available glucometer

COVID-19 viral antigen binding into glucose production, they have exploited the native catalytic properties of invertase and engineered a novel aptamer-based competitive assay. Under the assay, anti-S (or anti-N) protein aptamers conjugated to the enzyme invertase through a small oligonucleotide (15–25 base pairs antisense) that is complementary to a portion of the aptamer sequence. The biotinylated aptamer-oligo-invertase complex is pre-assembled on magnetic beads, and in the presence of respective target anti-N or -S aptamer undergoes conformation change. Hence, complementary strand along with invertase enzyme was displaced from MB, thus creating an antigen-sensitive switch for signal production. The incubation of released invertase enzyme with sucrose for unit time converts it into glucose, thus providing necessary amplification. The formed glucose is readout with a glucometer; the amount of glucose formed is directly proportional to the viral antigen (Fig. 6.3). The core advantage of this approach relies on distributed devices that were already ubiquitous in the market today, rather than developing custom hardware or expensive instruments.

6.3.2 Recent Challenges with SARS CoV-2 Diagnosis

A kind of new mutation in the genetic material in SARS CoV-2 was reported in reference to the genetic sequence of Wuhan-Hu 1 or USA-WA1/2020. The mutation in RNA viruses was up to million times higher than their host, as it supported their survival by enhancing their variability and evolvability. The probable reason for high mutation in the viruses is faster replication rate through faster polymerase, and high-rate kinetics polymerases make more mistakes (Duffy 2018). The list of SARS CoV-2 variants with their respective mutations is given in Table 6.1**.** These mutants have high transmission and virulence and lessen the effort of the social health measure. The SARS CoV-2 delta variant is a major concern due to its higher transmission rate, pathogenicity, and ability to evade the immune system of the vaccinated person.

Moreover, the structural and functional mutation in SARS CoV-2 RNA or protein imposes a serious threat on an effort to curb the challenges caused by SARS CoV-2 infection. The presence of new SARS CoV-2 variants in patent samples can possibly impact the performance of diagnostic tests; for example, several FDA-approved diagnostic tests such as Linea, Taq Path, and X-pert showed slightly reduced sensitivity against SARS CoV-2 variants B.1.1.7. (Health 2021). Some other potential challenges become apparent by different SARS CoV-2 variants such as the following:

Table 6.1 List of SARS CoV-2 variants and respective mutation

WHO label	Pango lineages	Additional amino acid changes monitored[a]	Earliest documented samples	Date of designation
Alpha	B.1.1.7	+Spike:484 K +Spike:452R	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351 B.1.351.2 B.1.351.3	+Spike:L18F	South Africa, May-2020	18-Dec-2020
Gamma	P.1 P.1.1 P.1.2 P.1.4 P.1.6 P.1.7	+spike:681H	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2 AY.1 AY.2 AY.3 AY.3.1	+Spike:417 N	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021

[a]*VOI* variants of interest (need to be monitored and characterized repeatedly), *VOC* variants of concern (need to be monitored and characterized by central agencies). Table adapted from WHO SARS CoV-2 declaration

1. As mentioned above, mutation in the genetic material of virus, especially primer binding region, can escape the detection by molecular diagnostic tests such as RT-PCR or LAMP. Hence, multiple target-based assay or multiplex assays are needed to minimize the chance of error.
2. Mutation in epitope (antibody binding region) or aptatope (aptamer binding region) can reduce the susceptibility and sensitivity of therapeutic agents, for example, aptamer and monoclonal antibody.
3. Having the ability to evade polyclonal antibody, an immunized person with natural infection or vaccine of SARS CoV-2 generates a polyclonal response that recognizes the receptor-binding domain (RBD) or other parts of the spike protein. Hence, any mutation in spike protein can evade the immunity of a person or any diagnostic assay based on the polyclonal antibody.

6.3.3 Dengue

Dengue is the fastest spreading viral disease by mosquitos. A nonstructural 1 (NS1) protein is a particular and sense biomarker for the detection of dengue. The detection methods of IgM- and NS1-based diagnostic tests for dengue are most widely used in many countries. The chemically modified peptide approach was used to design an electrochemical sensor for the diagnosis of dengue virus protein NS1. Young et al. (2000) reported the clinical test on arrest antigen in an infected patient by ELISA. This approach reveals a better understanding and significant detection of protein NS1 in the serum from dengue virus-infected specimens. The sensitivity of detection was approximately 4 ng/mL. ELISA targets NS1 protein, using the antigen in the bloodstream in clinical samples of infected patients at a critical stage of dengue. Infected patients with dengue fever contained NS1 protein level in their serum with 0.04–2.00 μg/mL in the initial stage of infection and 0.01–2.00 μg/mL in the later stage (Alcon et al., 2002). Cui et al. (2020) reported voltammetric electrochemical activities of synthetic dengue virus RNAs detected by indium tin oxide sensing electrode, and the limit of detection was shown to be 2 Amol. Cecchetto et al. (2020) reported serological point-of-care of free-label electrochemical capacitive identification for dengue virus infection. The modified approach employed a ferrocene-flagged peptide surface that contained anti-NS1 as the receptor. The assay capacitive had a limit of 1.36%, with an interval confidence of 99.99% (Cecchetto et al. 2020).

6.3.4 Encephalitis

An amperometric biosensor was constructed by sandwich gold label immunoassay for detecting the forest spring encephalitis antibodies' concentration maintained between 10^{-7} and 10^{-2} mg/mL with a sensor limit of recognition of 10^{-7} mg/mL (Brainina et al. 2003). A label-free amperometric immunosensor specifically detects Japanese B encephalitis (JBE) in the range of 1.1×10^{-8} to 1.9×10^{-6} lg pfu/mL. The correlation data coefficient was found as 0.995. A biosensor was

established for the detection of JBE using the label-free Fe^{2+}/Fe^{3+} target of immunoassay, and the detection limit of the device was 6×10^{-9} lg pfu/mL (Yuan et al., 2005). Similarly, a potentiometric biosensor was designed for JBE as an immunoassay approach, and the device limit was 6×10^{-9} lg pfu/mL (Jinchi et al., 2004). The light potentiometric biosensor was designed for the detection of Venezuelan equine encephalitis by using sandwich enzyme-label immunoassay, and the detection limit of the sensor was 30 ng/mL (Weaver et al., 2004). An extremely sensitive detection of JBE and avian influenza virus (AIV) by a field-effect transistor-functionalized graphene sensor has been reported. An antigen–antibody interaction assay was observed in both cases, and the current signal in the sensor analyzed it. These sensors showed the detection range of 1 fM to 1 μM for both cases. The detection limit of 1 fM for JBE and 10 fM for AIV was seen (Roberts et al., 2020). Lai et al. (2017) reported another way of detection of JBE by a carbon nanoparticle-based electrochemical biosensor. Immobilization of JBE antibody was done through carboxylic group linkage with the nanoparticles' amide group. The electrochemical biosensor showed a linear way observation range of data of 1–20 ng/mL with a lower recognition limit of 0.36 ng/mL, and detection sensitivity was 0.024 ng/mL for JBE analysis obtained in 10 min.

6.3.5 Hepatitis

Chronic hepatitis increases the risk of developing hepatocellular cancer, chronic hepatitis, and liver cirrhosis. Hepatitis B virus diagnosis kit was designed by Uzun et al. (2009) using surface plasmon resonance-based assay. The assay exhibited a detection limit of 208.2 mIU/mL and showed 0.015 mIU/mL association constant (K_A) and 66.0 mL/mIU dissociation constant (K_D). Seroprotection showed levels of 10 mIU/mL reported earlier in the case of HB surface antibody. The electrochemical biosensor for the HBV and TT virus detection by DNA amplified from polymerase chain reaction with clinical samples was reported. The biosensor was immobilized with 21–24 single-stranded oligonucleotides as a probe for HBV and TTV sequences and paste carbon electrode (Meric 2002). The detection of the hepatitis A virus by PCR using ssDNA as the probe was designed and tested. The fabrication of the electrochemical biosensor, HAV cDNA synthesis, which is complementary to ssDNA using the gold as the electrode, was tested. This device showed a limitation of signal cut edge to 0.65 pM for the ssDNA and 6.94 fg/μL for viral cDNA (Manzano et al., 2018). Another design approach established hybridization of DNA on a piezoelectric sensor for the detection of HBV. This is known as HBV DNA biosensor, which is more reliable and more sensitive. HBV DNA probe was crippled with gold electrodes with a frequency up to the range of 9 MHz. The quartz crystal in the piezoelectric sensor forms the adhesion cross-linking of glutaraldehyde and polyethyleneimine. The probe frequency shifts showed the significant linearization relation of hybridization with HBV DNA. The amount of HBV DNA showed better results between 0.02 and 0.14 μg/mL (Zhou et al., 2002). The identification of the hepatitis E virus using the pulse-electrochemical approach was reported. The

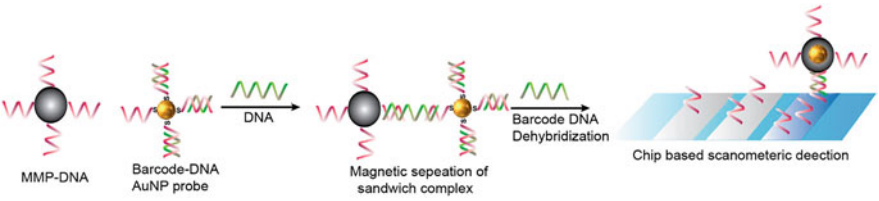

Fig. 6.4 Barcode-based detection of hepatitis B virus (HBV) (Wang et al. 2003)

sensor was fabricated using the combination of graphene quantum dots and nanowires of polyaniline embedded with gold. The device linearity was a concentration range of 10^2–10^7 copies/mL and HEV concentration in between 1 fg/mL and 100 pg/mL with a low-signal limit point of 0.8 fg/mL reported earlier (Chowdhury et al., 2019). A recent report on the design of biosensors with an electrochemical approach with nucleic acid combined with a new indicator of quercetin electroactive has been reported. It detects both ssDNA and dsDNA with the signal limit of 83 pM with a standard deviation of 4.6%. This study reports the fabrication of a biosensor as a successful technique for the detection of the hepatitis C virus with short sequences (Alipour et al., 2020).

A biobarcode amplification strategy was adapted by Wang et al. (2003) for detection of HBV DNA. This gold nanoparticle (AuNP) biobarcode scanometric assay was based on gold nanoparticle-enhanced reduction of silver ions into metallic silver, which is emerged as a visible black spot. In detail, it employed two different sets of DNA-functionalized AuNP and magnetic particle to capture and detect the HBV signature DNA sequence, respectively. The AuNPs modified with ssDNA specific to a target strand (barcode DNA) provide amplification and detection, while the second set with magnetic particle (MP) with ssDNA is specific to the target HBV strand. The presence of released complementary strand supports the aggregation of AuNP/DNA/MP conjugate. After washing, barcode DNA was substituted from nanoparticles and hybridized with a capturing DNA probe immobilized over the chip. Later silver staining reagents were used to amplify the detection signal (Fig. 6.4).

6.3.6 Human Immune Deficiency Virus (HIV)

Acquired immune deficiency syndrome is a severe transmissible immune disease caused by HIV. There have been many novel approaches of nanomaterial-based biosensors for HIV diagnosis. HIV infection response to CD4+ cells, CD4+ T lymphocytes, p24 core protein, HIV gene, p17 peptide, virus-like particles, and HIV-related enzymes and a viral duplicate within the host cell have been studied (Farzin et al., 2020). The new sandwich HIV p24 immunosensor based on chronoamperometry was developed. The electrochemical signal showed the concentration of p24, ranging from 0.01 ng/mL to 100 ng/mL, with a detection limit of 0.008 ng/

mL (Zheng et al., 2012), which was more sensitive than ELISA (1 ng/mL) and enhanced the reversibility and conductivity of the electrode. Shafiee et al. (2014) reported a nanostructured optical photonic crystal biosensor for HIV-1 viral load measurement with concentrations ranging from 10^4 to 10^8 copies/mL. It has been a rapid and sensitive optical detection method for biomolecules, cells, and viruses by monitoring the dielectric permittivity changes at the interface of a transducer substrate and a liquid media. An electrical sensing mechanism was also developed to detect captured HIV-1 on magnetic beads conjugated with anti-gp120 antibodies through impedance spectroscopy of viral lysate samples. Gray et al. (2018) reported the dual-channel surface acoustic wave biosensor, a pilot clinical sample study to diagnose HIV. This biosensor is a small lab prototype, portable, functionalized with ink-jet printing and dual-channel biochips, miniaturized, and requiring 6 μL of plasma. It can detect anti-p24 or anti-gp41 antibodies, with sensitivities of 100% (anti-gp41) and 64.5% (anti-p24) within 5 min. Amperometric sensors were also utilized to measure the concentrations of zidovudine fabricated using silver nanofilm and multiwalled carbon nanotubes immobilized on glassy carbon electrodes. This amperometric reported a linear range for zidovudine (0.37 μM–1.5 mM) concentrations from 0.1 to 400 ppm with a detection limit of 0.04 ppm (0.15 μM) (Rafati and Afraz 2014). Tombelli et al. (2005) reported the aptamer-based biosensor, which was immobilized on the gold surface of piezoelectric quartz crystals for the diagnosis of HIV-1 tat protein, based on the binding of a biotinylated aptamer on a layer of streptavidin. Two aptamer-based sensors have been established, and RNA aptamer specific for HIV-1 tat protein ranges from 1.25 to 2.5 ppm. Recently Yeter et al. (2021) reported an electrochemical label-free DNA impedimetric sensor with gold nanoparticle-modified glass fiber/carbonaceous electrode for the detection of HIV-1 DNA. The ssDNA was assessed using an electrochemical impedance biosensor. The correction of the sensor was achieved between 0.1 pM and 10 nM. The limit of detection was calculated using signal-to-noise ratio of 3 (S/N = 3) as 13 fM. An electrochemical label-free DNA impedimetric sensor was successfully developed, which is highly sensitive and relatively low-cost. A sensitive electrochemical assay was developed to monitor the electrophysiology of HIV-infected cells and treated cells with anti-HIV drugs (Kaushik et al. 2016).

6.3.7 Zika Virus

Zika virus infection is spread by the mosquito bite; it is a viral infection related to neuro-disorders and microcephaly. It is a most severe medical problem globally. Zika virus is similar to that of other flaviviruses (Kostyuchenko et al., 2016). The current approaches to diagnosis of Zika are testing the nucleic acid level at RNA, RT-PCR, and IgM Zika antibody arrest ELISA in serum, cerebrospinal fluid, or urine samples (Huzly et al., 2016). The electrochemical immunosensor made by layer by layer immobilization, of ZIKV-engulf protein antibody together as a single layer of dithiobis deposit on interlinked with microelectrode for the detection of ZIKV. The signal was recorded using the gold electrode, and concentration range

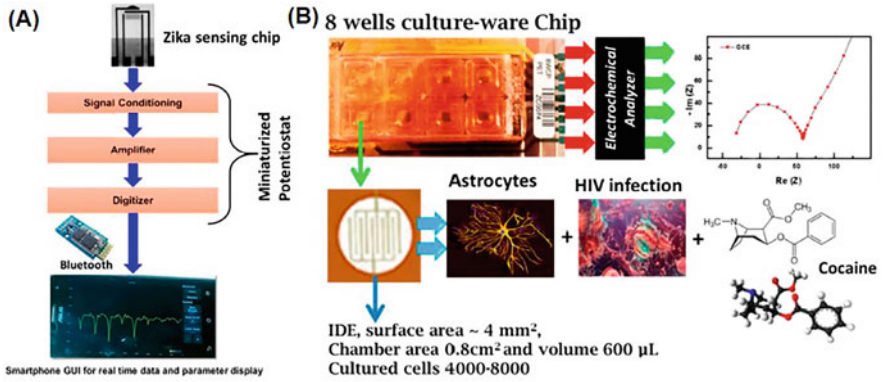

Fig. 6.5 The electrochemical Zika virus immunosensing chip for the detection of Zika-virus envelope protein at 10 pM level (**a**) (Kaushik et al. 2018). The chip-based electrochemical system monitors the electrophysiology of cells during infection and treatment (**b**) (Kaushik et al. 2016)

was between 10 pM and 1 nM. Kaushik et al. (2018) designed a biosensor for diagnosis of ZIKV with the detection limit of 10 pM and has been successfully integrated within the mobile for storage data and at point-of-care data analysis. The ssDNA-based biosensor was designed to detect ZIKV using an oxidized form of glassy carbon electrode adapted with silsesquioxane function immobilized with gold nanomaterial. The biosensor showed a detection limit of 0.82 pmol/L, with a linear way of 1.0×10^{-12}–1.0×10^{-6} mol/L with actual human serum samples (Afsahi et al., 2018). Tancharoen et al. (2019) reported that the complete structure virus is used for immobilization on a gold nanoparticle interlace electrode with graphene oxide and forms a gel polymer. The device detection limit was 1.0×10^{-20} mol/L. Recently, a biosensor based on synthetic ZIKV DNA oligonucleotide immobilization with gold-adapted polyethylene terephthalate (PET) electrode was reported. It showed a limit of 25×10^{-9} mol/L (Faria and Zucolotto, 2019). Steinmetz et al. (2019) reported a similar sensor to Kaushik et al. (2018) using a label-free impedimetric DNA biosensor for ZikaV diagnosis. Again, a similar report of label-free biosensor showing a limit of detection of 25 nM was reported by Faria and Zucolotto (2019). A mobile-based testing approach for quick recognition of Zika, dengue viruses, and chikungunya has been reported. Direct collection of ZikV from human clinical samples such as blood, urine, and saliva was performed by Priye et al. (2017). An effort was made to detect the Zika virus over an electrochemical immunoassay platform (Fig. 6.5) with a real sample for early-stage diagnostic on the site of the epidemic (Kaushik et al. 2018).

6.4 Conclusion and Future Direction

The application of electrochemical assay over miniaturized platform offers several advantages, such as inexpensive and label-free signal amplification. Since the commercial point-of-care application of glucometer, there has been extensive growth in the field of biosensors followed by their utility in various virus sensing applications. Despite the numerous advantages over other probes, the downside of the electrochemical assay, such as poor stability and variability using an antibody as a probe, cannot be neglected and needs to be resolved to have better activity in future research and development. Although engineered and some of the advanced bioprobes such as aptamers, affimers, or small fragment antibodies have remarkable stability, enhanced activity, and low cost compared to other probes for sensing, there is still a need to improve their specificity for high performance. In recent years, nanozyme (nanomaterial with enzymatic properties), DNAzyme, and ribozyme have emerged as suitable and novel systems for sensing applications, but still, these are in their naïve stage and need extensive effort to flourish in the field of biosensing. The functional groups on the surface of enzymes play a vital role in the catalytic activity, especially in the electron transfer process. Modification of their surface properties using different strategies can be used as a target binding without affecting their native properties. Another essential feature of the sensor for virus detection is a better understanding of the underlying mechanism of catalytic activity. The currently available techniques for detecting virus infection are time-consuming or unsuitable as a point-of-care system to fulfill WHO ASSURED guidelines. POC systems have been a much-needed diagnostics approach because of their user-friendliness, easy operation, accessibility in disease sites, and quick diagnostic in the remote areas that lack suitable clinical laboratory setup and expertise. This attribute can be attained by prudently understanding the current clinical need and selecting the suitable bioprobe and transducer platform for sensor design followed by novel surface modification strategies. The toxicity of various components (redox mediators, dyes, or nanomaterials) of the sensor is one of the main challenges that need to be addressed, especially for biomedical applications and health, environmental, and safety concerns. For the implantable and wearable sensor, despite the study in an animal model, its suitability should be carefully tested in the human model with respect to time frame.

The improvement in the current virus sensor by integration with integrated circuits and cutting-edge technologies will enable them for a better point-of-need or point-of-care system for detection of virus infection. The advancement of cutting-edge technology such as artificial intelligence (AI) and the Internet of things (IoT) has also been unified with biosensors nowadays, has enabled the sensors for real-time monitoring of biomarkers to generate bioinformatics needed for disease monitoring, and has provided therapy to optimize in time treatment.

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Continuous Glucose Monitoring for Diabetes Management Based on Miniaturized Biosensors

7

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Abstract

Diabetes is a major global health concern, with 422 million people affected worldwide and more than one million deaths annually. The continuous monitoring of blood glucose levels is one of the most effective ways to treat and manage diabetes and reduce the complications due to this. Nowadays, various laboratory-based technologies are replaced with advanced biosensors to accurately monitor glucose levels in blood samples. The biosensor technology has been continuously developed over the last 50 years to be a frontline diagnostic method, and its further development requires it to perform even more complicated task of monitoring particular analytes in complex biological fluids. For the commercial success of such sensing systems, use of nanomaterials and miniaturization of sensing systems can be two viable options. Selection of proper nanomaterial can enhance the selectivity and sensitivity of the biosensor, and its nonenzymatic behavior can increase its efficiency. Miniaturization of the sensing system makes it affordable and mass producible to use by a wider population. In addition, the development of such miniaturized systems is easy to fabricate and can produce

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_7

portable systems with ease of operations. In this chapter, various miniaturized optical, electrochemical, and wearable continuous glucose biosensors are discussed with the basic mechanism of glucose sensing.

Keywords

Glucose monitoring · Healthcare technologies · Point-of-care · Devices

7.1 Introduction

Diabetes is a health condition that arises due to the insufficient production of insulin in the human body or lack of receptors to use the insulin for glucose breakdown leading to very complicated body metabolism. Diabetes is associated with a hyperglycemic condition (a higher sugar level than the normal 80–120 mg/dL (4.4–6.6 mM) in blood) and can lead to cardiovascular diseases, vision impairment, stroke, nerve damage, foot ulcer, and even death. According to the International Diabetes Federation (IDF), 537 million adults (aged 20–79) are now diabetic, and the number of new cases has been tripled in the last 20 years. A significant proportion of the population is now diabetic (10.5%), and it is estimated that the global diabetic population will be 643 million by 2030 (www.diabetesatlas.org). A changed lifestyle with high calories/processed food intake, less physical activity, and hereditary characters are believed to be the main reason for diabetes. Achieving and maintaining the blood glucose level in the body at a certain threshold is the most important way to achieve a healthy life for diabetes patients and to avoid serious health conditions. According to Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), a regular test to tightly maintain the glucose level at a prescribed level leads to a reduced risk of major vascular and neurological diseases (Diabetes Control and Complications Trial Research Group et al. 1993; Holman et al. 2008). For that, a regular measurement of glucose level is important, and a self-test is more convenient than a physician-assisted test on a daily basis. Self-monitoring of blood glucose (SMBG) devices have been an important part of diabetes management for the last 20 years, and it helps not only monitoring the glucose level but also to prescribe insulin dose and frequency, nutrition, and food uptake. The SMBG is recommended for patients who undergo at least three-time insulin uptake daily, and it is found to be effective for glucose level management (Rodbard 2007). Though SMBG devices are clinically and commercially highly successful, the major disadvantages of these systems are that they provide information on isolated glucose levels, have painful invasive procedures of using a needle, and are time consuming. The continuous glucose monitoring devices (CGM) are now used more than SMBG due to noninvasive procedures and the ability to provide glucose level information throughout the time worn by the individual (Klonoff 2005; Girardin et al. 2009; Vazeou 2011).

The major advantages of using a CGM device over SMBG devices are as follows (Patton and Clements 2012):

(a) Most of the commercial CGM devices take a reading every 5 min (up to 288 test a day), enabling the fluctuation in blood glucose levels monitored in real time.
(b) The required sample volume for analysis is less than for SMBG, and mostly ISF fluid is used for analysis offering a noninvasive/minimally invasive solution.
(c) CGM devices can be attached to the upper arm, abdomen, or thigh, and a person can do his daily work without worrying about the devices.
(d) Hypoglycemic condition during a prolonged hyperglycemic condition, which is usually gone undetected by SMBG, can be detected here and can be treated in time.
(e) An alarm can be set for the hypo- or hyperglycemic condition.
(f) Accuracy of CGM, especially in normal glucose levels, is very high.

The working principle of all types of glucose biosensors, including the CGM devices, can be grouped on the basis of how the reaction center interacts with the electrode surface. The first-ever glucose biosensor, proposed by Clarks and Lyons, is a first-generation biosensor, where they described the use of glucose oxidase and oxygen as the final electron acceptor (Clark and Lyons 1962). In the first-generation biosensors, hydrogen peroxide is monitored, which is produced as a byproduct of the conversion of glucose to gluconic acid. The next phase of development, the second generation of glucose sensors, uses a mediator or an artificial electron acceptor for the oxidative reaction of glucose to gluconic acid. The second-generation sensors are based purely on the reduction potential of the analytes and do not require a large surface area nor a large sample amount to detect the glucose. This development enabled the use of a disposable electrode and made glucose sensing possible by a truly miniaturized device. The most advanced type, third generation of glucose biosensors, is based on the concept of the direct electron transfer between the enzyme reaction center and electrode. The concept is based on the involvement of flavin adenine dinucleotide (FAD)-glucose dehydrogenase, which is a three-subunit enzyme with a catalytic center, a small subunit, and an electron transfer subunit (Lee et al. 2021). Electrons produced at the reaction center by the oxidation of glucose are transferred to the electron transfer subunit and finally to the sensing electrode surface via FAD, 3Fe-4S cluster, and heme subunits. The third-generation biosensors are ideal for measuring glucose in various body fluids as they do not require any external mediator or chemicals, require a very small amount of sample volume, and can generate a signal at very low potential eliminating the chances of interference from co-existing molecules (Teymourian et al. 2020). The types of glucose biosensors based on their sensing mechanisms into various generations are summarized graphically in Fig. 7.1. The reaction mechanism at the working electrode for first-generation biosensors is

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\text{GOx} - \text{FADH}_2 + \mathbf{O}_2 \rightarrow \text{GOx} - \text{FAD} + \mathbf{H}_2\,\mathbf{O}_2$$

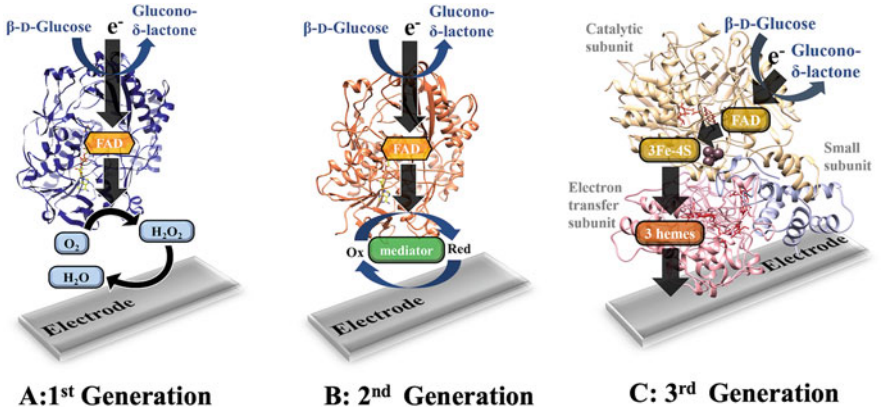

Fig. 7.1 The working principles of (**a**) first-, (**b**) second-, and (**c**) third-generation biosensors grouped on the basis of requirement of oxygen, mediator (or artificial electron acceptor), and path of the electron from reaction center to the electrode surface (reused with permission from Lee et al.)

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e$$

and for second-generation biosensors is

$$\text{Glucose} + \text{PQQ(ox)} \rightarrow \text{Gluconolactone} + \text{PQQ(red)}$$

From the Clarke–Lyon proposal on the early biosensors to today's wireless sweat glucose monitoring devices, the glucose biosensors have seen some of the biggest milestones that reshaped the next decades of research and development. Glucose biosensors are the most successful commercial biosensors, and they contribute close to 80% of the total biosensor market. The discovery of glucose-1-oxidase by Muller in 1928 prompted subsequent additions of electron mediators and other enzymes and their cofactors for further development in glucose sensing (Wilson and Turner 1992). Of the four types of enzyme reported to oxidize glucose as a principal substrate, (a) glucose dehydrogenases, (b) quinoprotein glucose dehydrogenases, (c) glucose-1-oxidases (GOx), and (d) glucose 2-oxidases, GOx is most commonly used in biosensor development. The subsequent important developments includes the following: 1956, the first biosensor (Clarks oxygen electrode); 1957, glucose oxidase used in CLINISTIX test strip by Ames; 1962, amperometric glucose biosensor was conceptualized; 1967, first practical enzyme electrode developed by Updike and Hicks; 1973–1975, Yellow Springs Instruments developed the first commercial glucose biosensor (Model 23A YSI analyzer); 1980, whole blood standard dextro meter by Ames with a digital display; 1982, Shichiri developed the first needle-type enzyme electrode for subcutaneous implantation; 1982, first fiber-optic-based biosensor for glucose developed by Schultz; 1984, first ferrocene-mediated amperometric glucose biosensor; 1992, i-STAT hand-held blood analyzer launched; 1999, third-generation glucose biosensors by Medtronic; 2000, wearable

noninvasive glucose meter GlucoWatch was launched; 2002, first implantable continuous glucose monitoring device "MiniMed" by Medtronic was launched; and 2013, smartphone integration into self-monitoring blood glucose devices (adapted from Yoo and Lee 2010; Teymourian et al. 2020).

The first CGM device to be approved by FDA is Medtronic Minimed Gold, a wired type first-generation electrochemical biosensor inserted under the skin (https://www.medtronicdiabetes.com/about-medtronic-innovation/milestone-timeline). Dexcon Inc. released its first CGM to market in 2006 with wireless transmission (https://www.dexcom.com/about-dexcom). Abbott in 2008, released the first second-generation CGM device, which uses a hydrogel with an osmium compound as the redox mediator molecules and immobilizes it with GOx for the glucose level determination. Most of the CGM devices use first- or second-generation biosensor principle for the detection of glucose level, with no third-generation biosensor yet in the market. The CGM biosensors are superior in detecting the glucose trend in the blood or other body fluids, which can be used for the recommendation of nutrition uptake or a period of no food uptake for the glucose level to come down (like Abbott FreeStyle Navigator II), as shown in Fig. 7.2 (Vashist 2013). The Medtronic's MiniMed 670G hybrid closed-looped system became the first FDA-approved glucose monitoring–insulin pump-integrated system for the insulin injection to the body based on the trend of basal glucose level. This became the first biosensor to be used for automated administration of medication solely based on the finding of the biosensor and opened a new era for more such developments (https://www.fda.gov/medical-devices/consumer-products/artificial-pancreas-device-system).

7.2 State of-the-Art Miniaturized CGM Sensors

Biosensors are bioanalytical devices comprising of three important units, i.e., a bio-recognition element (BREs), a transducer, and a processor that collectively converts a biochemical reaction to a measurable signal (Mahato et al. 2017; Mahato et al. 2018b; Purohit et al. 2019a; Purohit et al. 2020d). The BREs such as enzymes, peptides, DNA probes, or aptamers recognize the target molecule by their affinity or catalytic activity based on their inherent properties and lead to a change in reaction parameter (Baranwal and Chandra 2018; Mahato et al. 2018a). The transducer converts the change to a measurable signal, and based on transduction type, it is mainly grouped into optical, electrochemical, or mechanical biosensors, and the processor converts the signal into a digital output (Kumar et al. 2019c; Mahato et al. 2019; Vernekar et al. 2021). The advancement in nanomaterial synthesis and their exponential characterization in recent years have made them an integral part of biosensors (Mahato et al. 2020c; Purohit et al. 2020a). The role of nanomaterials as a signal-generating molecule (acting as an enzyme mimic) and signal amplification have been widely used in making the biosensors more sensitive toward the target molecules (Purohit et al. 2019b; Kumar et al. 2019b; Mahato et al. 2020b; Purohit et al. 2020b; Kumar et al. 2020b; Divya et al. 2021). In addition, the surface engineering and surface chemistry of these nanomaterials play an important role in

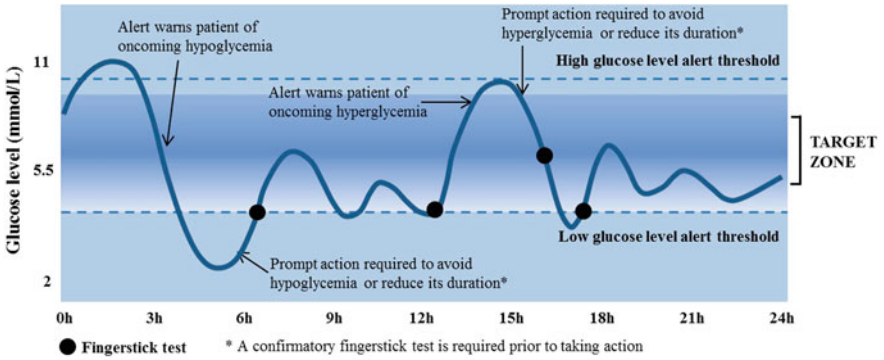

Fig. 7.2 Blood glucose level trends detected by CGM device and its implication for further clinical interventions (Reproduced with permission from Vashist 2013)

the proper arrangement of the BREs and to create an antifouling surface (Kumar et al. 2019a; Kumar et al. 2020a; Mahapatra et al. 2020; Mahato et al. 2020a; Mahato et al. 2021). The recent trends and developments in continuous glucose biosensors can be discussed under electrochemical, optical, and wearable sensors.

7.2.1 Electrochemical Systems

Electrochemical biosensing allows miniaturization of the devices and a robust detection of target molecules at the electrode surface, which selectivity and sensitivity can be used by using various functional nanomaterials and surface chemistry (Prasad et al. 2016; Chandra and Prakash 2020). Electrochemical sensors have been used for glucose detection based on the nonenzymatic property of the nanomaterial-modified surfaces and/or the catalytic activity of GOx (Noh et al. 2012; Kadian et al. 2018). The reaction of GOx with glucose generates hydrogen peroxide, and several reports have used the degradation of peroxide radical as a signal-generating step, which can be further catalyzed by various nanomaterials.

Chen et al. developed a needle-based CGM using a PVDF and Nafion-coated two-layered selective membrane for prolonged in vivo analysis (Chen et al. 2015). The fabricated electrode and its working mechanism are shown in Fig. 7.3a, where they used Au and Pt nanoparticles to create a good conducting layer between the electrode and the GOx for selective glucose detection and the polyaniline-Pt scaffold to efficiently immobilize the enzyme on the surface. They hypothesized that the bilayered membrane would inhibit the movement of interfering molecules, limit the excess glucose, and compensate the oxygen deficiency for the reaction. The CGM device was able to detect glucose levels in the 0–20 mM range and a shelf-life of 21 days. Ribet et al. reported another minimally invasive method by inserting a three-electrode system in the lumen of a single hollow microneedle to detect glucose in ISF fluids (Ribet et al. 2018). Bae et al. developed a fully stretchable

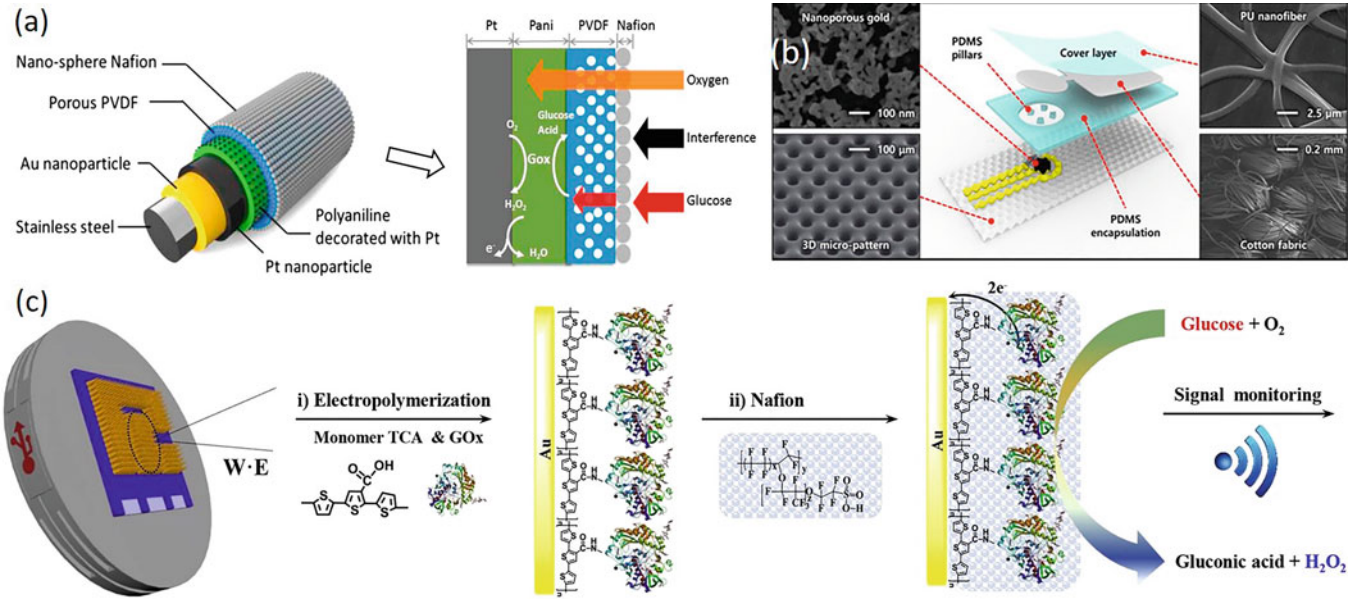

Fig. 7.3 Electrochemical CGM devices. (**a**) A PVDF–Nafion-coated selective membrane for long shelf life of the electrochemical CGM sensor (reproduced with permission from Chen et al. © Elsevier); (**b**) A stretchable electrochemical glucose sensor using a nanoporous gold electrode (reproduced with permission from Bae et al. © American chemical society); (**c**) A conducting monomer and Nafion-modified microneedle-based CGM device with wireless monitoring (reproduced with permission from Kim et al. © Elsevier)

electrochemical sensor using a nanoporous gold electrode for nonenzymatic continuous detection of glucose in sweat samples, as shown in Fig. 7.3b (Bae et al. 2019). Sharma et al. reported a microneedle array electrode-based CGM sensor, where the epoxy-based microneedle was metalized with platinum or silver to get a working or reference electrode (Sharma et al. 2016). Then the electrode was functionalized with a polyphenol layer to capture GOx and shown to detect glucose in 0.5–30 mM. The sensor was stable for 3–6 h in vivo diagnosis. Kim et al. developed another microneedle-based assay by immobilizing GOx on a terthiophene carboxylic acid-electrodeposited AuNP-modified microneedle (Kim et al. 2019). Nafion was used as an antifouling layer, and the sensor was able to detect glucose in the range of 0.05–20 mM. The sensor was able to detect glucose in finger-pricked blood, and the response can be monitored wirelessly (Fig. 7.3c). Zhao et al. used a silk-D-sorbitol composite-modified microneedle-based sensor to create a biocompatible sensor (Zhao et al. 2020). The Pt working electrode and Ag wire counterelectrode detect Gox-generated hydrogen peroxide to measure the glucose concentration in the ISF. Pu et al. improvised such systems by using a cylindrical electrode to get a bigger working electrode surface and modified it with graphene–Pt nanoparticles for improved sensitivity (Pu et al. 2018). The printed and flexible electrode was able to detect glucose continuously in the subcutaneous region of the rat model. Xiao et al. developed another radiofrequency identification-based wireless implantable sensor system that can detect glucose in the range of 0–30 mM (Xiao et al. 2015). The major parts of the devices were a ferrite antenna, a system on chip, and an electrochemical sensor encapsulated inside a biocompatible membrane. Hossain et al. reported a Pt nanoparticle and glucose-treated reduced graphene oxide-based glucose detection system, where chitosan was used to immobilize GOx enzyme and increase sensitivity toward glucose (Hossain and Park 2016). The sensor was able to detect glucose in the range of 0.02–10 mM. Pu et al. reported another CGM using a graphene–AuNP–Gox-modified electrode in a microfluidic system, where the microfluidic system was used to extract ISF noninvasively (Pu et al. 2016). The sensor achieved an LDR of 0–162 mg/dL and an LOD of 1.44 mg/dL and can be used continuously for 48 hours. Although the electrochemical sensors are sensitive and selective, they is prone to biofouling in the presence of complex body fluids, for which Nafion and other coatings are applied over the electrode surface to maintain their stability.

7.2.2 Optical Systems

Optical detection of glucose levels in blood, urine, and tear samples was a standard clinical procedure before the invention of biosensors and other sensitive techniques. All the early detection methods (Benedict's copper reagent, copper reagent tablet developed by Ames, the first biosensor with glucose oxidase developed by Ames) monitored the change in color as a result of the breakdown of glucose are optical detection methods of glucose. Later, several advanced biosensors based on mostly near-infrared (NIR), mid-infrared (MIR), Raman, photoacoustic sensing,

fluorescence, and optical coherence tomography (OCT) were developed to measure glucose in body fluid (Jernelv et al. 2019). The naked eye detection-based optical biosensors are developed due to their ease of detection and simple working principles. Gabriela et al. developed a paper-based colorimetric detection device by using a 3,3′,5,5′-tetramethylbenzydine (TMB) as a chromogenic substance (Gabriel et al. 2017). GOx, HRP, and TMB was immobilized in the chitosan-modified μPADs, and in the presence of glucose in tear, the reaction will follow a oxidation–reduction cycle to generate a visible blue color (the sensor fabrication and working principle are shown in Fig. 7.4a). The color change can be quantified by using an office scanner, and the biosensor can detect tear glucose linearly in the range of 0.1 and 1.0 mM and an LOD of 50 μM. Zhang et al. developed another visual glucose sensor, where they monitored the color change due to the etching of gold nanorods by hydrogen peroxide released due to GOx reaction. They used MoO_4^{2-} as a catalyst in this indirect measurement of glucose, and this can be quantified by monitoring the peak shift of longitudinal LSPR bands (Zhang et al. 2017). Karim et al. developed another glucose sensor by using an enzyme mimicking silver nanoparticle-modified cotton fiber, termed Ag@Fabric, using TMB as the chromogenic agent (Karim et al. 2018). Surface plasmon resonance (SPR), another optical technique, is a commonly used method to detect various analytes based on the change in the local refractive index at the metal interface and does not damage the target analytes. Yuan et al. developed a fiber-optic-based SPR biosensor sensitive for detecting glucose in the range of 0.01–30 mM and with an LOD of 80 nM (Yuan et al. 2018). They used a p-mercaptophenylboronic acid (PMBA) SAM over an AuNP-modified fiber-optic surface that can interact with glucose and used Au NPs/AET-PMBA as a signal amplification tag to cause a significant SPR shift for the low level of glucose detection. Lin et al. used a temperature tunable poly (N-isopropylacrylamide) (PNIPAAm) hydrogel-based fiber-optic biosensor to develop GOx pockets to detect glucose when a particulate temperature is applied (Lin et al. 2019). The activity of the sensor can be controlled by applying an external temperature and can be multiplexed with other target analytes. Lobry et al. developed a glucose biosensor based on the interaction of concavaline A (Con A) with saccharides, where they immobilize Con A on a polydopamine layer-modified AuNP U-shaped fiber-optics, as shown in Fig. 7.4b (Lobry et al. 2019). The polydopamine layer results in increased insensitivity toward changes in pH, temperature, and interfering molecules bringing a stability to glucose sensing. The biosensor achieved an LDR and LOD of 10^{-8} to 10^{-2} M and 10^{-8} M, respectively. Chen et al. combined a 2D photonic crystal layer (shown in Fig. 7.4c) with 4-boronobenzaldehyde (4-BBA) and poly(vinyl alcohol) (PVA) to form a hydrogel-based matrix and achieved an LDR of 0–20 mM for tear glucose monitoring (Chen et al. 2018). Glucose binds to boronate, altering the volume of PVA, changing the Bragg's diffraction, and can be used for the detection in the physiological range. Lin et al. reported a smartphone-integrated CGM for tear glucose monitoring by using a phenylboronic acids (PBA) and poly(2-hydroxyethyl methacrylate) (HEMA) hydrogel-based contact lens, where the interaction of glucose with PBA swells the contact lens (Lin et al. 2018). This change in thickness can be detected and analyzed using Gullstrand's equation by the smartphone. The working

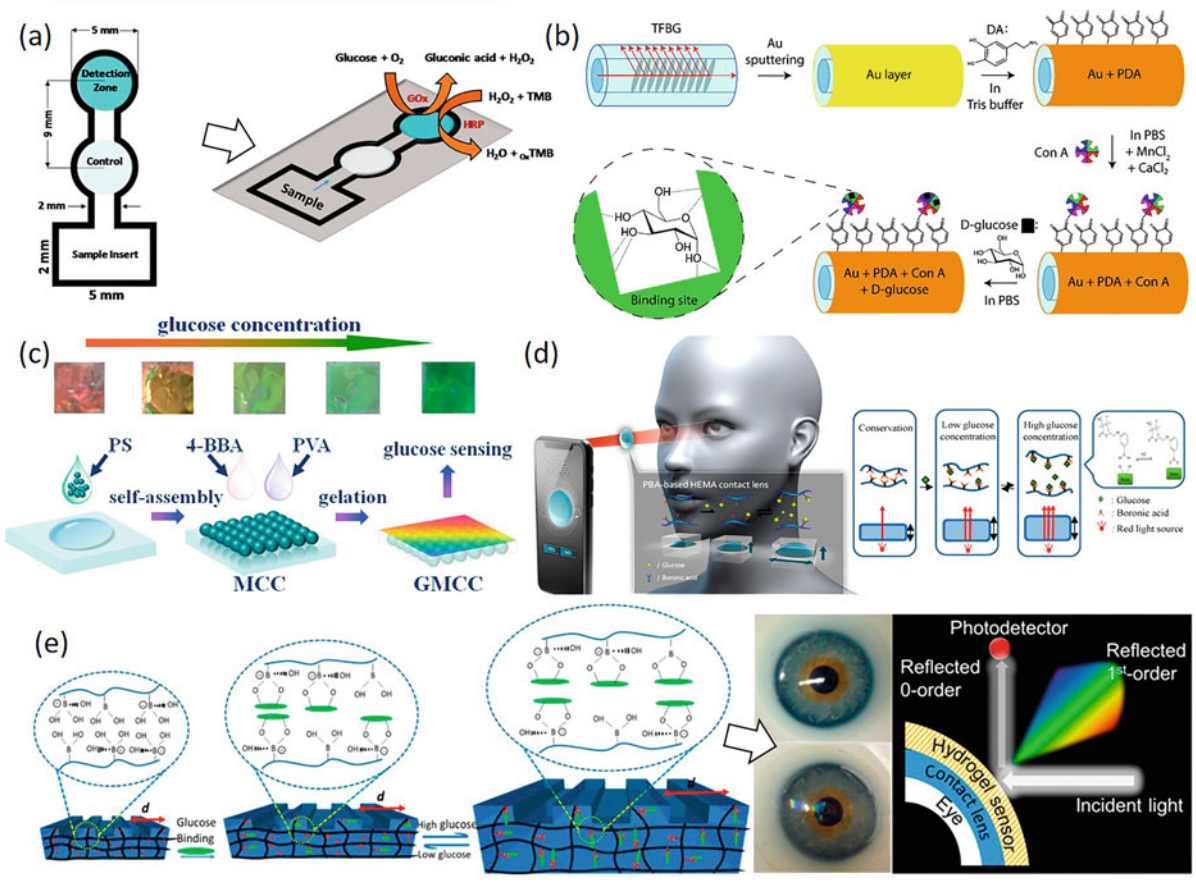

Fig. 7.4 Optical continuous glucose monitoring devices. (**a**) A paper-based colorimetric sensor for tear glucose monitoring (reproduced with permission from Gabriel et al.); (**b**) Con A functionalized fiber-optic biosensor (reproduced with permission from Lobry et al. © Elsevier); (**c**) 2D crystal photonics-based optical

principle and sensing mechanism of the biosensor are shown in Fig. 7.4d. Elsherif et al. developed a tear glucose monitoring sensor by printing a layer of photonic crystal hydrogel of certain periodicity on a contact lens, and when the hydrogel swells in the presence of glucose, it changes the overall periodicity (Elsherif et al. 2018). The change in glucose levels in the range of 0–50 mM can be correlated by monitoring the space between zero- and first-order spots in Bragg's diffraction. The change in periodicity of hydrogel in the presence of glucose and sensing mechanism is shown in Fig. 7.4e.

7.2.3 Wearable Systems

Wearable point-of-care devices offer continuous sensing of the target analytes found in various noninvasive fluids (Teymourian et al. 2021; Mahato and Wang 2021). The wearable sensors do not come in direct contact with blood to induce any contamination, and most of them are painless and noninvasive. However, the sensors are more prone to sensor instability and biofouling and receive inadequate sample volume for diagnosis. The sensor design and fabrication are also more complex than traditional electrodes and need person-to-person calibration (Purohit et al. 2020c). However, the wearable sensors are more personalized than other sensors and can be automated and integrated with other diagnostic methods for effective sensing. Gao et al. reported a fully integrated wristband multiplexed glucose sensor for the detection of glucose in perspiration on a flexible platform (Gao et al. 2016). The sensor was constructed on a PET substrate and a silicon-based fully integrated circuit for the data analysis, where the working electrode was fabricated by using a chitosan–carbon nanotube composite with GOx. The sensor was capable of autonomous sample collection and analysis of the analytes in the sample. Emaminejad et al. later incorporated reverse iontophoresis into the fully autonomous glucose sensor platform for effective sweat extraction, as shown in Fig. 7.5a (Emaminejad et al. 2017). They used various sweat-inducing chemicals in the iontophoresis hydrogel for the collection of sweat, and the release of the chemicals was controlled externally. Both these works involved the development of a wireless communication of the sensor with the other devices and possible cloud storage of the data. Zhao et al. reported a self-powered fully automated sweat glucose sensor, where they used flexible photovoltaic cells for solar energy harvesting and Zn-MnO_2-based aqueous electrolyte for energy storage (Zhao et al. 2019). The device was capable of sensing without any external charging or battery. He et al. reported another fully integrated multiplexed device, which was entirely fabricated on a textile platform termed silk fabric-derived intrinsically nitrogen (N)-doped carbon textile (SilkNCT) (He et al. 2019). Recently, Yu et al. developed a biofuel-powered wireless wearable device

biosensor (reproduced with permission from Chen et al. © American Chemical Society); and glucose-responsive hydrogel-based biosensors printed on contact lens for tear glucose monitoring based on (**d**) PBA and HEMA (reproduced with permission from Lin et al.); (**e**) PBA and photonic crystal (reproduced with permission from Elsherif et al. © American Chemical Society)

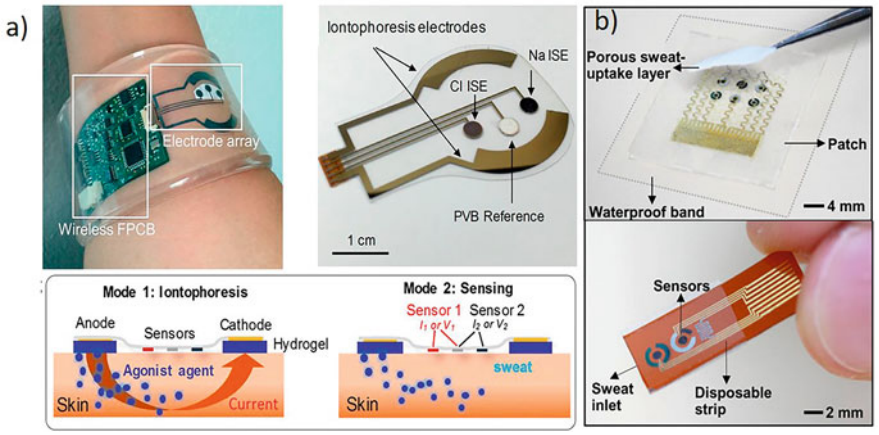

Fig. 7.5 Wearable devices for continuous glucose sensing based on a stretchable electronic platform. (**a**) Wearable CGM with multiplexed features and sweat extraction module injecting agonist agent (reproduced with permission from Emaminejad et al. © 2017 National Academy of Sciences). (**b**) Wearable CGM patch with porous material for sweat uptake and the electrode for glucose sensing (reproduced with permission from Lee et al. © 2017 American Association for the Advancement of Science)

capable of multianalyte detection (Yu et al. 2020), where they used a lactate reaction-based perspiration-powered integrated electronic skin (PPES) for power generation. The PPES was capable of generating 3.5 mW cm^{-2} and continuous measurement for 60 h without external power uptake. Lee et al. developed a disposable multiplexed system capable of glucose sensing in sweat with real-time optimization of the value in relation to the changing temperature, pH, and other parameters (Lee et al. 2017). Figure 7.5b shows the microneedle-based sweat extraction patch and the working electrode design, where the porous gold-based sensing surface was modified with GOx. The wearable sweat sensors are prone to errors due to the external parameters, and this work tried to minimize that error by using a multilayered patch, and the sensor was integrated with a drug-releasing module. The GlucoWatch® biographer was a commercial wearable CGM device based on a wristwatch-like platform and uses reverse iontophoresis technique to detect glucose by the amperometric method (Tierney et al. 2001). The device was able to detect glucose every 20 min and was based on the first-generation principle of sensing. Another wearable noninvasive device is GlucoTrack®, where the sensor detects glucose level when placed on the ear lobe. An innovative solution developed by Glucowise™ is currently under human trial (www.gluco-wise.com), where a 40-Hz radiofrequency is used to monitor the capillary-level glucose concentration, and its noninvasive, cost-effective, and needle-less technique and wireless data transmission can be used successfully in the commercial market. A detailed account on the commercially available wearable noninvasive CGM devices is discussed in the next section. A comparative account on the development of different types of CGM devices is summarized in Table 7.1.

Table 7.1 Electrode configuration and sensing parameters of a recently developed continuous glucose sensor

SI. no.	Sensor matrix	Enzyme/sensing method	Testing model	Sensing performances	References
1	PtNP/graphene-modified cylindrical flexible sensor	Electrochemical—GOx in a Nafion layer	In vivo (rat model)	LOD: 3.54 mg dL^{-1} LDR: 0 to 570 mg dL^{-1} Sensitivity: 0.354 nA/(mg dL^{-1})	Pu et al. (2018)
2	Wirelessly powered RFID	Electrochemical—GOx	Subcutaneous skin	LOD: 0.75 nA/mM LDR: 0 to 30 mM Sensitivity: 0.01 μA·mm^{-2}	Xiao et al. (2015)
3	Terthiophene carboxylic acid (TCA)/gold-coated microneedle array	Electrochemical—FAD-GOx	Finger pricked blood	LDR: 0.05 to 20.0 mM Sensitivity: 0.22 μA/mM^{-1} cm^{-2}	Kim et al. (2019)
4	Microneedle array electrodes (MICoMS) with polyphenol (PP) film	Electrochemical—GOx	In vivo (inserted into the forearm)	LOD: 0.5 mM LDR: 10 to 1 mM	Sharma et al. (2016)
5	RGO/activated carbon (AC)/PtNP and chitosan	Electrochemical—GOx in chitosan and Nafion layer		LOD: 2 μM LDR: 0.002 mM to 10 mM Sensitivity: 61.06 μA/$mMcm^2$	Hossain and Park (2016)
6	PVDF with AuNP, PtNP, and porous polyaniline layer	Electrochemical—GOx	Mice	LDR: 0 to 20 mM Sensitivity: 0.23 μA/mM	Chen et al. (2015)
7	Hollow microneedle lumen with platinum working electrode	Electrochemical: GOx in a BSA-GA layer	Forearm skin	LDR: 0 to 14 mM Sensitivity: 1.5 nA/mM	Ribet et al. (2018)
8	Silk/D-sorbitol pyramidal microneedles	Electrochemical—GOx	Artificial ISF	LDR: 1.7 to 10.4 mM	Zhao et al. (2020)

(continued)

Table 7.1 (continued)

SI. no.	Sensor matrix	Enzyme/sensing method	Testing model	Sensing performances	References
9	Graphene/AuNPs	Electrochemical—GOx	ISF	LOD: 1.44 mg/dl LDR: 0 to 162 mg/dl	Pu et al. (2016)
10	Stretchable nanoporous gold	Electrochemical	Sweat	LDR: 0.01 to 1 mM Sensitivity: 253.4 μA cm^{-2} mM^{-1}	Bae et al. (2019)
11	UV–vis–NIR spectrophotometer	Optical	In vitro phantom	LDR: 0 to 400 mg/dL	Park et al. (2020)
12	Photonic crystal (PC) with 4-boronobenzaldehyde-functionalized poly (vinyl alcohol) hydrogel	Optical nonenzymatic sensor monitoring Bragg diffraction	Tear samples	LDR: 0 to 20 mM	Chen et al. (2018)
13	Paper-based colorimetric sensor using TMB as colorimetric agent	Optical—GOx and HRP	Tear samples	LDR: 0.1 to 1.0 mM LOD: 50 μM Sensitivity: 84 AU/mM	Gabriel et al. (2017)
14	2D polystyrene CCA with 3-APBA functionalized hydrogel	Optical nonenzymatic	Human urine	LDR: 0 to 10 mM	Yan et al. (2016)
15	Molybdate (MoO_4^{2-}) and gold nanorod	Optical—GOx	Human urine	LDR: 0 to 30 μM LOD – 0.1 μM	Zhang et al. (2017)
16	Ag@fabric	Optical—GOx	Urine sample	LDR: 0.1 to 2 mM LOD: 0.08 mM	Karim et al. (2018)
17	Epidermal microfluidic device	Optical—GOx	Sweat	LDR: 0 to 20 mM LOD: 200 μM	Koh et al. (2016)
18	μPAD-based colorimetric sensing	Optical nonezymatic sensor using 4-AAP and DHBS as chromogenic solution		LOD: 27 μmol L^{-1}	de Castro et al. (2019)
19	PBA-based HEMA contact lens	Optical with boronic acid in HEMA and glucose	Tear glucose	LDR: 0.1 to 0.6 mM	Lin et al. (2018)

7.3 Commercialized CGM Devices

There is a huge economic potential of CGM devices in the management of both type 1 and II diabetes as they help in managing it better than other blood glucose sensors. The market value of the CGM is expected to see a growth of 10% in North America, 20% in Asia Pacific and 19% in Middle-East and Africa, and 16% in Europe in that period. In 2020, North America contributed to 93% of the global market value with an anticipated market value of USD 4.5 billion in 2026 (https://www.mordorintelligence.com/industry-reports/continuous-glucose-monitoring-market). Currently, four manufacturers, i.e., Abbott (FreeStyle Libre and Freestyle Libre 2), Dexcom (G6), Medtronic (Guardian Connect and Guardian Sensor 3), and Senseonics Eversense, are the major manufacturers of CGM devices.

Medtronics uses a three-electrode-based sensing principle with a wired first-generation sensor, where platinum-based working and counter electrodes and Ag/AgCl reference electrode are used. GOx is used as the enzyme, and glutaraldehyde is added as the cross-linker to avoid enzyme leaching. Polyamine and siloxane-based membrane is used to create a diffusion layer for selective glucose permeability and prevent glucose saturation.

Daxcom sensor is a two-electrode-based sensor with platinum/platinum-iridium working electrode and Ag/AgCl reference electrode and monitors hydrogen peroxide oxidation. Polymers are used to cover the sensor surface for selectivity, and GOx is used as the catalytic enzyme, which is again protected with a polymer layer such as perfluorocarbons or silicone-doped polyethylene glycol, which ensures oxygen availability for the reaction and reduces the leaching of the enzyme.

Abbott CGM devices use a second-generation glucose-sensing principle and uses a GOx–osmimum complex in a three-electrode-based detection system. The electron mediator osmium complex eliminates the requirement of oxygen in the reaction and can be operated at a lower potential than other devices. Abbott also uses a semipermeable membrane based on poly-pyridine or poly-imidazole for effective glucose flux control.

Senseonics is the only commercially available nonenzymatic/optical CGM device with a fluorescent-based sensing principle, where the interaction of glucose with fluorophore-linked boronic acid is used for quantification of glucose. An increase in glucose concentration leads to the inhibition of the fluorescent quenching by boronic acid, and PMMA layer is used for glucose flux control.

The commercial CGM devices face the problem of interference from molecules such as acetaminophen and ascorbic acid, need to be calibrated regularly (for some, twice a day), need to be replaced after a maximum of 14 days, and need to face the issues of accuracy. The commercialized sensors have been used in various clinical studies and found to be more efficient in controlling the glycemic condition than the blood glucose monitoring devices. Recently, the CGM devices are more progressively being integrated with insulin pumps to create a close-loop system for better diabetes management. The major products of these manufacturers are shown in Fig. 7.6, and their sensing parameters are compared in Table 7.2.

Fig. 7.6 Commercial CGM devices. (**a**) FreeStyle Libre Pro sensor (https://www.freestyle.abbott/in-en/products/freestyle-libre-pro); (**b**) Dexcom G6 CGM system (https://www.dexcom.com); (**c**) the MiniMed™ 770G System; (**d**) MINIMED™ 640 g INSULIN PUMP SYSTEM and THE GUARDIAN™ CONNECT SYSTEM (www.medtronicdiabetes.com/treatments/continuous-glucose-monitoring); and (**e**) the Eversense CGM System (https://www.contournext.com/eversense/eversense-cgm-system)

7.4 Close-Loop CGM-Insulin Pump Integration

After the success of the early CGM devices, FDA in 2006 approved the testing of a close-loop system (an insulin delivery system controlled by glucose-sensing data) with a goal to make diabetes management more advanced than ever before (Mastrototaro et al. 2006). The major components in this are the CGM device, a transmitter, and the insulin pump. The major insulin pump manufacturers are Medtronic Inc., Roche Diagnostics, Animas®, and Insulet Corporation (Walsh et al. 2015). As one of the most successful products, the MiniMed™ 670G system from Medtronic (https://www.medtronic-diabetes.co.uk/insulin-pump-therapy/minimed-780g-system) is such a CGM integrated insulin pump system that the system automatically adjusts itself to avoid blood glucose level high and lows. The insulin pump is integrated with Guardian™Sensor 3 CGM system, and the glucose level can be viewed in CareLink™ Connect app. The pump gets the glucose

Table 7.2 Commercially available CGM devices and their sensing parameters

Product	Fingerstick calibration	Transduction method	Age of use	Warm up	Wear length	Alarm	Data display	On-body form and transmitter design	Cost
Freestyle Libre	0—Factory calibrated	Amperometric—second generation with GOx	18+ years	1 h	14 days	None	Reader, Android and iPhone apps (FreeStyle Libre Link)	~2 stacked quarters, one-press insertion, fully disposable transmitter integrated with sensor patch	$60 for the sensor scanner, $40 per sensor
Freestyle Libre 2	0—Factory calibrated	Amperometric—second generation with GOx	4+ years	1 h	14 days	Yes	Reader	~2 stacked quarters, one-press insertion, fully disposable transmitter integrated with sensor patch	$60 for the sensor scanner, $60 per month
Dexcom G6	0—Factory calibrated	Amperometric—first generation with GOx	2+ years	2 h	10 days	Yes	Receiver, Android and iPhone apps, smartwatches	Eraser-sized transmitter, one-button inserter, three-month use transmitter separate from sensor	$300 for the transmitter, $600 for the receiver, and $80 per sensor
Medtronic Guardian Sensor 3	2/day minimum	Amperometric—first generation with GOx	7+ years 14+ years (Guardian Connect)	2 h	7 days	Yes	Guardian Connect Android and iPhone apps	~2 stacked quarters (clamshell), one-button inserter, rechargeable transmitter separate from sensor	$1000 for the transmitter, $80 per sensor

(continued)

Table 7.2 (continued)

Product	Fingerstick calibration	Transduction method	Age of use	Warm up	Wear length	Alarm	Data display	On-body form and transmitter design	Cost
Senseonics Eversense (US)	2/day minimum	PET fluorescence with boronic acid	18+ years	24 h after implantation	90 days	Yes	Android and iPhone apps	Sensor inserted by a healthcare professional, rechargeable, watchface-sized transmitter separate from sensor	$1400 for the initial package, $200 to $300 for insertion (90-day life)

level signal from the CGM through the transmitter, and the pump maintains a steady glucose level. The plastic cannula of the pump is replaced every 2–3 days, whereas the sensor is replaced every 7 days. The device is recommended for people above 7 years of old and a minimum daily dose of 8 units. Similarly, Daxcom CGM system is integrated with t:slim X2 insulin pump for type-1 diabetes management, and Omnipod DASH® for the management of both type-1 and -2 diabetes management. Tandem's Control-IQ technology launched the first FDA-approved algorithm to be used in insulin dosing devices, and after this, many other manufacturers have developed their own algorithm for optimal insulin release. In future, new combinations of these algorithms and devices can be used for better management of diabetic healthcare.

7.5 Challenges and Opportunities

7.5.1 Sensor Error Measurements

The variation in the real-life sensor measurement and the reference measurement is known as the error in CGM monitoring. Though it can be measured in terms of standard deviation or variance, an error grid with color code is typically followed by clinicians to measure the error in personalized devices. The Clark Error Grid or the Surveillance Error Grid is followed for the accuracy of the data and monitors the extreme states of glucose level in a patient (Klonoff et al. 2014). International Standards Organization (ISO) in 2013 developed the ISO 15197–2013 standards for glucose monitoring device manufacturers to follow a set of regulations based on the error grid: "(a) 99% of results must fall within zones A + B of the Consensus Error Grid (CEG) for T1; (b) 95% of results >5.5 mmol/L must fall within 15% of the reference method; (c) 95% of results <5.5 mmol/L must fall within 0.83 mmol/L of the reference method" (https://www.iso.org/standard/54976.html). The time of calibration of these sensors is important to minimize the errors in calculations. Calibration should be avoided, while the glucose level is fluctuating fast, and should be done when the glucose level is steady. Also, based on the algorithm to minimize the errors, Bequette has suggested using raw glucose level as the independent variable in the linear regression analysis of the CGM can yield lower noise (Bequette 2010).

7.5.2 Stability of the Sensor

The enzymes used in the sensor tend to degrade or lose their function after several cycles of sensing due to their exposure to hydrogen peroxide and other radicals (Tse and Gough 1987). In addition, enzyme leaching, instability, and membrane degradation lead to the loss of signal. Several strategies are followed to ensure sensor stability like cross-linking of enzyme with BSA, covalently binding to polymers, or direct fixing on the sensing surface (Wisniewski and Reichert 2000). In addition, the

sensing membrane or the biocompatible layer is prone to calcification and delamination, which contributes to the sensor instability.

7.5.3 Interferences from Small Molecules

Though the above-mentioned parameters are important for the CGM, interference from a number of analytes is usually neglected while measuring the glucose level. Molecules from the body as well as administered from outside can cause the interference, like drugs or their metabolites (acetaminophen, dopamine, ibuprofen, methyldopa, salicylic acid, tetracycline, tolbutamide, etc.), and endogenous species (L-ascorbic acid, bilirubin (unconjugated), cholesterol, creatinine, galactose, triglycerides, urea, uric acid) can cause the deflection in the signal (Basu et al. 2016; Basu et al. 2017). Some of these interfering molecules are electroactive at the potential range used by glucose monitoring devices to oxidize hydrogen peroxide (+0.6 V vs. Ag/AgCl). To remove the interference from electroactive species, a peroxidizing layer can be coated with biological elements like ascorbate oxidase (AOx) or strong oxidants (e.g. PbO_2, BaO_2, CeO_2 and MnO_2) over the electrode surface with GOx to peroxidize other molecules (Jia et al. 2010; Njagi and Kagwanja 2011). To minimize the effect of these molecules in signal generations, extra attention should be given during the calibration step where both the minimum and maximum (4.4 and 6.7 mM) concentrations should be tested with high concentrations of these molecules. The use of a suitable mediator molecule to oxidize hydrogen peroxide at a lower potential can also minimize the risk of oxidizing other molecules to affect the signal generation by glucose (Mao and Yamamoto 2000). In addition, the use of permselective membranes such as cellulose acetate films can be used to improve the selectivity of the sensor. Membranes such as electropolymerized films of poly(phenylenediamine), polyphenol, poly (aminophenol), and various substituted naphthalene help in size exclusion, whereas Nafion and its hybrid membranes are used for the electrostatic repulsion of interfering molecules (Jia et al. 2010; Kulkarni and Slaughter 2016). Nafion is a negatively charged perfluorinated ionomer capable of restricting the anionic interference and is also used for the effective immobilization of enzymes on the electrode surface (Lim et al. 2005). Such membranes are usually hybridized with other cross-linking membranes for enhanced stability, sensitivity, and low interference.

7.5.4 Biofouling

In case of in vivo or implanted biosensors, the nonspecific adsorption of proteins on the sensor surface known as biofouling is the major concern for its stability. The tissue damage caused by the insertion or position of the sensor leads to a host immunological response involving the adsorption of various plasma proteins and cells covering the sensor surface (Wisniewski et al. 2000). To mitigate these problems, antifouling surfaces with good histocompatibility with host systems

PEG, PHEMA, or modified phospholipid-based biomimicry polymers are used (Nichols et al. 2013). Nafion is also used as a membrane to restrict the movement of proteins and other cell particles to reach the electrode surface reducing the effect of biofouling. Another method followed to reduce the effect of biofouling is the use of anti-inflammatory drugs like dexamethasone (Ward et al. 2010) and vasodilators such as nitric oxide releasing substrate on the sensing surface. Such studies on the nitric oxide-releasing sensing surface have shown reduced bacterial, cell, and platelet adhesion (Gifford et al. 2005; Gifford 2013; Malone-Povolny et al. 2019).

7.5.5 Oxygen Dependence

In the subcutaneous and interstitial fluids, the concentration of oxygen drops significantly affecting the performance of enzymatic glucose sensors (Zhang and Wilson 1993). Oxygen is required to measure the concentration of hydrogen peroxide, which is a cofactor for GOx. The amount of oxygen for reaction becomes limited in a solid–liquid two-phase zone due to the slow diffusion of oxygen in the liquid phase losing the sensitivity and restricting the linearity range of the bioassay. Polyurethane, Nafion, silicone elastomer, polycarbonate, hydrogels, etc. are used to reduce the diffusion rate of glucose, or other electron mediators are used to normalizing the oxygen to glucose imbalance (Bornhoeft et al. 2017). Superhydrophobic electrodes such as MXene and superhydrophobic carbon fiber substrate are used to mitigate oxygen deficiency by forming air pockets on the electrode surface (Lei et al. 2016; Lei et al. 2019).

7.5.6 Patient-to-Patient Physiological Variation

The percutaneous/subcutaneous layer of an individual shows tissue heterogeneity, and the temperature fluctuation in that region causes a difference in patient-to-patient fluctuation in the sensing signal. Also, the pressure applied on the sensing region, mostly due to the movement or posture of the individual leading to increased/decreased blood flow, may also contribute to this change in signal (Nichols et al. 2013).

7.6 Conclusion

The CGM devices can provide the data of glucose concentration throughout the day for a better understanding of the personalized glucose profile of an individual and can be used for recommending the right nutrition and medication. The working principle of the CGM devices can be optical or electrochemical and can be integrated with a wearable device. The state-of-the-art CGM devices are now more focused on the development of wireless wearable devices with innovation in self-power generation and multiplexing with other analyte detection. A host of commercially

successful CGM devices are now available with good accuracy and are trying to integrate them with a close-loop insulin pump to avoid the hyperglycemic condition. The biggest advantage of the CGM devices is its warning for individuals when they are approaching a hypoglycemic or hyperglycemic condition and the detection of hidden hypoglycemic conditions in between prolonged hyperglycemic conditions. Integration of the sensing module with wireless data transmission module, cloud storage of data, stretchable electronics, automated insulin pump releasing based on sensor responses is going to be a major goal for further development of CGM devices.

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Transforming Healthcare Technologies with Wearable, Implantable, and Ingestible Biosensors and Digital Health

8

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Abstract

Remote monitoring and ubiquitous digital health systems are becoming pervasive among patients and healthcare practitioners. With the explosion of the amount of digital data from such systems, and the ability to analyze and glean insights at large scales, there is substantial demand for innovative sensor technology that would be unobtrusive, highly precise, and easy to use. Digital health systems can provide vital information to healthcare professionals when critical clinical events occur. Accurate and high signal-to-noise ratio measurements are indispensable in this regard. As a secondary yet essential attribute, ease of use is necessary for such systems, especially for unskilled or elderly patients. Ultimately, whether critical or chronic monitoring, the intended clinical application will drive the requirement for different sensor technologies and biosensors' embodiments in medical devices. Wearable, implantable, or ingestible biosensors accordingly serve applications requiring different combinations of a trade-off between accurate and timely monitoring and long-term ease of use. This chapter describes the diseases that have the most critical need for innovation in biosensor systems, the essential components required for such systems, and how they are integrated into healthcare service delivery. It will further describe the types of considerations

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_8

needed as we transition from designing wearable to ingestible and finally implantable biosensors in the order of their potential risk of harm to the patient. Finally, it explains the intricacies of an end-to-end solution that includes devices, data integration, and analytical capabilities needed to transform healthcare technology.

Keywords

Wearables · Ingestibles · Wireless electronics · Biosensors · Healthcare

8.1 Introduction

Digital health was first described by Seth Frank (Frank 2000). At the time, it was perceived as an internet-connected group of applications and media that could connect patients with commerce. However, recently both the WHO (WHO 2019) and the FDA (FDA 2020) have evolved the definition of digital health to include several healthcare aspects, including telemedicine, genomics, artificial intelligence, wearable medical devices, and mobile applications. eHealth (electronic Health) and mHealth (mobile Health) have been topics of extensive research over the past two decades. The inclusion of these technologies in digital health by both the WHO and FDA marks a transition to clinical adoption of these technologies and the vital role that digital health will play in the future of healthcare.

8.1.1 Digitization of Patient Data

8.1.1.1 The Electronic Health Record (her) and Electronic Medical Record (EMR)

Before the emergence of EHR and EMRs, the accumulation of longitudinal data required for causal inference in epidemiological research was demanding in time and resources. EHRs now allow collation of information across different modalities, including unstructured text data, claims information from payors, and quantitative results inclusive of vital statistics and lab results for each patient over the entire continuum of care (Casey et al. 2016). Recent studies show that EHR adoption continues to grow in the United States (Adler-Milstein et al. 2017).

Decision support tools are an essential part of EHR systems. They have been proven to reduce the likelihood of errors with drug allergies, drug–drug interactions, and drug dosing errors (Atasoy et al. 2019). EHRs are also set up through interoperability standards to accept data from additional sources like mHealth devices, capturing time series longitudinal data. These independent and significant data sources are ideally suited for the development of novel decision support tools leveraging the latest advances in machine learning (ML) and artificial intelligence (AI), such as natural language processing (NLP), AI-based classification, and image segmentation algorithms used in radiology.

8.1.1.2 Smart Devices, Social Networks, Wearables, and Internet Applications

In 2016 alone, global smartphone sales reached close to 1.5 billion, one for every fifth person on earth (Carton et al. 2018). An additional source of digital health data is personal devices, patient interactions on social media, and internet applications. Social support, self-care, and psychological health are known to produce better outcomes for patients. It can be a critical addition to the standard of care (Lin and Kishore 2021). There are several consumer devices by companies like Apple Inc., Fitbit (currently owned by Google Inc.), and Samsung in the market with form factors like wrist-worn, ring, and necklace-style, which can collect physiological data like heart rate and photoplethysmography. Data from the Apple Watch device have been used to detect atrial fibrillation (Perez et al. 2019). However, they must be used with care as recommended by Seshadri et al. (2020).

The evolution of biosensor technology coupled with the pervasive use of digital records in US hospitals has created the opportunity for novel analytics that could provide more specific and sensitive clinical decision support for clinicians. Therefore, there is a critical need for devices that are noninferior and on par with traditional medical devices used in hospital settings.

8.1.2 Common Diseases in Need of Novel Biosensor Systems

Over the past decades, researchers have proposed several novel devices and systems for the diagnostics therapeutics and theranostics of diseases such as cardiovascular diseases (CVDs), cancer, Alzheimer's disease (AD), and multiple sclerosis (MS). Biosensors can play a crucial role in improving the timeliness and efficiency of clinical management of these diseases and improving the overall quality of life for patients.

8.1.2.1 Cardiovascular Diseases (CVD)

Globally, the leading causes of mortality and disability are ischemic heart disease and stroke (Roth et al. 2020). The prevalence and mortality of CVDs have increased from 271 million to 523 million and 12.1 million to 18.6 million, respectively, from 1990 to 2019. In the United States, a conservative projection by Pearson-Stuttard et al. (2016) estimated that the total coronary and stroke deaths by 2030 will increase by ≈18% and 50%, respectively. Total costs (direct and indirect costs) of CVD were estimated to be $555 billion in 2015. These costs are expected to double to $1.1 trillion by 2035.

Acute coronary syndrome (ACS) is a class of CVD that includes myocardial infarction (MI) and unstable angina. MI is further divided into ST-segment elevated MI (STEMI) and non-ST elevated MI (N-STEMI). STEMI indicates complete blockage of the coronary artery, whereas N-STEMI indicates partial blockage. Both STEMI and N-STEMI result in heart muscle damage, which in turn produces biomarkers that can be detected. Unstable angina means that clots are formed but not large enough to cause blockages, so heart muscle damage may not occur (Ouyang

et al. 2021). CVDs are the most prevalent cause of mortality. They have received the most attention in terms of the development of novel devices. Examples of these devices are described in Sect. 1.3.

8.1.2.2 Cancer

Cancer is the abnormal growth of groups of cells. It can start in any organ and spread to any part of the body. It is benign if it does not spread and metastasized if it has begun to spread. There are several subtypes of cancer classified based on which organ system is affected or where it originates. Globally, cancer is the second leading cause of death, with 9.6 million deaths as of 2018. In terms of mortality, the leading types of cancer are lung, colorectal, stomach, and breast (Collaboration GBoDC 2019). In 2017, the cost burden of cancer was $177 billion in the United States.

The progression of cancer is stratified into stages, and the treatment plans are formulated depending on the stage of cancer. Biomarkers for cancer play an essential role in the potential for early detection and formulation of prognosis for cancer. Early diagnosis of cancer before it manifests clinically can significantly improve survival rates. The estimated cost savings among the leading cancer subtypes by incidence, breast, lung, prostate, and colorectal cancers, and melanoma could be as high as $67 billion (Kakushadze et al. 2017).

8.1.2.3 Alzheimer's Disease

AD is a neurodegenerative disorder that manifests as the gradual loss of memory and mental function, also known as dementia. Globally, AD is the leading cause of dementia (DeTure and Dickson 2019). Over the past decades, researchers have gathered evidence to show that the leading causes for AD are the accumulation of extracellular formation of beta-amyloid plaques or amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau protein in the brain. There are no known cures for AD. There is a critical need for an early diagnostic tool for managing AD and providing the best quality of life for patients (Carneiro et al. 2020).

8.1.2.4 Multiple Sclerosis

MS is a chronic neurological disease that falls under the category of autoimmune diseases in which the myelin sheath that insulates individual nerves is gradually depleted by the patient's own immune response. MS is predominantly diagnosed in patients 20–40 years of age. The economic burden of this disease has high indirect costs as the progression of the disease in young populations results in fatigue, pain, paralysis, double vision, and inability to perform studies and work (García-Domínguez et al. 2019). Several advancements have been made in treating MS, and early diagnosis is vital to the long-term management of this disease.

8.1.2.5 Viral Infections

Over the last few decades, several viruses have emerged and spread to the scale of epidemics or pandemics. Some examples are human immunodeficiency virus (HIV), avian influenza (AIV), Zika, human papillomavirus (HPV), Chikungunya virus

(CHIKV), rabies virus (RABV), Japanese encephalitis, and human norovirus. Historically, the mortality associated with viral infections has been low compared to other noncommunicable diseases. However, rare occurrences can lead to substantial mortality and infection rates, such as the severe acute respiratory syndrome caused by the novel coronavirus SARs-CoV-2 in 2019. Public policy decisions on containment and management of outbreaks of this nature require accurate infection rates and assessments of populations affected. There is a dire need for advancements in biosensor systems with high accuracy, low complexity, fast response, and low cost to address such crises promptly (Saylan et al. 2019).

8.2 Biosensor Systems

Biosensor systems have several embodiments depending on the application. The broad categories are point-of-care (POC), wearable, implantable, and ingestible devices. Biosensor devices consist of a biorecognition element and a transducer, which can be used to detect the presence of a biomarker for the diagnosis of specific disease conditions.

8.2.1 Biosensor System as a Medical Device

Biosensor systems can be categorized as invasive and noninvasive types. Invasive includes point-of-care technology (POCT) devices that require any incision or blood draw to extract a sample, as well as implantable and ingestible devices. Noninvasive includes POC devices that use any external body fluids and wearables. Figure 8.1 illustrates the categorization of biosensors.

8.2.1.1 Point-of-Care

POC diagnostics has emerged as a fast, portable, and cost-effective method for the early detection and diagnosis of several debilitating diseases. POC devices consist of three components—a biorecognition element, a transducer, and an amplifier or output conditioner that presents the measurement, qualitative or quantitative. POC devices detect biomarkers of four types: genomic, transcriptomic, proteomic, and metabolomic. Researchers over the past several decades have developed several transduction techniques. Electrochemical transduction remains the most commonly used. One of the most successful uses of electrochemical-based biosensors is in POC glucose monitoring. Mahato et al. provide a comprehensive history of the evolution and current state-of-the-art electrochemical biosensors (ECBs) (Mahato and Wang 2021).

Table 8.1 summarizes some of the recent advances in POC biosensor technology for the common diseases described in Sect. 1.2. An exhaustive list of biomarkers for multiple conditions is beyond the scope of this chapter, so a summary is provided. A substantial body of work in detecting viral infections is based on ECBs (Goud et al. 2021; Khan et al. 2020).

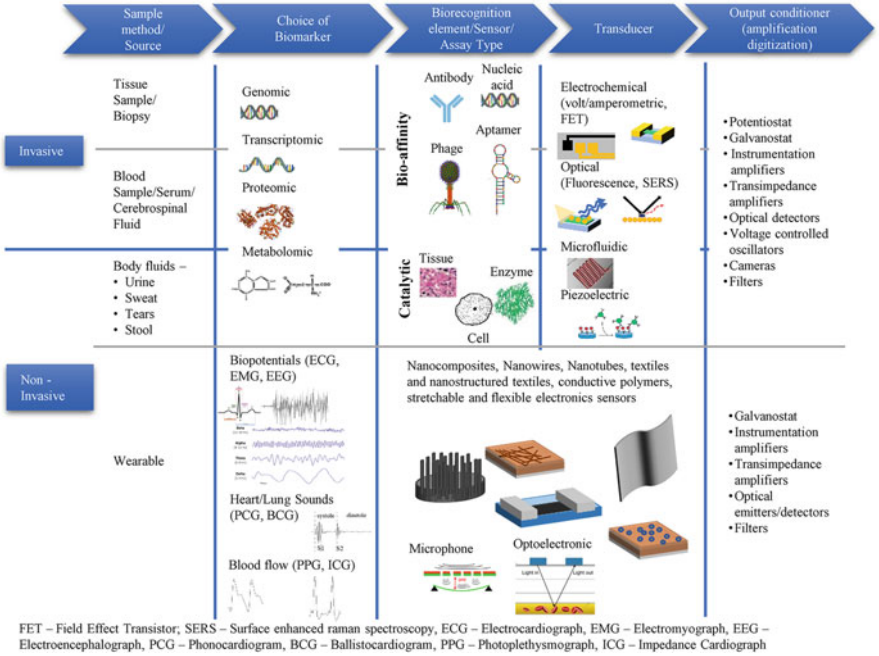

Fig. 8.1 Types of biosensor systems and their subcomponents

An emerging mechanism in the operation of ECB is direct electron transfer (DET). DET-based ECB is gaining attention because it does not require leachable mediators, and they are active in the same redox potential window as the biorecognition elements (Goud et al. 2021).

8.2.1.2 Implantable Devices

Implantable devices are widely used in the treatment of cardiac diseases. Pacemakers, implantable cardioverter defibrillators (ICDs), cardiac resynchronization therapy—biventricular pacemaker (CRT-P), and cardiac resynchronization Therapy—defibrillator (CRT-D) devices are the most prevalent types. They are used to assess and treat different cardiac diseases ranging from arrhythmia to heart failure with a high risk of sudden cardiac death. In heart failure patients, ambulatory pressure measurement at the pulmonary artery using an implantable wireless pressure sensor has been proven to improve clinical management by reducing the risk of re-hospitalization (Desai et al. 2017).

Several promising implantable technologies are under development and have reached the stage of bench validation on animal models. Researchers using pulse transit time directly measured blood pressure on an artery, and Fiala et al. validated this on animal models (Fiala et al. 2013). Vennemann et al. recently described blood flow sensors with the ability to gather data on a patient's smartphone (Vennemann et al. 2020). Marlan et al. described real-time monitoring of lung tumor hypoxia

Table 8.1 Common diseases and the biomarkers associated with diagnostic, prognostic, or therapeutics

Disease	Disease subtype	Diagnostic/ prognostic/ therapeutic	Embodiment of device	Required biosample	Biomarker	Detection mechanisms/operating principle and biomarker
Cancer	Breast cancer (Sharifi et al. 2020; Ranjan et al. 2020; Falkowski et al. 2021)	Diagnostic	POCT	*Tumor* BRCA1/BRCA2, HER-2, EGFR, KRAS *Blood* BRCA1/BRCA2, KRAS, miR-21, miR-155, miR-222 *Serum* HER-2, CA, CEA, PR, ER, p53 *Urine* ER, PR, CEA	CEA, BRCA1/BRCA2, EGFR, KRAS, HER-2, CA, miR-21, miR-155, miR-222	*Surface plasmon resonance (SPR)* CEA, miR-122, PSA, CA125, HER-2, 5LOX, collagen IV *Differential pulse voltammetry (DPV)* miR-155, HER-1, HER-2, CA 15–3, CA125, miR- 21, CEA, CA242, BRCA1, H1047R, VEGFR2 *Microfluidic* EGFR2, glypican-1, CA15–3, CA125, CEA, ErbB2, PTK7, HER-2, PSA, IgG, AFP *Electrochemical impedance spectroscopy (EIS)* HER-1, CA125, miR34a, miR-155, p53 *Cyclic voltammetry (CV)* HER-1, HER-2, HER-3 *Square wave voltammetry (SWV) and linear sweep voltammetry (LSV)* CA242, CA125, p53, miR-21, miR-155, HER-2, IL6, CA15–3 *Amperometry* CA242, CA125, miR, TP53, CD9, CD24, CD44, CD54, CD63, CD81, CD326, CD340, p53 *Capacitive* HER-4, CA19–9 *Electrochemiluminescence* CA15–3, MUCIN-1, CA19–9 *Field effect transistor (FET)* miR-155, HER-1, HER-2, CA125 *Chronocoulometric* miR-21, CA15–3, p53, HER-2, BRCA1 *Fluorescence* CYPIA-1, miR-21, mRi-1246, p53
		Prognostic	POCT		HER-2, BRCA1/BRCA2, ER, PR, Ki67, p53, HSP, GIPC-1, c-myc, c-myb, cyclin D1, cyclin B1, RS/ DJ-1, oncotype DX RS, CK, miR-148, miR-210, miR-21, miR-221, and miR-652.	
		Therapeutic	POCT		HER-2, BRCA1/BRCA2, ER, PR, CA, Ki67, miR-21, and CTC	

(continued)

Table 8.1 (continued)

Disease	Disease subtype	Diagnostic/ prognostic/ therapeutic	Embodiment of device	Required biosample	Biomarker	Detection mechanisms/operating principle and biomarker
	Lung cancer (Yang et al. 2019)	Diagnostic	POCT	*Tumor* EGFR *Serum* CA125, NSE, CYFRA21-1, TPA	NSE, SCCA, TPA	*Electrochemical* NSE, CYFRA21-1, SCCA, CA 125, TPA, VEGF *Optical* NSE, CYFRA21-1, CEA, SCCA, CA 125 *FET* CA 125
		Prognostic	POCT		CYFRA21-1, CEA	
		Therapeutic	POCT		VEGF, EGFR	
	Colorectal cancer (Zhang et al. 2021; Falkowski et al. 2021; Shirafkan et al. 2018)	Diagnostic	POCT	*Tumor* EGFR, IL-6 *Blood* MUC1 *Serum* CEA, CA199, IL-6, p53, miR-92a/miR-29a, let-7 g/miR-21/ miR-31/miR-92a / miR-181b/miR-203 *Urine* P53 *Stool* miR-451, miR-223/miR-92a	CEA, CA199	*Electrochemical* CEA, CA199, MUC1, IL-6, p53, KRAS *Surface plasmon resonance (SPR)* CEA
		Prognostic	POCT		CA199, IL-6, p53, KRAS	
		Therapeutic	POCT		MUC1, p53, miR-143, miR-145	
Alzheimer's disease (Carneiro et al. 2020)	–	Diagnostic	POCT/assay	*Serum* Aβ42, tau protein, ApoE, ApoE4 *Plasma* Tau protein, ApoE *Buffer*	Aβ42, tau protein, ApoE, ApoE4	*Electrical conductance* Aβ42 *Differential pulse voltammetry (DPV)* Aβ42 *Electrochemical impedance spectroscopy (EIS)*

				Aβ42, tau protein, ApoE, ApoE4 *Cerebrospinal fluid (CSF)* Aβ42 *Brain tissue sample* Aβ42		Aβ42, tau protein *Square wave voltammetry* Aβ42, Aβ42/Aβ40 *Surface plasmon resonance (SPR) and localized SPR* Tau protein, Aβ42/Aβ40/tau protein *Linear sweep voltammetry (LSV)* Aβ42/Aβ40
Multiple sclerosis (MS) (Can Demirdöğen 2021)	–	Diagnostic/ prognostic	POCT	*Serum* MS-specific antibodies, miR-145, miR-422/miR-223/ miR-126/miR-23a, anti-myelin basic protein (MBP) autoantibody *Cerebrospinal fluid (CSF)* Anti-myelin basic protein (MBP) autoantibody	Interleukin-12 (IL-12), MBP, MS-specific autoantibodies, miR-145, miR-17, miR-422, miR-223, miR-126, miR-23a	*EIS* IL-12, matrix metalloproteinase-9 (MMP-9) *AC impedance measurement* Myelin basic protein *Cyclic (CV) and square wave voltammetry (SWV)* MS-specific autoantibodies, anti-MBP autoantibody *SPR and localized SPR* MS-specific autoantibodies, miR-422/ miR-223/miR-126/miR-23a *Fluorescence spectrophotometer* miR-145
Cardiovascular diseases (Ouyang et al. 2021)	Risk of CVD	Prognostic	POCT	*Serum/plasma*	cTnI, BNP, TNF-α, SAA, IL-6, fibrinogen	*Field effect transistor (FET)*
	Acute myocardial infarction (AMI)	Diagnostic	POCT	*Serum*	cTnI/Myo/CK-MB, cTnI/h-FABP/copeptin	*Surface-enhanced raman spectroscopy (SERS)* cTnI/Myo/CK-MB *Chemiluminescence* cTnI/h-FABP/copeptin
	Cardiorenal syndrome (CRS)	Diagnostic	POCT	*Plasma*	cTnI/NT-proBNP/NGAL	*SPR and localized SPR*
	Myocardial infarction (MI)	Diagnostic	POCT	*Diluted serum*	cTnI/CK-MB	*Near-infrared enhanced fluorescence*

(continued)

Table 8.1 (continued)

Disease	Disease subtype	Diagnostic/ prognostic/ therapeutic	Embodiment of device	Required biosample	Biomarker	Detection mechanisms/operating principle and biomarker
	Heart failure (HF)	Diagnostic	POCT	*Diluted serum*	BNP/ST2	*Fluorescence*
Viral infections (Goud et al. 2021; Khan et al. 2020)	HPV	Diagnostic	POCT	*Serum/blood* Anti HPV-16-L1: anti-HPV complex, DNA, PT DNA	Anti-HPV-16-L1:anti-HPV complex, DNA	*SWV* Anti-HPV-16-L1:anti-HPV complex, DNA
	HBV	Diagnostic	POCT	*Serum/blood* HBsAb, peptide aptamers, RNA aptamer, DNA	HBsAb, peptide aptamers, RNA aptamer, DNA	*DPV* RNA aptamer, DNA *EIS* *Peptide aptamers*
	HIV-1	Diagnostic	POCT	*Serum/blood* RNA aptamer, HIV-1 p24 capsid protein	RNA aptamer, HIV-1 p24 capsid protein	*FET* RNA aptamer, HIV-1 p24 capsid protein *EIS* Antibody *DPV* RNA aptamer
	Zika	Diagnostic	POCT	*Serum/blood* ZIKV-specific envelop protein antibody, ZIKV-NS1 antibody, antigen ZIKV-derived proteins, aptamer	ZIKV-specific envelop protein antibody, ZIKV-NS1 antibody, aptamer, antigen ZIKV-derived proteins	*EIS* Aptamer, ZIKV-NS1 antibody, antigen ZIKV-derived proteins *SWV* Antigen ZIKV-derived proteins *DPV* Aptamer
	Avian influenza virus	Diagnostic	POCT	*Serum/blood* Antibody horseradish peroxidase–streptavidin conjugate, DNA aptamer (H5N1)	Antibody horseradish peroxidase–streptavidin conjugate	*DPV* DNA aptamer (H5N1)

	COVID	Diagnostic	POCT	*Serum* S-RBD protein, IgG, nucleocapsid phosphoprotein of SARS-CoV-2 *Nasal secretion and saliva* S-RBD protein *Blood/saliva* Viral antigen nucleocapsid protein/IgM/IgG/ C-reactive protein *Saliva* Nucleocapsid protein, (nCovid-19Ab)/SPE, SARS COVID antibody	S-RBD protein, IgG, nucleocapsid phosphoprotein of SARS-CoV-2, IgM, C-reactive protein, SARS COVID antibody	*Chronoamperometry* S-RBD protein, viral antigen nucleocapsid protein/IgM/IgG/C-reactive protein, SARS COVID antibody *EIS* S-RBD protein, IgG *Voltammetry/CV* SARS COVID antibody

using a microfabricated oxygen sensor (Marland et al. 2020). Researchers have also described implantable sensors for multifunctional multianalyte sensing and delivery of drugs or chemicals (Wang et al. 2020). Implantable glucose sensors have reached a commercial stage, and clinical validation is ongoing (Kropff et al. 2017).

Pressure measurements in different organs and blood vessels such as the brain, eye, pulmonary artery, bladders, and orthopedic implants would be valuable for the assessment of several diseases and conditions. The assessment and care for traumatic brain injury, hypertension, heart failure, glaucoma, hydrocephalus, and orthopedic implant effectiveness can be improved by offering greater insight to clinicians. Traditional implantable devices require surgery to implant and then are removed after the desired monitoring period has elapsed. Bioresorbable devices are an emerging technology that could obviate the need for removal. These devices are constructed using materials that biofluids can consume over a pre-defined duration of time. Shin et al. have demonstrated a bioresorbable pressure sensor intended for monitoring healing and chronic diseases (Shin et al. 2019). They used a thermally grown SiO_2 layer to encase the sensor. Si and SiO_2 materials have a slow dissolution rate dependent on the thickness of the layer, temperature, and pH. A Si nanomembrane is used as a piezoelectric sensing element in most pressure sensing applications (Mohankumar et al. 2021). Yang et al. recently described a wax encased pressure sensor using a Si-nanomembrane for sensing intracranial pressure (Yang et al. 2020).

8.2.1.3 Wearable Devices

Wearable devices described in the literature fall under two categories: *clothing and accessory wearables*—sensors integrated into clothing or other accessories such as gloves, watches, armbands, rings, chest straps, headbands, or helmets, or jewelry such as necklaces, and *skin integrated wearables*—sensors integrated into adhesive patches or tattoo-based printable embodiments. The wearables that belong to the first category most often measure heart rate, galvanic skin response, electrocardiogram (ECG) and photoplethysmograph (PPG), blood pressure based on pulse transit time, and actigraphy. Actigraphy includes step counts and calories burned. The second category of wearables most often measures analytes present in sweat, tears, saliva, interstitial fluids, and wound fluids (Mohankumar et al. 2021).

Pertinent to clothing and accessory wearables, several wearable devices have reached a commercial stage and are undergoing various clinical validation studies. Zio Patch, NUVANT MCT, Apple Watch, and Masimo Personal Health are examples of cardiovascular health monitoring devices (Sana et al. 2020). The Cyrcadia breast cancer monitor uses temperature sensors incorporated in wearable adhesive patches and AI methods for diagnostics (S et al. 2020).

Pertinent to skin integrated wearables, saliva is an information-rich body fluid with several valuable biomarkers for disease diagnosis and monitoring. Some examples of biomarkers are CVDs (cardiac troponin I (cnTl), cholesterol), heart failure (tumor necrosis factor-α), stress levels (cortisol, α-amylase), and neurodegenerative diseases (glutamate). Recent trends in research are towards the

implementation of sampling of saliva and embedding biosensors in pacifiers for babies and dentures or mouthguards for adults (Mani et al. 2021).

Sweat-based sensors are convenient because they are noninvasive, and sweat can be sampled from several sites on the body, which offers design flexibility. Sweat can be secreted through exertion or through electrical (iontophoresis) or chemical stimulation. Sweat-based sensors have been demonstrated for several analytes such as lactate, pH, alcohol, sodium, glucose, urea, chloride in a tattoo form factor (Bandodkar et al. 2015), and wrist-band or patch made of textiles or flexible polymers (Mohan et al. 2020). Moon et al. have recently demonstrated touch-based sweat measurement for tracking pharmacokinetic profiles of levodopa which is used in the symptomatic management of Parkinson's disease (Moon et al. 2021).

Tears are rich in protein biomarkers and contain the third-highest concentration of proteins among biofluids, blood being the richest, followed by interstitial fluid (ISF). Several biomarkers in tears have been correlated with ocular diseases (trachoma, glaucoma, keratoconus, and dry eye syndrome) and systemic diseases (diabetes, cystic fibrosis, multiple sclerosis, and Parkinson's disease) (Bandodkar and Wang 2014). The challenges with biofouling and sampling of tears have been a topic of research for several years. A capillary-based sampling of tears at the corner of the eye has emerged as a viable method. Successful integration of tear-based sensors has been demonstrated in contact lens form (Iguchi et al. 2007). Glucose and lactate sensing from tears is the most mature among analytes in the literature thus far.

ISF biosensing is an emerging area of research. ISF is a rich source of protein biomarkers that is similar in proteomic concentration to plasma and serum. ISF is sampled through the skin using minimally- or non-invasive microneedles (Samant and Prausnitz 2018). Lactate and glucose sensing has been demonstrated thus far with microneedle sampling (Bollella et al. 2019). ISF analysis on the microneedle tip is another recent research area that could obviate the need for on-chip analysis or off-chip instrumentation (Teymourian et al. 2021).

The analysis of fluids secreted at wound sites is vital to both the assessment of healing and the development of a closed-loop system for therapeutic management. The wound site should be monitored for changes in blood pressure, oxygen, temperature, pH, microbial activity, and interleukin-6 (IL-6), among other analytes (Brown et al. 2018). Individual physical measures and biomarkers have several candidate biosensor systems proposed in the literature. However, there is still a critical need for a fully integrated wound management solution incorporating all the required biosensors and closed-loop therapeutics.

8.2.2 Biosensor Systems in Pharmaceutics

8.2.2.1 Ingestible Sensors for Monitoring and Diagnosis

The measurement of medication compliance for patients who need to take prescribed medication regularly and the monitoring of the progression of ulcers among patients are two areas where biosensors incorporated in a pill have proven effective. Medication compliance products have received approval from FDA recently and are being

used in the field. Proteus and etectRx (ID-Cap System (FDA 2019)) are recent products with applications in patient compliance. The pills or ingestible event markers, once ingested, transmit signals to a wearable lanyard or patch, which in turn communicates with a mobile app on a smartphone and makes the compliance information like time of pill ingestion available to the patient and physician. These technologies are the most recent advances to reach commercialization following the more mature technology of capsule endoscopy, which facilitates imaging of the upper gastrointestinal tract, small bowels, and colon for diagnosis and prognosis of small bowel bleeding and tumors, Crohn's disease, celiac disease, ulcerative colitis, and colorectal neoplasia (Melson et al. 2021).

8.2.2.2 Closed Loop Continuous Drug Monitoring (CL-CDM) and Therapeutics

Monitoring of physiological status and compliance is an essential part of standard clinical care. However, ultimately, the insights gleaned from monitoring data should drive decisions on how to intervene therapeutically so that the patients may benefit in a timely fashion. Research efforts seek to address two types of challenges in CL-CDM: *first*, to gain a deeper understanding of the variations in pharmacokinetic profiles of drugs along with its inter- and intrasubject variability, and this will lead to the ultimate goal of personalized medicine where drugs and dosages can be prescribed with high specificity for each patient; *second*, improving the ability to quickly and automatically, i.e., without any intervention from the patient or clinicians, adjusting dosages of drugs in response to physiological changes, drug concentration, or a specific analyte reflecting physiological status, measured continuously. The most prominent clinical application for CL-CDM is glucose monitoring and insulin infusion devices (Scholten and Meng 2018).

In typical implementations of CL-CDM, the device includes (a) a continuous real-time implantable biosensor, (b) an external control system device that measures and then computes the necessary dosage of the drug, and (c) a mechanical device, usually an infusion pump, that provides the calculated dosage.

A Continuous Real-Time Implantable Biosensor

Continuous real-time biosensors can consist of bioaffinity sensors or enzymatic sensors and a transduction mechanism that is usually electrical or optical. The most common and mature form of the sensor for glucose monitoring is enzymatic and amperometric. The reason is that enzymatic amperometric sensors have a fast response that is required for a real-time sensor. However, enzymatic sensors require the formulation of specific enzymes that are cumbersome to synthesize for arbitrary analytes (Li et al. 2020a). On the other hand, the advantage of bioaffinity sensors is the availability of several types of biorecognition mechanisms (e.g., DNA, RNA, and aptamers). Several bioaffinity sensors are being developed and, coupled with advances in miniaturization, can lead to multianalyte continuous monitoring systems in the future.

Two recent studies in rat models have demonstrated highly precise feedback-controlled delivery of vancomycin (Dauphin-Ducharme et al. 2019) and tobramycin

(Arroyo-Currás et al. 2018). Electrochemical aptamer-based implantable biosensors were used in these studies. Furthermore, these sensors can be incorporated in flexible and stretchable form factors leading to wearable noninvasive or implantable devices (Zhao et al. 2019).

A Control System Device

The control mechanism's role is to maintain the signal level or, in this context, the analyte of interest. The controller's essential goal is to use the current measurement from the biosensor to predict the dosage of the drug required to maintain the analyte concentration at the desired level. Commercially mature controllers in devices like artificial pancreas may use blood glucose levels or additional parameters and implement proportional integral derivative (PID) controllers or model-predictive control (MPC) designs. In 2016, the OpenAPS (Open Artificial Pancreas System) community used a Do-It-Yourself system to improve the overall percentage time in the normal glucose range and reduce A1C from 7.3% to 6.79% (Wu et al. 2020b). Artificial intelligence (AI)-based approaches to control algorithms may potentially achieve greater accuracy of control but have not yet proven to outperform PID or MPC controllers. As the amount of data available for training AI systems increases, they may become desirable for controller implementations in the future.

An Actuator

One of the essential parts of a CL-CDM is a precise and titrable mechanism to administer the drug. Mechanisms for drug delivery have progressed from requiring invasive cannulae in the early insulin pumps to minimally invasive and pain-free microneedles. Emerging transdermal drug delivery systems use electroporation, where an electric field is applied to increase cell permeability, or iontophoresis, where a voltage gradient is used to deliver drugs. Recently, combinations of microneedles and iontophoresis have been demonstrated (Donnelly et al. 2014), and wearable systems that use iontophoretic methods have also been developed (Wu et al. 2020a).

Materials' advances primarily in nanomaterials and polymers have provided the impetus for improved biocompatibility, specificity, and lower power requirements for these systems. Electroactive biomaterials are an example of such an emerging class of materials based on conductive polymers. These materials are capable of transducing electrical signals into physicochemical signals and vice versa. They can be activated electrically to drive redox reactions and also result in cyclical volumetric expansion and contraction. These physiochemical responses could be used for controlled drug release (Olvera and Monaghan 2020).

8.3 Need, Risk, and Regulation of Medical Devices and Drugs

The regulatory pathways for medical devices and drugs begin with establishing two attributes of the medical device or drug under consideration, namely, intended use and risk and type of harm to the patient. The Food and Drug Administration (FDA)

classifies devices as Class I, II, and III. Regulatory oversight increases from I to III. The FDA provides clearance to market products that fall under Class II, known as a 510(k) clearance, and approval to market for Class III devices, known as premarket approval (PMA). Class III devices have the following definition according to the FDA guidance: "(devices) that support or sustain human life, are of substantial importance in preventing impairment of human health, or which present a potential, unreasonable risk of illness or injury." In the European Union (EU), the Medical Device Regulation (MDR) classifies devices with more granularity (Class I nonsterile and sterile, Class IIa, Class IIb, and Class III). MDR has a separate directive for in vitro diagnostic devices. Similarly, regulatory oversight increases with class.

Regulatory frameworks embody best practices for manufacturers to follow to create devices and drugs proven to be safe and effective and ultimately improve the quality of life for patients and help clinicians care for their patients. In this sense, the following steps can be treated as a harmonization of the regulatory framework's requirements with the caveat that the requirements will change based on the specific use intended for the device or drug and the reason or indication for when it should be used.

- **User Needs:** The process of uncovering user needs entails market research and technological comparisons of the proposed device or drug and the current standard of care. It is vital to establish why this device or drug is necessary and how it is likely to benefit patients or healthcare in general. This process will reveal requirements both technological and market-related for the proposed device.
- **Risk Management:** The overall risk management procedure is detailed in ISO 14971: Medical Device—Application of risk management to medical devices. The risk management process is active for the entire life cycle of the device or drug and should be periodically evaluated and updated to account for any new information that is uncovered regarding the device or drug throughout its use by clinicians and patients. The process consists of four main steps.
  - **Risk Analysis:** This is the process of uncovering risks by systematically evaluating all foreseeable harm to patients from device-related failures, user-related failures, or unintended use-related failures. For internet-connected devices and drugs, cybersecurity, HIPAA, and GDPR considerations must be included.
  - **Risk Assessment:** This is the process of evaluating each identified risk item and assigning a risk score that would stratify the item as requiring mitigation, justification of how the benefits outweigh the risks, or no mitigation required.
  - **Risk Control:** This is the process of identifying methods and mechanisms that could be used to mitigate the potential harm to patients resulting from the foreseeable harm to patients. Following risk control measure implementation, the risks are reevaluated.
  - **Residual Risk Analysis:** After the implementation of the risk control measures, the risks should be reevaluated, and the residual risk should be determined. If the residual risks are acceptable, then the design and

development process can move forward. This is the final step in the design and development phase.

- **Analytical Validation:** This is the process of verifying and validating that the device or drug performs the functions or interacts with the patient in the manner that was laid out through the process of user needs and risk management. Each requirement is verified against predefined acceptance criteria. In addition, depending on the type of device or drug, there are international standards published by organizations such as the American Association of Medical Instrumentation (AAMI), International Standards Organization (ISO), and International Electrotechnical Commission (IEC) that establish performance and safety-related criteria that the proposed device or drug should satisfy. Compliance with such standards follows best practices. Some are recognized by the FDA and required for obtaining CE mark in the EU. Analytical validation may also be referred to as nonclinical bench testing because it does not involve human subjects or clinical trials.
- **Clinical Validation:** In cases where the device or drug is considered high-risk or requires evidence for claims of efficacy, clinical validation is required. Clinical validation involves the enrollment of human subjects belonging to the intended population of patients who will use the device or drug. Clinical trials should be done following Good Clinical Practices guidelines and conform with the regional regulatory laws (Drago et al. 2018; Richmond and Tatavarty 2018).
- **Commercialization and Clinical Implementation:** This is the final step in the development lifecycle for any medical product. In a general sense, this step will involve establishing good manufacturing practices (GMP), complete traceability and documentation of quality-related operations, and an ability to continuously monitor and react to new findings of safety or effectiveness with corrective or preventive actions. These activities need to be in tandem with clinical adoption and implementation. This step involves patient and clinician education, proving effectiveness so that payors, either private or governmental, will reimburse the cost of use of the product. Higher-risk products will likely require continuous post-market surveillance involving continued clinical studies.

Specifically, pertinent to in vitro diagnostics devices or POC devices, the FDA has additional guidelines and requirements based on whether it is classified as a high-complexity device. In the case of high-complexity, Clinical Laboratory Improvement Amendment (CLIA) will apply, and the testing laboratory must meet the CLIA quality system standard.

An overview of the process from lab-scale proof of concept to commercial deployment and reimbursement was described in this section. Figure 8.2 illustrates some example activities in each of the steps that were outlined.

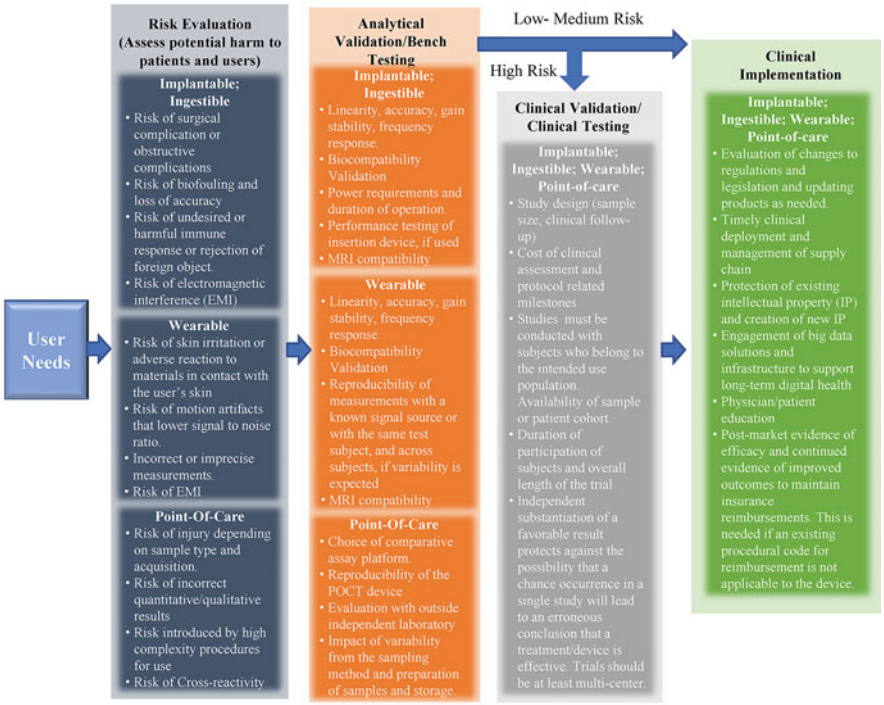

Fig. 8.2 Examples of typical considerations while navigating the path to regulatory clearance for biosensor systems

8.4 System-Level Architectures for Biosensors

As described in the introduction, digitization of patient data is an emerging trend that has great promise in transforming healthcare. A generic system-level architecture is one that involves the following layers:

1. *The sensor layer* consists of the sensing device, which may be wearable, ingestible, and implantable, POCT device, or a smart device.
2. *The network layer* consists of a method to transfer data from the sensing device and digitize the data if needed. The network layer also offers the means for internet connectivity, which may be through a smart device or directly to a mobile network through a gateway.
3. *The data layer* consists of cloud computing and storage resources. These resources generally include the ability to process, transform, and store the data from several devices while maintaining the security of data and Health Insurance Portability and Accountability Act (HIPAA) compliance due to the presence of Personal Health Information (PHI).

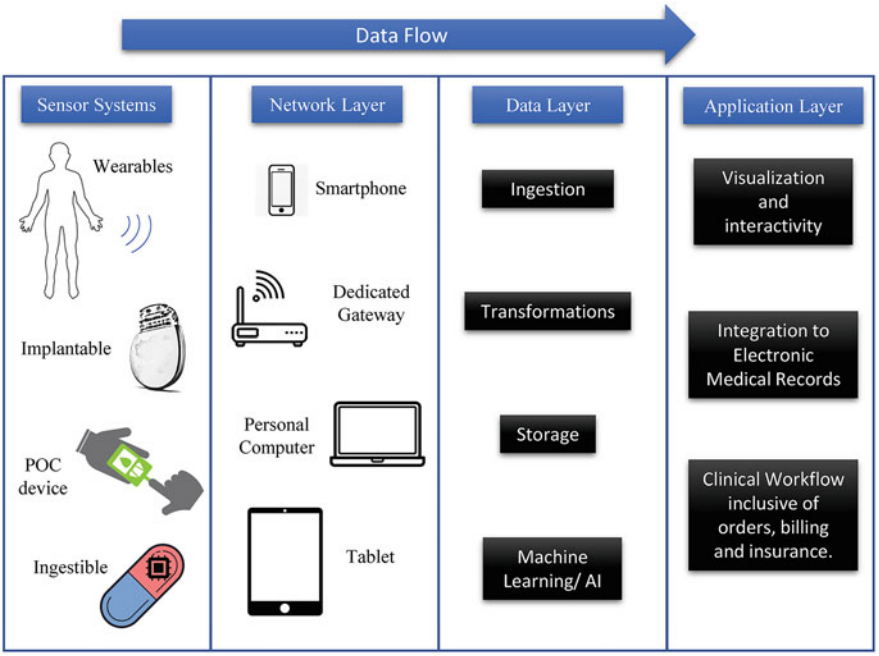

Fig. 8.3 Illustrating the layers of the data flow architecture

4. *The application layer* consists of a cloud-based client or user-facing applications. These applications, in addition to the previously stated HIPAA compliance, will include software implementations that support access, visualization, and integration of healthcare data across different devices and the hospital's information system. The application layers could implement or integrate into an existing medical coding and billing system that is used for medical claims and insurance reimbursements (Fig. 8.3).

8.4.1 Data Flow Architectures

8.4.1.1 Smart Devices as Sensors and Transducers—Local Processing

Smartphones are equipped with a wide range of sensors that are used for various consumer applications. A subset of these sensors can be used as biosensors. Cameras, microphones, light sensors, and force sensors have been demonstrated as viable biosensors for several applications. Chandrashekar et al. have shown that blood pressure can be estimated using the front-facing camera and force sensor. The user presses their index finger against the sensor. A custom application displays the photoplethysmography (PPG) and the estimated blood pressure (Chandrasekhar et al. 2018). Nemcova et al. demonstrated the use of a smartphone's camera and microphone to monitor heart rate, blood oxygen, and blood pressure (Nemcova et al.

2020). Oculocare's Alleye uses smartphone cameras to assess vision and progression of conditions like macular degeneration, which is a debilitating complication from aging and diabetes that can result in blindness (Faes et al. 2021).

The availability of high-quality cameras even in mid-range smartphones has made smartphones a viable tool to replace microscopes and spectroscopy in point of care applications. Several applications of smartphones in sensing and processing data acquired by POCT devices that use optical transduction have been demonstrated (Chen et al. 2021).

8.4.1.2 Medical Device as a Smart Device Accessory

Medical devices can be designed as accessories that connect to a smart device either wirelessly through near-field wireless communication or Bluetooth, coupled through the microphone or a wired connector interface available on the smart device. The data acquired by the medical device can be processed locally on the smart device or sent to the cloud for processing. The result of the processing can be presented on the smart device again or available through a client application that can be accessed from any internet device.

Several smartphone applications that use the in-built camera with an attachment, such as Molescope, SkinVision, and UMSkinCheck, have been commercialized. They use the attachment-enhanced camera to image and algorithmically classify skin lesions as benign or malignant. The accuracy of these classifiers is low and still an area of research, but coupled with the latest advancements in AI-assisted classifications and improvements in the optical components of the accessories; these applications can improve in the future (Davis et al. 2019). Smartphone-based fundus imaging for monitoring and diagnosing diabetic retinopathy has shown great promise as a tool that is both accurate and economical in middle- and low-income countries (Wintergerst et al. 2020). Several researchers have demonstrated microscopy using a smartphone over the past decade. Different microscopy types, including bright field, darkfield, phase, fluorescence, and reflectance confocal microscopy, have been demonstrated (Zhu et al. 2020). Researchers have demonstrated several smartphone-based microfluidic devices for POC applications (Hasanzadeh 2021) and flow cytometry (Li et al. 2020b).

The microphone in smartphones has been used by several researchers to demonstrate monitoring of pulmonary health. Thap et al. used a smartphone to perform lung function tests to determine its effectiveness in a 26 subject cohort with 13 healthy and 13 chronic obstructive pulmonary disease (COPD) patients. The authors concluded that a ratio of forced expiration volume in 1 s to forced vital capacity could be estimated with a high clinical correlation (Thap et al. 2016). Zhou et al. have demonstrated a precise spirometer comparable to a laboratory spirometer by designing a handheld flow head with Bluetooth connectivity. The flow head communicates the flow data to the smartphone, and the smartphone has an app that displays the results (Zhou et al. 2019).

Purohit et al. describe recent advances in smartphones as an optical, electrochemical, and wearable sensing interface (Purohit et al. 2020).

8.4.1.3 Direct Cloud Connectivity—Medical Internet of Things

The data collected in these medical device embodiments are transferred directly to the cloud for processing and analytics. In many cases, a smartphone application may accompany the device only to serve a user interface for data entry-related tasks or only available to clinicians who may use them to access the data from the cloud. Several commercialized devices fall within this category of Medical IoT with direct cloud connectivity. Some examples are Zio Patch by iRhythm Technologies (Yenikomshian et al. 2019), EarlySense (Breteler et al. 2020), and VitalPatch by Vitalconnect (VitalConnect). Although there are several commercialized devices, concerns regarding the security of information, privacy, and data use are ongoing areas of debate and research (Ray et al. 2020).

Remote patient monitoring with implantable device data interrogation at the patient's home has been commercially available from Boston Scientific, known as the Latitude system (Scientific), and Medtronic is known as MyCarelink (Medtronic) (Medtronic MyCareLink 2021; Scientific n.d.; Vinitha Sree et al. 2020; VitalConnect VitalPatch 2021).

8.5 Human Factors and Usability Engineering (HF/UE) Considerations

The risk of harm to the patient due to device failure or incorrect use is a possible outcome of user errors. It behooves the medical device designer or manufacturer to ensure that user errors are either wholly avoided or, if they occur, the consequences should be minor. Human factors engineering is a multidisciplinary field that combines expertise in human behavioral analysis with engineering principles for device-user interface design and strategies to mitigate the potential for harm to a patient due to behavioral patterns. HF/UE is a risk management activity that deals with the analysis and design of the interactivity between the intended user and the device (Borsci and David 2020).

8.5.1 Device Users, Environment, and Interface

A user's interaction with a device typically involves iterative cycles of the following steps: (a) users perceive information about the state of the device; (b) users cognitively accept and process the information and make a decision on what they would like to accomplish; (c) users translate that goal into a sequence of inputs that are compatible with the device's user interface, for example, changing the settings navigating to a different function; (d) the device accepts the user's input and processes it internally to change the state of the system; and (e) the device presents feedback to the user that reflects the change that was performed (Redmill and Rajan 1996). Within this interaction cycle, there are three attributes to consider: (a) the user's characteristics are reflective of their knowledge, education, and cognition; (b) the usage environment considers the circumstances under which the user must

perform these tasks, for example, at home, in a hospital, special environments that may limit their ability to interact with the device; and (c) complexity and type of user interface, for example, does the interface include alarms, displays, buttons, and specific sequence of inputs to function correctly?

8.5.2 User Flow and Task Analysis

During the design and development of a medical device, every step in the flow of interactions with a medical device must be documented. This flow will help identify the specific tasks that the intended end-user must perform. The list of tasks is then analyzed to reveal tasks, if performed incorrectly, that could lead to serious harm to the patient or compromised medical care. These tasks are known as critical tasks. If critical tasks were identified, then mitigation of harm due to failure of these tasks is required. Additionally, usability validation testing must be performed to validate that there are no critical tasks remaining after mitigation. Typically, the risk assessment may be performed using methods like failure mode effects analysis (FMEA) or fault tree analysis (FTA).

Task analysis may be performed analytically with heuristic analysis or expert analysis. Empirical task analysis could be performed by involving a representative group of the intended end user known as focus group. The type of exercise performed with the subjects may include contextual inquiry where the users interact with an existing device that is marketed, interviews to determine the user behavior in hypothetical use scenarios and their attitudes, beliefs, and perceptions, a cognitive walkthrough, or simulated tests with mock devices that may only implement the device user interface.

8.5.3 Mitigation of Use-Related Risk

Risk mitigation can be of three types: (a) inherent safe design, (b) protective measures, and (c) information for safety. Inherent safe design renders the risk highly improbable. Examples include using a connector mechanism that will allow only one orientation for a connection and hiding features that can be mistakenly chosen on the user interface. Protective measures may include warning signs to draw the user's attention if they follow a path that seems incorrect, using alerts or physical safety mechanisms that force the user to deliberate more before choosing a course of interaction with the device. Finally, information for safety involves all risk control measures that are the last resort if inherent safe design and protective measures are infeasible. It includes additional safety training for the user or written warnings and caution statements.

8.6 Challenges and Future Trends

Biosensor development is a rapidly growing area of research driven by the demand for a patient-centric personalized healthcare system. The need for this paradigm shift has been reinforced by the challenges posed by the recent COVID-19 pandemic. Healthcare service delivery needs to shift from hospitals to the patient's home and everyday life. Several challenges and opportunities for continued research and development exist among the biosensor systems described in this chapter.

Challenge I: Biocompatibility: This challenge applies specifically to implantable and wearable devices that require either implantation or direct contact with the skin for prolonged durations of time. The foreign body response is triggered by the body's immune system as soon as an external object comes into contact with the body. There exists a steep trade-off between biocompatibility and selectivity, and sensitivity of biosensors. Future materials innovation may hold the answer to maintain sensor performance while maintaining biocompatibility. *Challenge II: Sample separation:* Biosensor readings may be confounded by the presence of interfering cross-reactive species. Methodologies like microfluidic separation of analytes need to be developed further to improve the separation of the analyte of interest from other biofluid products. In wearables, measurands may be confounded by movement and other artifacts that manifest in the same frequency range as the signal. Better signal and noise separation techniques are needed to overcome this source of incorrect measurements. *Challenge III: Biosensor performance:* Currently, there are no FDA-cleared biosensors for the biomarkers described in this chapter. Biosensors are not yet on par with clinical laboratories in terms of selectivity and sensitivity. Although selectivity may be achieved with bioaffinity-based sensors, sensitivity requires more material innovation. Nanomaterials may hold the solution to this problem. *Challenge IV: Biofouling*: Biosensors rely on surface interaction of the recognition elements and the analyte. Biofouling is the mechanism by which a film of unwanted cells or organisms may accumulate on the surface containing the biorecognition elements. More research is needed to discover antifouling strategies, which may involve material innovation or mechanisms using existing materials. *Challenge V: Intermediate Sample Storage:* Biological samples are highly perishable, so the time duration between extraction and application to the biosensor system should be as short as possible. This may be mitigated by parallel multianalyte testing with biosensors. However, more research is needed to evaluate such multianalyte systems under real use-case scenarios. *Challenge VI: Digital Data:* Data privacy, security, and storage are a significant cause for concern as the digital health era emerges. Cybersecurity is an ongoing effort that must take place in parallel with any new sensor system development. Even mature technologies may face new threats from cybersecurity, so it is important to have constant surveillance, detection of any breaches, and timely responses with patches and updates.

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9 Multiplexed Biosensors for Efficient Diagnosis of the Clinical Conditions toward Health Management

Ravindiran Munusami and Mouli Ramasamy

Abstract

Multiplexed biosensors for detecting and diagnosing several clinical conditions have been advancing rapidly due to the continual research progress. Biosensors are analytical devices that convert or transduce a biological response into a quantifiable signal. Multiplexed biosensors expedite the detection of multiple conditions resulting in a more agile and better disease diagnosis, monitoring, and management. Simultaneous and synchronous detection using multiplexed biosensors provides information beyond what a single sensor or device could render. This chapter describes the potential opportunities and challenges in multiplexed biosensors for the efficient diagnosis of clinical conditions and health management. Some of the major applications covered in this chapter include glucose, drug, and infectious disease monitoring using multiplexed biosensors, along with the most recent advancements and materials considerations in the field of multiplexed biosensors.

Keywords

Multiplexed biosensors · Healthcare · Biosensing materials

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
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9.1 Introduction

Biosensors and biosensing play a vital role in healthcare diagnostics and monitoring in recent years due to advancements in technology and multidisciplinary research approach. Due to nanotechnology and microtechnology advancements, healthcare monitoring and diagnostic systems have become very compact and portable. On-the-go diagnostic systems are the new methods adopted worldwide. The wearable electronic system can continuously monitor anyone's health when a person is performing his/her day-to-day activity without the need to go to a hospital for routine health inspection. A multiplexed biosensor is the next step in the advanced diagnostic and monitoring technique adopted in healthcare research to give precise information of diagnostic value. Multiple sensors are used to get vital health information on various detections from different bio-analytes, as described in Fig. 9.1. The information from the multiple sensors is multiplexed to get the healthcare information for monitoring and diagnosis.

The biosensor classification is based on paper, array, beads, and microfluidics, as shown in Fig. 9.1. Each of the types has different detection techniques, as listed in Table 9.1. As depicted in Table 9.1 (Vadgama and Crump 1992; Dincer et al. 2017), each biosensor type has different detection schemes such as calorimetric, optical, electrochemical, and amperometry. Some of the systems are commercially available for use in the market, whereas a few systems are under research and development.

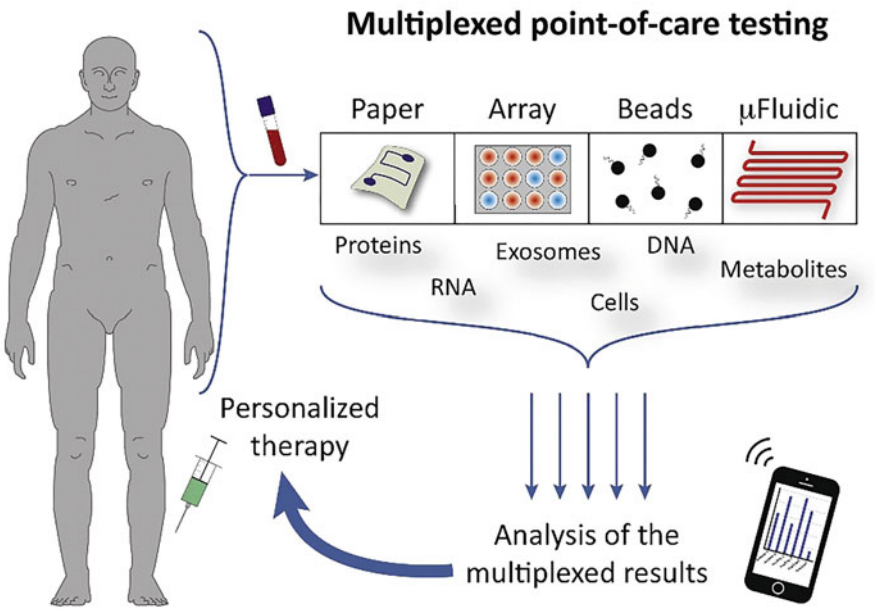

Fig. 9.1 Illustration of the multiplexed biosensor in healthcare

Table 9.1 Biosensor systems classification

Biosensor systems	Detection schemes	
Paper-based systems	Calorimetric detection	Optical detection
Array-based systems	Optical detection	Electrochemical detection
Bead-based systems	Flow calorimetry detection	Real-time PCR detection
Microfluidic-based systems	Calorimetric detection	Amperometry detection

Multiplexed biosensors can be made of more than one of these types of biosensor systems combined for healthcare applications.

The complete system here involves biosensors, bio-analytes, nanomaterials, and bioelectronics. The present chapter gives a detailed insight into the multiplexed biosensor systems and their applications in healthcare. Biosensors get the biological information and convert it into electrical signals to support the bioelectronic system to give healthcare information. The transduction mechanism in the biosensor is the chemical transduction method and physical transduction method. Most of the biological information will be collected from the bio-analytes such as an antibody, DNA, receptor, enzyme, tissue, cell, and organelle. The biosensor transduction system's physiochemical response will be ions, electrons, holes, light, mass, and heat, which will be amplified and communicated to the external system (Vadgama and Crump 1992; Teymourian et al. 2021). Biosensors can be applied on both invasive and non-invasive applications depending on the need of the end-user. As the in vivo applications suggest, these types of biosensors are used on long-term applications such as artificial organs and long-term monitoring of a person's clinical conditions. When the same is applied with multiple sensors in a system for monitoring a person's clinical condition, then the multiplexed biosensors are used. Multiplexed biosensors simultaneously give healthcare information from multiple bio analytes, giving precise information on a person's health condition (Mohanty and Kougianos 2006).

9.2 Multiplexed Biosensors and System Considerations

Multiplexed biosensors are of different types based on the structure and applications such as multiplexed nano-biosensor, soft and fibrous multiplexed biosensor, wearable multiplexed biosensor, multiplexed nano-plasmonic biosensor, multiplexed Point of Case Testing Device (xPOCT), multiplexed label-free biosensor, and Field Effect Transistor (FET)-based multiplexed biosensor. Multiplexed Nano Biosensors use low-dimensional nanostructures such as quantum dots with better photoluminescence property based on the size and the nanoparticle placement on the quantum dots such as Au, Ag, and rare earth materials (Purohit et al. 2019). All these particles have different adhesion with the bio analyte, where it can be used to detect different analytes at the same time. Based on the accurate assessment of the expression of the biomarkers, these type of biosensors offer the flexibility in identifying the disease at the early stage. These types of multiplexed nano biosensors

have a single sample that can deliver multiple results simultaneously (Purohit et al. 2019; Mahato et al. 2021). Flexible and helical bundles of functionalized carbon nanotube (CNT)-based biosensors are used as electrochemical sensors for long-term invasive monitoring of disease biomarkers in the human biological systems (Feiner and Dvir 2020). Wearable multiplexed biosensor systems are used to give the in-vitro biomarker information to get insights into the human system's health conditions on a day-to-day basis. These wearable multiplexed sensors work as a biomarker for simultaneous glucose, pH, temperature, and lactate measurement. Since the multiplexed biosensor is made with a flexible substrate, it can be affixed to any part of the human body for continuous monitoring (Yokus et al. 2020).

Nanoplasmonics-based multiplexed biosensors are used to identify more than one bacterial infection related to sexually transmitted diseases (Chlamydia trachomatis and Neisseria gonorrhoeae) from a single bio analyte such as urine. Plasmonic microarray-based gold nanohole sensors are used to identify Chlamydia trachomatis and Neisseria gonorrhoeae (Soler et al. 2017). Point-of-care diagnostics is another critical area in healthcare monitoring. Multiplexed point-of-care devices are becoming increasingly popular in clinical diagnostics to identify the presence or absence of any disease without any need for time to analyze the same. Such devices have multiple detection schemes at the same time. Various multiplexing technologies in the biosensor systems such as paper, bead, array, and microfluidics are used to detect various clinical conditions at the same time by using a single analyte or multiple analytes (Dincer et al. 2017; Park et al. 2016). Label-free biosensors are made of physical-type detections such as mechanical defection due to the antigen–antibody binding on the sensory elements such as nano/microcantilever beams. When these structures are made as matrix structures, they can detect multiple biomarkers simultaneously (Luo and Davis 2013). Hydrogel-based gate materials are used in the FET, where bio-specific receptors are placed to make the bioanalytes settle over the receptor to work as biomarkers (Bay et al. 2019). Similar to the discussed types of multiplexed biosensors, there are yet more multiplexed biosensor types present, based on their applications, such as drug screening, protein profiling, immunology, genomics, proteomics, and metabolomics (Zhao et al. 2013). Figure 9.2 gives a brief insight on the different body fluids used as analytes in biosensors to study the heal condition. Each fluid has unique biomarker nature such as metabolites, proteins, antibodies, lactate, and ions. The same can be applied to multiplexed biosensors for studying health conditions. For example, a multiplexed biosensor can understand the metabolite of tears, saliva, and sweat to get precise health conditions.

Electrochemical multiplexed microfluidic biosensor with CRISPR-powered approach was used to amplify the micro-RNA biomarkers used as a point-of-care diagnostic for identifying the disease in patients. A multiplexed version of the electrochemical microfluidic biosensor was made by making four novel chips for simultaneous quantification of a maximum of eight micro RNAs from pediatric medulloblastoma blood patients for the detection of multiple nucleic acids parallelly (Bruch et al. 2021). Electrochemical-based multiplexed biosensors are used as point-of-care cardiovascular disease biomarkers for C-reactive protein (CRP), troponin I (cTnI), and procalcitonin (PCT). The fabricated point-of-care device is made by a

Fig. 9.2 Biofluids and detection scheme for multiplexed biofluidic sensors

wax printing technique, with multiple detection zones and working electrodes, simultaneously detecting the three cardiovascular biomarkers using a single sample with better sensitivity (Boonkaew et al. 2021). Like cardiovascular disease identification and detection, some diseases are more dangerous, killing the human population. HIV infection and its related diseases are becoming very common in most countries. Array-based sensing is being used for detecting bio analytes recently. A similar approach is adopted for a multiplexed biosensor made of array-based serology assay for detecting HIV and related infections with the waveguide illumination approach (Myatt et al. 2009).

Study of the metabolic change and related health issues is essential in recent years due to the unestimated changes in the chemicals with different age groups due to the lifestyle. Continuous measurement of glucose, lactate, pH, and temperature using a wearable multiplexed biosensor system is much needed to understand the changes in a person's metabolites. In vitro measurement of glucose, lactate, pH, and skin temperature from the sweat gives the metabolites changes (Yokus et al. 2020). Multiplexed glycan microarray biosensor fabricated with glycan probes with 2.6 and 2.3 linkages is used for influenza virus detection based on capturing lectins (carbohydrate-binding proteins) (Zhang et al. 2021). A sepsis biomarker is fabricated

with the graphene-based detection from whole blood with the surface chemistry approach. Three sensors were used in multiplexed approach by modifying the approach in a single chip with different capturing probes (Zupančič et al. 2021). As an approach to have multiple detections on the same analyte, different materials have been used in the biosensors. Recent research on the materials has made various materials used for sensing applications with excellent mechanical, electrical, optical, magnetic, and thermal properties. Synthesis methods have made low dimensional structures such as 0-dimension, 1-dimension, and 2-dimension structures with better sensing properties.

9.3 Materials for Multiplexed Biosensors

Different types of materials such as carbon-based, metal-organic, metal oxides, polymer, and composites are used in various sensing applications. Materials used for biosensing are based on the sensing capability and the selectivity of the application. Table 9.2 describes the various biosensing principles and their respective materials. The same materials or different materials can be used in multiplexed biosensing applications based on the device's functional requirement.

The materials used on a particular sensing modality can be used for sensing multiple analytes simultaneously. This can be achieved based on the different nanoparticle distribution over the surface for better adhesion to keep the analyte-binding property better. For instance, any low-dimensional structure can have different metal nanoparticles deposited over it in groups to have sensed on different analytes simultaneously. Hence the materials used in various classes of biosensors can be used in multiplexed biosensors. Materials used in multiplexed biosensors based on their application prospects are discussed in detail in the following section.

Omni dispersible Hedgehog Particles algometry nature has improved surface-enhanced Raman scattering effect with higher intensity than smooth surface colloids. This nature helps the simultaneous detection of multiple targets in complex medium with higher ionic strength. These omni dispersible active colloids help in the multiplexed biosensing capability of biological fluids. Based on multiple sensing, single or multiple biofluids help in precise healthcare monitoring (Montjoy et al. 2018). Quantum dots are the most promising low-dimensional structures beneficial for biosensing application due to the antibody conjugates available for better labeling. Novel bio-functional quantum dots bioconjugate are used to detect the multiple bio-analyte simultaneously and are very useful for multiplexed biosensing applications (Hildebrandt 2011). Nanostructured conductive hydrogel-based multiplexed sensing is another simple and effective approach where the paper substrate can be used as point-of-care diagnostics. Fabrication of the hydrogel-based biosensing is also straightforward, where an inkjet printer is used for depositing the material. A fabricated sensor array and the working electrode in a single page can be used for multiplexed assays to detect glucose, lactate, and triglycerides with outstanding sensitivity. Fabricating a multiplexed sensor device was done with the three rounds of printing on a paper substrate (Valente et al. 2018).

Table 9.2 Biosensor materials

Sensing principle	Materials
Electrochemical biosensing	Magnetic nanoparticles
	Quantum dots
	Polypyrrole nanotubes
	Metal-organic frameworks
	DNA nanomaterials
	Metal nanoparticles
Photoelectrochemical biosensing	TiO_2
	Nano silk
	Quantum dots
Colorimetric biosensing	Metal oxides
	Gold nanoparticles
	Carbon nanomaterials
Fluorescence biosensing	Quantum dots
	Gold nanoparticles
	2D carbon nanomaterials
	Metal oxides
	Zirconium phosphate
Chemiluminescence biosensing	Superparamagnetic iron oxide particles
	CuS nanoparticles
Electrochemiluminescence biosensing	Quantum dots
	Carbon nanomaterials
Surface plasmon resonance biosensing	Titanium nitride nanomaterials
	Gold nanoparticles
	Magnetic nanoparticles
Surface-enhanced Raman scattering biosensing	Metal nanomaterials

ZnO-based sensors have been in research over some time on various bio analyte detection and are used as biomarkers due to the ability of the material's physical and chemical properties. ZnO-based nanoparticles and nanostructures (Nanorods, Nanospheres, Nanoflowers, etc.) were fabricated using different techniques. Due to the better electrical, mechanical, optical, and physical properties, ZnO has been an excellent material for biosensing applications. Nanostructured ZnO can also be used as a multiplexed biosensor to detect cardiac biomarkers cardiac troponin-I, cardiac troponin-T, and B-type natriuretic peptide. The fabricated sensor's response time was recorded better, which can be very useful for quantifying the multiple biomarkers. Sensor response time was also recorded to be very good with a better signal-to-noise ratio and improved signal response using electrochemical detection. High specificity and selectivity were also recorded with the target biomarkers due to the nanostructured ZnO (Shanmugam et al. 2018). Multiplexed immunoassay sensor with modified ZnO nanorods using reduced graphene oxide paper electrode and silver deposition was fabricated with amplification strategy for chorionic

gonadotropin to detect prostate-specific antigen and carcinoembryonic antigen (Sun et al. 2015).

Graphene-based biosensors are widely used due to their better physical and chemical properties when used independently or doped with other materials. It is a complex, single-layer, two-dimensional network of carbon atoms having excellent electrical sensing capability. Like hydrogel ink, graphene nano inks are used to fabricate the multiplexed nano array–based biosensors for metabolite detection. These multiplexed biosensors were fabricated with graphene ink on a microfluidic paper to detect the metabolites such as glucose, lactate, xanthine, and cholesterol with better sensitivity. The fabricated biosensor's significant properties include very low measuring time with a broad detection range at a very low sample rate (Labroo and Cui 2014). Similarly, Alzheimer's detection can also be carried out with the graphene oxide material with different bio analytes. A similar approach can be adopted with different bio analyte detection simultaneously with a multiplexed biosensing approach (Wang et al. 2018; Zhou et al. 2018; Jin et al. 2018).

9.4 Bioelectronics for Biosensor Systems

When sensing the bio-analytes, biosensors will have the information about the intermolecular electron transfer found in the biological system in the form of voltage or current with distortion at the output, providing a synergy between the electronics and biology (Szent-Györgyi 1968; Turner 2005). In some cases, the intermolecular interaction may be in some other forms, such as heat and mass. Bioelectronic circuits are used in healthcare systems to perceive and analyze the sensed data. Bioelectronic systems are classified based on the function of micro/nano bioelectronics, plastic bioelectronics, hydrogel bioelectronics, organic bioelectronics, and molecular bioelectronics. Micro and nano bioelectronics use micro and nanostructures such as silicon nanowires, carbon nanotubes, and graphene in conjunction with biology and electronics toward the miniaturized transducers nanoscale with better sensitivity and biocompatibility for healthcare monitoring applications. Table 9.3 lists various nanostructures, nanoparticles, and nanomaterials that are commonly used as bioelectronic components.

The nanostructures and nanomaterials discussed in this section are used in the bioelectronic application as sensors and nanodevices for healthcare monitoring (Zhang and Lieber 2016; Li et al. 2021). Wearable bioelectronic systems are essential due to the dire need for real-time healthcare monitoring. Plastic (polymer and organic)-based wearable and implantable bioelectronic systems are becoming increasingly popular because of the flexible nature of the substrates that could offer better conformity. They are also 3D curved and dynamically mobilizable resulting in a seamless electronic–biological interface that is soft, stretchable, and comfortable. These polymer bioelectronic systems provide outstanding physical, mechanical, electronic, and biological functions such as thermal, acoustic, photonic, chemical interactions, adhesiveness, electrical sensing, and biocompatibility (Someya et al. 2016). Hydrogel-based bioelectronics is used by having hydrogel as a potential

Table 9.3 Nanostructures and Nanoparticles in Bioelectronics

Type	Material structure
Metals/metal oxides	Au nanoparticles
	Ag nanoparticles
	Ag nanospheres
	ZnO nanoflakes
	ZnO nanorods
	Co3N nanowires
	HfO2 nanoparticles
	Ti3C2Tx nanoparticles
	Co3O4 nanoparticles
	NiO nanoparticles
Carbon	Graphene
	Graphene oxide
	Reduced graphene oxide
	Carbon nanotube (CNT)
	Single-walled CNT
	Multi-walled CNT
Inorganic	Si nanowire
	Si nanorods
Nanocomposite	Graphene/Ag NW
	Ag-rGO
	Fe3O4/GO/MIP
	IrO2@NiO core-shell NWs
	CuO/GO/CNF
	Au/rGO/AuPt NP
	rGO-ZnO
Micro-pattern	PANI-Au hybrid nanostructure
	Microneedle
	Microfluidic structure

interface between biology and electronics due to the resemblance as a biological tissue with electrical, mechanical, and bio-functional engineering nature, described in Fig. 9.3.

Due to the tissue–electronics interface and bioelectrical interface, hydrogel-based bioelectronics is more suitable for healthcare applications (Yuk et al. 2019). Nanoscale electronics and biological structures' binding and adhesion are essential for biosensing application. Molecular-level interaction of the electrons and bio-analytes plays a vital role in molecular bioelectronics for healthcare applications (Davis et al. 2005). Organic bioelectronics is similar to another approach where organic compounds and semiconductors such as poly(3,4-ethylene dioxythiophene):polystyrene sulfonate PEDOT:PSS, polypyrrole, horseradish peroxidase (HRP), and glucose oxidase (GOx) are used in bioelectronic applications (Rivnay et al. 2014; Berggren and Richter-Dahlfors 2007). Hence there have been various approaches adopted in bioelectronics based on the materials as discussed.

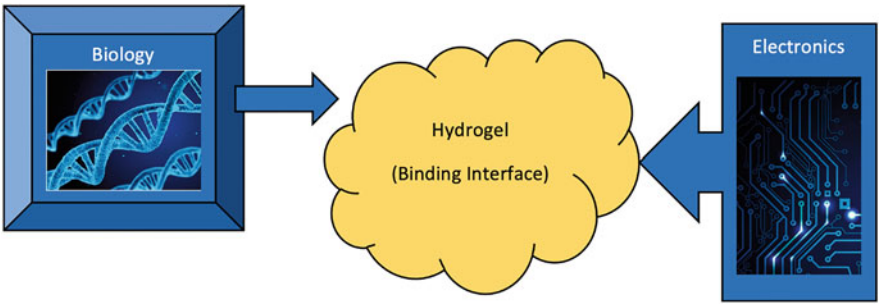

Fig. 9.3 Hydrogel interface

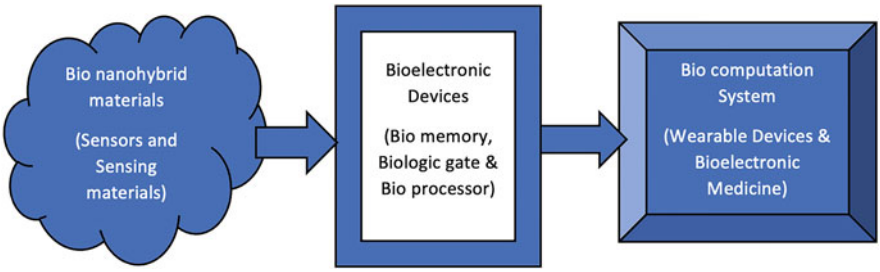

Fig. 9.4 Bioelectronics system

Bioelectronics has wide healthcare applications such as information storage, biosensing, diagnostics, organism mimicking, and neural mimicking, which will have various electronic devices involved (Yoon et al. 2019) (Fig. 9.4).

Figure 9.5 provides insights about the bioelectronic system's detailed information as a block diagram where there are the sensors and sensing materials in contact with the bio analytes on the left-hand side. The next block is the signal conditioning block, where the signal received from the sensor will be conditioned with the bioelectronic devices. The signal conditioning performed here involves analog to digital, digital to analog conversion, filtering, and data conditioning for wired or wireless transmission. Then the information is received by the biocomputation system block for diagnostics and monitoring as in vivo or in vitro. The whole bioelectronic system can be classified concerning the materials used in any of the blocks discussed in the above sections. If the plastic substrate is used on the sensing part, bioelectronic device part, and biocomputation part, it is called a plastic bioelectronic system. Most of the wearable devices used as part of the bioelectronic systems involve plastic electronics due to the device's ability to be mounted or placed on an uneven surface for continuous monitoring. Recent progress on the same can be addressed with tattoo electronics—recent technology development for healthcare monitoring. Similarly, depending on the type of bioelectronic system, it is used for varying healthcare applications.

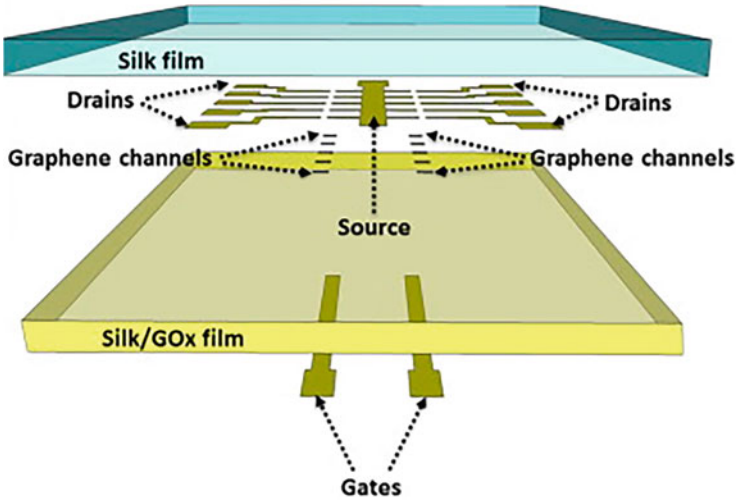

Fig. 9.5 Spatial multiplexed glucose biosensor (You and Pak 2014)

9.5 Multiplexed Biosensor Systems and Applications

Multiplexed biosensing systems have been used for disease detection and healthcare monitoring with various approaches based on the architecture and the materials used. The present chapter will focus more on the metabolites marker and drug delivery approaches using multiplexed biosensing principle. Approaches adopted in sensing includes quantum dots, nanoparticles, microfluidics, lab on a chip, micro/nanoneedle, and many more. Glucose marker is a significant breakthrough in recent years without invasive approaches, whereas the metabolites of a person are identified through various biosensing methods. Paper-based multiplexed electrochemical and glass fiber strip-based biosensor was fabricated with carbon ink, resulting in a wide range of measurements with reproducibility (Amor-Gutiérrez et al. 2019). Similar to the paper based multiplexed biosensor, microfluidic paper-passed electrochemical biosensor array with eight sensors for detection of more than a few analytes such as glucose, lactate, and uric acid in urine to identify the diabetic level of a person more precisely (Zhao et al. 2013). Like the non-invasive approach for diabetic detection, an invasive approach with microneedles was also adopted in the multiplexed biosensing. Acrylate polymer-based microneedle was fabricated to detect metabolic acids, tumors, and chemistry changes over selective detection of pH, glucose, and lactate with changing physiological conditions (Miller et al. 2012). Carbon-coated, stainless-steel pins were used as three-electrode configurations in the glucose biosensing with enzymatic sensor phase and ferrocyanide electron transfer mediator (Rama et al. 2017). Multiplexed marker array can be achieved by using multiple antibodies placed over the Au nanoparticles, which can detect interleukin-12 and

Table 9.4 Electrochemical glucose sensor progress (2009–2020)

Year	Sensor type	Sensor subtype
2009	Paper-based sensor	Not applicable
2010	Implantable sensor	Not applicable
2011	Nanomaterial-based sensor	Engineered GOx
2012	Wearable non-invasive sensor	Contact lens
2013	Enzymatic sensor	Not applicable
	Self-powered sensor	Self-powered continuous sensor
		Self-powered tear sensor
2014	Flash glucose monitoring	Not applicable
2015	Tattoo ISF sensor	Not applicable
2017	Multiplexed	Fully integrated sensor
2017–2018	Sweat sensor	Drug delivery patch
		Microfluidic sweat patch
		Skin-like ISF sensor
2018	Nanomaterials of wearables-based sensors	Not applicable
2020	Glucose insulin chip sensor	Not applicable
	Flow-through sweat sensor	Not applicable
	On-body MN patch	Not applicable

tumor necrosis factor-a in impedance spectroscopy-based biosensor for detection of diabetes with the information availed from glucose and hemoglobin (La Belle et al. 2011). Glucose and diabetes are very closely related, and hence glucose is used as an effective biomarker for determining diabetes in a biological system. Glucose sensor has been on a progressive arena over the years with different architectures over the decade. Table 9.4 (Amor-Gutiérrez et al. 2019; Miller et al. 2012; Rama et al. 2017; La Belle et al. 2011; Barbee et al. 2010; Ng et al. 2008; You and Pak 2014; Zhu and Trau 2012; Werley et al. 2020; Márquez et al. 2019; Sridara et al. 2020; Wang et al. 2011; Hossain and Park 2017; Shu et al. 2015; Yin et al. 2016; Mei et al. 2016; Bao et al. 2008; Teymourian et al. 2020) illustrates the progress on the electrochemical glucose biosensor over a decade from 2009 to 2020.

Glucose detection with multiplexed biosensing has different approaches adopted recently such as:

- Spatially multiplexed glucose biosensor.
- Reconfigurable multiplexed glucose biosensor.
- Multiplexed biosensor with different materials for glucose sensing.

Spatially multiplexed biosensing is a technique used to detect glucose in a biological system where numerous identical sensors are placed in a coordinate system to record the information simultaneously. Multiplexed sensing systems adopt the spatial approach by pacing the micro/nanoarray/beads in a spatial coordinate on a substrate so that the multiple sensors will give the needed information at the same time simultaneously. This spatial multiplexed sensing system is made more

reliable by advancing the micro/nanofabrication techniques available (Barbee et al. 2010; Ng et al. 2008). Spatially multiplexed sensing technique is widely used for various biomarker detection for understanding the biology of a person. Glucose detection using a spatially multiplexed biosensor is one such approach to understanding the change in metabolites in understanding the diabetes condition. Different techniques have been used for the glucose biomarker using the spatial multiplexed sensing approach, such as flexible substrate-based spatial multiplexed system, optical-based spatial multiplexed system, and microfluidic-based spatial multiplexed system. Flexible substrate–based spatial multiplexed glucose sensors have the uniqueness of the sensor's placement on any uneven substrate of a human body, which can function as a wearable system to read the information continuously by using sweat, temperature, and other physiological conditions as bio analytes. Carbon ink on a piece of silk fabric with the thread coated with carbon ink and potassium ferricyanide over which GOx is deposited to detect the bio analyte's glucose is used as a wearable spatial multiplexed glucose biosensor system (You and Pak 2014; Mahato and Wang 2021).

Figure 9.5 shows the structure of the glucose-sensing mechanism of a field-effect transistor (FET)-based sensor. Similar sensor devices are arranged spatially to form multiplexed sensors for glucose sensing. A similar arrangement can be modified with different types of sensors for glucose detection. Spatially encoded microarrays are used for glucose detection with the bio-functional microparticles arrays placed on a gel-based microstructure combined with microfluidics. The proposed mechanism has spatial encoding microbeads with fluorescent dye to identify the tumor using a fluorescence microscope and arrays with enzymes having microparticles to detect glucose (Mahato and Wang 2021). Multiplexed optical biosensor array with similar fluorescent spectra arranged in cells is an approach adopted for detecting glucose. The FLII12Pglu-700μδ6 sensor detects glucose in a cell with an excitation wavelength of 440 nm and an emission wavelength of 480,530 nm (Werley et al. 2020). Reconfigurable point-of-care sensing systems is much in need for the present healthcare monitoring. A reconfigurable, smartphone-interfaced electrochemical sensor for detecting two analytes simultaneously is used for identifying type-1 diabetes. Two electrodes are fabricated to detect two analytes simultaneously by depositing glucose oxidase and lactate oxidase for detecting glucose and lactate. Reconfiguration of the system can be achieved by removal of the membranes by calcium chelator phosphate buffer. Electrodeposition of a new membrane will detect a new analyte of the same type or different type (Márquez et al. 2019).

9.6 Multiplexed Biosensors for Glucose Monitoring

Different materials such as metals, metal oxides, polymers, and composites are used for sensing glucose in multiplexed biosensors approach based on the type of multiplexed sensor. A flexible electrode array-based multiplexed sensor is fabricated using functionalized PDMS film with gold nanoparticles, Prussian blue, GOx, and LOx to detect glucose and lactate as a multiplexed biosensor (Yokus et al. 2020).

Multiplexed paper-based glucose detection is done with paper and carbon ink for electrochemical processes with ferrocyanide used as a mediator. Fabrication of the paper-based multiplexed sensor is cost-effective with the capability of eight simultaneous measurements (Amor-Gutiérrez et al. 2019). Nanostructured conductive hydrogel is an alternative material used as electrodes to fabricate the sensor using an inkjet printer for multiplexed glucose sensing (Li et al. 2018). Graphene ink is used for the fabrication of multiplexed biosensor array for the detection of glucose. The graphene ink's biofunctionalization is done for better sensitivity when used for glucose-sensing applications (Labroo and Cui 2014). Multiplexing capabilities toward glucose sensing can be achieved with the array of sensors having multiple sensing capabilities with different materials having adhesion with the bio analyte to be used as a glucose marker. Different materials with the glucose-sensing capability can be used as a single multiplexed sensor with different arrays.

Electrode materials made of carbon dots and copper oxide composite are used for glucose detection having a three-electrode setup with Ag/AgCl as the reference electrode (Sridara et al. 2020). Similarly, various electrodes made with different materials, as listed below, are used for glucose sensing (Wang et al. 2011; Hossain and Park 2017; Shu et al. 2015; Yin et al. 2016; Mei et al. 2016).

Electrode materials made of carbon dots and copper oxide composite are used for glucose detection having a three-electrode setup with Ag/AgCl as the reference electrode (Bao et al. 2008; Teymourian et al. 2020). Similarly, different types of electrodes made with different materials as listed below are used for glucose sensing (Mei et al. 2016; Bao et al. 2008; Teymourian et al. 2020; Ngoepe et al. 2013; Bian et al. 2020; Garzón et al. 2019; Jarockyte et al. 2020).

- GOx/CdS/Gr on GCE.
- PDDA/Ch/GOx/PtAuNPs/PtZn on Pt.
- Au/GO on GCE.
- Cu/Cu2O/CSs on GCE.
- Nafion/NPC-CB on GCE.
- PDDA/Ch/GOx/PtAuNPs.

Tests such as amperometry and cyclic voltammetry were carried out for the glucose sensors to identify the sensitivity. The sensitivity of the listed glucose sensors ranges from 1.76 to 110 μA mM^{-1} cm^{-2}, in which the PDDA/Ch/GOx/PtAuNPs glucose sensor has a better sensitivity of 110 μA mM^{-1} cm^{-2}. Apart from the electrode-based glucose sensing, other biosensors are made with different materials of bulk and low-dimension nanostructures for better performance (Bao et al. 2008; Teymourian et al. 2020). Some of the materials include CNT-based composites, nanostructured TiO_2, carbon nanofibers/helical carbon nanofibers, Pt-polyaniline (PANI) hydrogel heterostructures, PANI-wrapped boron nitride nanotubes, organic ligand, and metal ions, 3D hybrid graphene–CNT structures, metal dichalcogenides, reduced graphene oxide, MoS_2, WS_2, WSe_2, $MoSe_2$, hollow sphere nanostructured poly(3,4-ethylene dioxythiophene) (PEDOT), AuNP conjugate, Co, Ni, Cu, Nanoporous PtAg, PtCu, Co@Pt core-shell NPs, hollow Ag/Pt

NPs, and Pt/Au nanowires. Different synthesis methods were adopted to manufacture the nanomaterials to get the required low-dimensional structure for improved property to be used as glucose biosensors. Multiplexed glucose biosensors have different architectures to get the multiplexed sensing capability. Micro/nanofabrication techniques such as deposition, lithography, and etching were used to obtain the needed architectures such as micro/nano arrays, microfluidic channels, immune assays, and membranes for multiplexed glucose sensors.

9.7 Multiplexed Biosensor for Drug Monitoring

Drug delivery systems utilize sustainable, responsive, and targeted drug delivery vehicles to offer illness management. Advanced drug delivery systems overcome traditional drug delivery limitations by enhanced bioavailability, therapeutic index, and reduced side effects with improved patient compliance or acceptance (Ngoepe et al. 2013). As the use of therapeutic drugs is constantly increasing, therapeutic drug monitoring (TDM) is clinically practiced for detecting given drug concentrations at fixed intervals in the bloodstream of patients (Bian et al. 2020). Various techniques have been employed in TDM, such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and immunoassays. These three techniques have been employed to investigate the minimum effective concentration of drugs in human saliva, urine, blood, plasma, and serum. However, these techniques involve a long time sample process, costly reagents and equipment, trained personnel, and a specialized laboratory for processing them (Garzón et al. 2019). On the other hand, biosensors are the most innovative yet straightforward techniques available today—a single device that meets all the requirements. These biosensors have been used to manage illnesses/diseases such as cardiovascular disorders and diabetes that require glucose and cholesterol levels maintenance (Ngoepe et al. 2013). Standard biosensors are used to detect a single analyte. However, this will not be enough for an early and accurate diagnosis to obtain. In this case, having multiplexed biosensors that involve a multitude of biomarkers capable of performing multiplexed biological detection can be employed (Garzón et al. 2019). Simultaneous detection of several biomarkers minimizes false positives and false negatives during clinical diagnosis, which readily occurs when measuring a single molecule. Hence, multiplexed biosensors capable of simultaneously detecting multiple analytes provide accurate data for therapeutic drug monitoring and diagnosis. Therefore, the multiplexed biosensors emerged as a valued tool for clinical diagnosis (Jarockyte et al. 2020; Yáñez-Sedeño et al. 2017).

9.8 Multiplexed Biosensor Systems for Infectious Diseases Monitoring

Infectious diseases caused by bacteria, virus, parasites, and fungi are the primary concern for the people and governments worldwide as they impact individual and public health. Notable outbreaks of infectious diseases enumerated by the World Health Organization (WHO) include malaria, tuberculosis, AIDS, hepatitis, chikungunya, the Ebola virus, and the recent outbreak of Covid-19 (Rodovalho et al. 2015). Lack of sanitary conditions, the flawed urbanization process, and lack of city planning and water supplies significantly contribute to the spread of infectious diseases (Rodovalho et al. 2015). In this case, a diagnostic test is essential to detect the presence or absence of infection. Standard diagnostic tests rely on laboratory-based techniques, including microscopy and microorganism culture, immunoassays, nucleic acid amplification, Enzyme-linked Immunosorbent Assay (ELISA), and PCR. These techniques lack sensitivity, and the culture process has a significant time delay. Also, techniques such as ELISA and PCR are highly sensitive but have complex sample preparation processes and are challenging to implement multiplex detection (Sin et al. 2014). Hence, the real-time identification of infection offers effective medical treatment and control over epidemic outbreaks. Therefore, appropriate diagnostic tools that are cost-effective, rapid, robust, and sensitive are in great demand (Jain et al. 2021). In recent years, biosensors are widely employed for fast and accurate diagnoses. A biosensor is an analytical device that gives measurable signals via a transducer by converting a target analyte's molecular recognition (Sin et al. 2014). Generally, these biosensors are classified into optical, electrochemical, and piezoelectric devices based on the way they transduce signals (Castillo-Henríquez et al. 2020). Jeong et al. studied bacterial detection using a fluorescent supramolecular biosensor. The pathogen's binding induces conformational changes in supramolecular state that emits fluorescence which can selectively detect E. coli (Jeong et al. 2019). In another study, Mathelie et al. developed a silica NPs-assisted electrochemical biosensor for the specific and sensitive detection of E. coli (Mathelié-Guinlet et al. 2019). Another type of piezoelectric biosensor refers to the ability of material that generates voltage under mechanical stress. Guo et al. employed a piezoelectric biosensor with antibody-functionalized AuNPs that enhance changes in detection signals. The results demonstrated that the developed biosensor could be a suitable real-time monitoring method for the particular pathogen (Guo et al. 2012). Despite the emerging sensor technology, these next-generation biosensors still need to overcome fundamental challenges in the particular case of infection detection, a fully integrated biosensor capable of multiplexed and label-free detection. The development of multiplexed biosensor is a top priority to address the current issue as it operates by detecting multiple analytes on one device (Soler et al. 2017). Soler et al. studied the multiplexed nanoplasmonic biosensor for the simultaneous detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine samples. The plasmonic microarray composed of gold nanohole sensor arrays exhibited excellent optical transmission and provided highly sensitive analysis and label-free configuration. Thus, the multiplexed biosensor employed in this study exhibited outstanding

sensitivities, with a limit of detection of 300 CFU/mL for C. trachomatis and 1500 CFU/mL for N. gonorrhea. It is successfully employed to identify and quantify the two bacterial levels in a one-step assay (Soler et al. 2017). In another study, Gao et al. investigated the performance of a multiplex electrochemical biosensor for pathogen identification. The study examined the feasibility of rapid diagnosis of bloodstream infections using a multiplexed electrochemical biosensor. The results demonstrated that the sensor successfully identified the bacterial species, such as Klebsiella, Enterobacter, Serratia, Citrobacter, and Enterococcus in spiked blood samples. Thus, the multiplexed biosensor improves clinical management by providing appropriate and timely antimicrobial treatment (Gao et al. 2017).

9.9 Conclusion

Technological growth of science and engineering has made various researchers and scientists work on cross-disciplinary and interdisciplinary research, consenting significant progress in different research areas. One of the promising research areas is biosensing and bioelectronics, where people from medicine, physics, engineering, material science, chemistry, and biology collaborate to give various healthcare application devices. Working on these advanced areas of research always has vast-spread opportunities and challenges. The same is applied to the most recent advancement in bioelectronic systems when more than one sensor is used as multiplexed biosensor systems. Precise diagnostics can be achieved with the multiplexed biosensing approach since multiple bio analytes can be studied to understand the physiological condition of a person. Single bio analytes on multiple sensors can also be applied to better understand the bio analyte nature with varying adhesions and physical properties. Various measurements such as glucose, lactate, pH, and temperature can be done at the same time parallelly. Multiple sensing principles such as optical, electrical, chemical, mechanical, and biological can be applied simultaneously to improve the point-of-care diagnostics. The multiplexed biosensor has significant benefits in healthcare applications due to its low measuring time with a broad detection range at a low sample rate. Overlapping symptoms between the diseases of the human systems have made increasing demand for multiplexed biosensor systems that can detect multiple biomarkers simultaneously. Multiplexed biosensor systems based on membrane-based lateral flow assays limit the biomarkers compared to the existing systems. Multiplexing multiple test lines in lateral flow assays strip has physical limits, which could be overcome with multiple strips. Fabricating multiplexed array for bio analyte detection is tedious due to the cost and fragile nature involved. The use of various nanomaterials such as gold and graphene in multiplexed biosensors has its limits and challenges in their ideal characteristics such as size, shape, and biocompatibility. These challenges have to be adequately addressed when using nanomaterials in multiplexed biosensing applications.

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Biocompatible Sensors Are Revolutionizing Healthcare Technologies

10

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Abstract

Sensors bridge the gap between disease diagnosis and the attainability of physiological indicators. The Internet of Things (IoT) and artificial intelligence algorithms have become the core of new-generation health technologies. However, some in-situ/in-vivo physiological indicators and intrinsic biological behaviors on the micro scale cannot be detected due to the loss of the spatial and temporal consistency of the samples by in-vitro detection methods that are widely used now. Recently, biocompatible sensors are developing rapidly and have provided the possibility for long-term and in-situ monitoring of physiological information. Here we review the biologically compatible sensors, including wearable, implantable, and ingestible sensors; outstanding features; design

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_10

strategies; and applications. Moreover, the detection targets of the sensors are further divided into vital life parameters (such as body temperature, heart rate, blood pressure, and respiratory rate), biochemical indicators, electrophysiological signals (such as electrocardiography, electromyography, and electroencephalography), and motion signals based on the clinical needs. Derived from the emerging concept of next-generation diagnostics and healthcare technologies, we discuss unprecedentedly how biocompatible sensors reform the state-of-the-art healthcare system. Finally, the prospects and challenges of biocompatible sensors have been explored.

Keywords

Biocompatible sensors · Human health · Miniaturized devices · Therapy

10.1 Introduction

Biocompatibility is a significant challenge in the application of sensors. Generally speaking, it includes blood compatibility, histocompatibility, immune-compatibility, mechanical compatibility, and many other different levels. Biocompatibility refers to materials that cause appropriate reactions in specific body parts (van Loon and Mars 1997). According to the interpretation of the International Standards Organization (ISO) meeting, biocompatibility refers to the ability of living organisms to react with inert materials and generally refers to the compatibility between the material and the host (ISO 2009). After the biomaterial is implanted in the human body, it will impact and affect the specific biological tissue environment. The biological tissue will also have an impact and influence on the biomaterial. The cyclic result of the two continues until equilibrium is reached or the implant is removed. Therefore, biocompatibility is an ongoing theme in biosensors research (especially those that require direct contact with the body).

A series of new advances in material science and processing technology have improved the biocompatibility of sensors, enabling sensors to interact with our bodies directly, thereby extracting more body health information in situ, which was previously unimaginable. These biocompatible sensors can be deployed on the skin, in the gastrointestinal tract, and even inside human tissues. At the application level, such sensors can be divided into three different types: wearable (usually functioning on the skin), implantable (which can play a role directly in-vivo, usually in direct contact with the tissue), and ingestible (usually introducing them into the gastrointestinal tract through swallowing or other actions).

Among three types of sensors, wearable sensors, especially skin-contact sensors (or epithermal sensors), are the fastest developing type of biocompatible sensors. Direct contact with the skin has relatively few restrictions than implantable and digestible sensors that may involve complex immunoreactions and tissue response in sterilization residue, biological toxicity, irritation, and hypersensitivity. The compact, integrated, and lightweight wearable sensor can acquire vital health parameters

such as breathing rate, heart rate, oxygen saturation (SpO_2), and blood pressure on the skin surface (Guo et al. 2021). Compared with traditional medical monitoring equipment, wearable sensors can achieve long-term health monitoring without affecting daily life, and they could be further developed to capture highly sensitive EMG or pressure signals that can reflect swallowing and voicing (Tao et al. 2017; Wei et al. 2019; Kim et al. 2019c). Meanwhile, more than skin-contact sensors, non-invasive biochemical molecular testing that could be embedded into wearable sensors may significantly reduce the pain of liquid biopsy, allowing home and remote testing through health Internet of Things (H-IoTs) techniques, and these on-site (or in-situ) physical health information can help us diagnose underlying diseases and even perform long-term health monitoring.

Although wearable sensors can obtain a considerable number of health parameters, some characteristics of internal lesions and infections are often difficult to detect in vitro at the early stage of the disease course. Therefore, the sensitivity of wearable sensors is limited or interfered with by various inherent physiological and pathological mechanisms. To get a more accurate indication of the onset of symptoms, the clinician usually uses a biopsy or an intubation endoscope. However, these methods have poor real-time performance and do great harm to patients. To solve these problems, miniaturization of devices developed from microelectronics plays an essential role, and applying low-toxicity, biodegradable materials in sensor design and fabrication is also essential, which, accompanied with microelectronics, paves the way for long-term in-vivo detection.

In this chapter, we provide a global, comprehensive, and forward-looking view of biocompatible healthcare sensors. First, we review the rules for designing the required functions of sensors and the latest research progress in specific medical applications for detecting physiological information, respectively. Then, based on the state-of-the-art technology, we discuss the role of biocompatible sensors in driving the next generation of integrated diagnostic and therapeutic equipment and the transformation of medical services and intelligent management. Finally, the future and challenges of biocompatible sensors have also been prospected.

10.2 Wearable Biosensors for Healthcare

10.2.1 Features of Wearable Biosensors and Designing Strategies

Traditional healthcare services require well-trained personnel and relatively larger equipment, which leads to real-time and long-term monitoring inconvenience. Wearable sensors are designed to solve this problem and revolutionize medical devices and traditional diagnostic methods with timely, remote, wearable, portable, and even artistic properties. Over the past few years, wearable systems have proven to be highly sensitive and can quickly and noninvasively acquire biophysical and biochemical signals such as body movement, blood pressure, body temperature, metabolites, and some biomacromolecules. However, compared with the medical detection equipment deployed in vitro, the complex sensing interface puts forward

relatively high requirements for the performance characteristics of wearable sensors. Therefore, in the design and fabrication of device structure, more consideration should be given to the desired characteristics, and appropriate functional materials should be selected comprehensively.

10.2.1.1 Stretchability and Conformality

The most significant difference between wearable systems and traditional healthcare sensing devices is their malleability or stretchability, which makes a conformal contact between the sensor and the skin interface. The feature of the extension is also referred to as mechanical biocompatibility. Good stretchability ensures continuous and steady signal acquisition while maximally not affecting arbitrary body movements. Generally recognizing, there are three main design approaches to obtain tensile properties: appropriate geometric layout, implementing thin layers, and applying flexible materials.

Devices made of hard or brittle materials usually have certain advantages in electrical performance and operating stability. One of the most immediate ideas is miniaturizing devices and obtaining flexibility through the carefully designed structural arrangement. The most prevalent strategy, "island-bridge" layouts, involves conductive traces (bridges) interconnecting high-performance but rigid functional components (islands) (Libanori et al. 2012). By comparison, several design strategies of wire layout have proved to be effective in converting rigid materials into tensile materials and maintaining electrical properties (Fig. 10.1a–d). For example, serpentine-shaped structures, consisting of periodic arcs and straight segments, have been widely adopted to connect rigid islands on top of soft elastomers[4]. Additionally, using pre-tensioned elastomer substrates or tiling techniques also has a good effect. In recent years, with the introduction of flexible materials such as organic polymers and gels, the mechanical mismatch of wearable devices has been solved to a large extent. However, the mismatch at the heterogeneous interface still exists, and structural optimization of device layout is still the critical strategy to improve the stretchability (Liu et al. 2017).

According to Euler−Bernoulli beam theory, implementing thin layers can also obtain the desired flexibility. It predicts that the flexural rigidity, defined as the resistance of a material to bending, is proportional to the object's thickness to the third power. For instance, single crystalline silicon nanomembranes (Si NMs), with thicknesses of 100–200 nm, can be transferred from silicon-on-insulator (SOI) wafers onto thin-polymer substrates (Fig. 10.1e). Such integration allows for bending to small radii of curvature without fracture due to the decrease in bending stiffness by several orders of magnitude (once again by the cube of the thickness) (Sun et al. 2006). Recent works have also reported methods to produce large-area organic and/or inorganic devices on ultrathin substrates, thus enabling bending radii as small as tens of microns, even when using materials with relatively large elastic moduli.

The application of elastic materials plays an essential role in the development of flexible electronics. Wearable devices made by low-dimensional materials represented by carbon nanotubes, graphene, MoS_2, and black phosphorus have

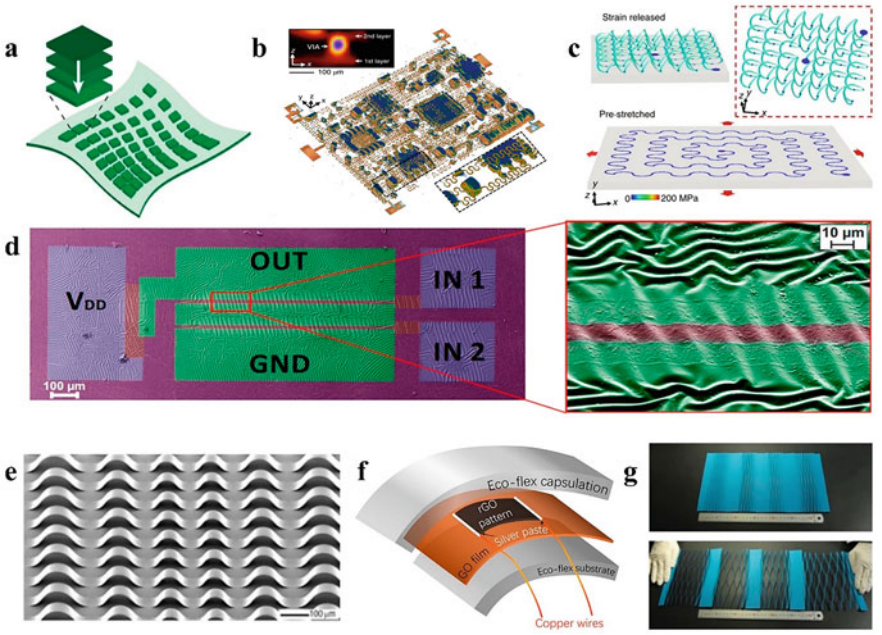

Fig. 10.1 Designing strategies for stretchability and conformality. (**a**) The schematic illustration of stiff patterning devices islands on soft substrates (Libanori et al. 2012). (**b**) Island-bridge stretchable electronics with serpentine interconnects (Huang et al. 2018). (**c**) Bi-axially stretched soft elastomeric substrate (pre-stretched). The color represents the magnitude of Mises stress in the metal layer (Jang et al. 2017). (**d**) Colored SEM image of a wrinkled unipolar logic NAND gate and the channel region (Cantarella et al. 2017). (**e**) Single crystalline silicon nanomembranes with thicknesses of 200 nm bent to radii of approximately 100 μm (Sun et al. 2006). (**f**) Schematic layout of a single strain sensor, consisting of a laser scribed graphene pattern, electrodes, and Ecoflex capsulation integrated vertically like a sandwich (Wu et al. 2020). (**g**) The unstrained and strained states of the SWCNT strain sensor, respectively (Yamada et al. 2011)

been developed. Their flexibility is actually due to the ultra-thin thickness of active materials. In addition, many commercial polymers and elastomers can be used as substrates for flexible and stretchable electronic products. Silicone materials (an example of the PDMS shown in Fig. 10.1f, g) as the earlier flexible material have an elastic modulus similar to skin, thereby enabling optimal conformal skin–device contact, adhesion, transpiration, etc. (Kim et al. 2011). Beyond silicone, additional polymeric materials such as poly(vinyl alcohol) (PVA) films and polyethylene terephthalate (PET) have been implemented as supporting substrates or protective layers for optimized skin–electrode contact. These materials have different thicknesses, elastic modulus, adhesive strength, and other physical properties. However, the complexity of fabricating high-performance integrated circuits has limited many advanced digital functions in epidermal devices, such as wireless communication, signal processing, and power transfer. With the development of technology, sensing devices have been gradually integrated into clothing (Yamada et al. 2011),

insoles (Wu et al. 2020), sweatbands, and other daily necessities (Zhu et al. 2019). Some biodegradable and biocompatible biomacromolecules have also been used in the preparation of wearable medical systems. This brings people closer to the latest technology and highlights the integration of wearable medical systems and daily life.

10.2.1.2 Immune Bio-Compatibility and Bio-Degradability

In point-of-care applications, the ideal situation is that wearable healthcare devices will not pose additional threats to health while avoiding restrictions on daily activities. Above, we discussed the mechanical and biological compatibility of wearable devices, and here we mainly introduce immune compatibility. For future healthcare applications of nanostructured materials, there is a great need for a deeper understanding of immune response and specific definitions of exposure criteria under various circumstances (Al-Qatatsheh et al. 2020). Previously mentioned flexible materials are biologically compatible and have low toxicity. Additional studies on both processed films and fabricated devices for cell cytotoxicity and in vivo immunological tests show promising results for immunologic and tissue biocompatibility.

Compared with those biocompatible synthetic materials, natural biomaterials have more excellent biological characteristics (such as renewability, low cost, water-solubility, bio-degradability, self-adherence and self-cleaning, etc.) (Zhou et al. 2020), and providing an essential platform for the production of various hybrid materials with certain functional active groups. In addition, it is more environmentally friendly and skin-friendly for long-term deployment and waste recycling. Some emerging studies have applied chitosan (Zhong et al. 2011), natural pollen (Arman Kuzubasoglu and Kursun Bahadir 2020), etc., to physical or chemical parameters–sensing applications. Although even biocompatible and biodegradable materials make long-term monitoring possible, all of these wearable devices have an upper limit application time due to the natural turnover cycle of epithelial cells (around 2 weeks).

10.2.1.3 Other Desired Features

Mechanical biocompatibility and immune biocompatibility are the basis of the safe and stable work of wearable healthcare systems. Based on realizing essential functions, some improved features have been gradually endowed to wearable biosensors. For example, some flexible patterning manufacturing methods can achieve tattoo-like electronic skin, which can be easily prepared, deployed, and aesthetically unified. Tang, et al. improved the electronic tattoo to make it more sticky and better able to hold shape, increasing the effectiveness of the electronic skin as an actual tattoo (Tang et al. 2021). Likewise, several research groups have developed materials with self-healing properties for flexible and stretchable electronics; details could be found in the topical review (Imani et al. 2016). These features together are pushing wearable healthcare systems toward practical applications.

10.2.2 Detectable Indicators of Physical Health

Researchers tried to put old sensors on people's bodies to get more accurate and effective information as an initial idea. This leap has revolutionized the model of health testing. Some wearable electronic devices (such as sports bracelets and stickers) detect them efficiently and in real-time. Understanding intrinsically related health parameters is essential when assessing the health status and diagnosis of an underlying disease. The research in the past decade has enriched the detection targets of the wearable medical system, and we summarized them in Table 10.1.

10.2.2.1 Long-Term Monitoring of Vital Health Parameters

Respiratory rate, heart rate, blood oxygen saturation (SpO_2), blood pressure, etc., also known as the vital health parameters, directly reflect the basic vital signs of the human body. Moreover, abnormal fluctuations in vital health parameters are associated with trauma, infection, or chronic disease. Therefore, monitoring vital health parameters simultaneously is very important in both daily life and medical care. However, general medical devices such as blood pressure monitors or stethoscopes cannot realize multi-functional detection and are inconvenient, mainly because of the different detection mechanisms. Fortunately, the wearable healthcare system provides a multi-functional platform, which can be equipped with multi-functional sensors and has the advantages of portability, comfort, and aesthetics. For example, the newest generation of wearable devices presented by Randazzo et al. can monitor patients' health parameters, including skin temperature, electrocardiograms, SpO_2, and physical activities (Randazzo et al. 2020). Similarly, many other multi-functional wearable systems monitor vital health parameters and communicate with data platforms such as mobile phones for real-time, long-term data acquisition.

10.2.2.2 Physical Physiological Parameters

Human motion monitoring is necessary for sports performance, medical treatment, personalized rehabilitation, and disabled people. Termly assessment of body movements can detect abnormal gait patterns, sudden tremors, and difficulty swallowing or vocalizing, which are the precursors of fatal diseases, including Parkinson's disease, Alzheimer's disease, and epilepsy. Thus, it contributes to the early diagnosis and treatment and can also be used for sign language recognition and human–computer interaction (Zhou et al. 2020). The body's movement usually manifests as a considerable change of strain on the skin and a small-scale strain recognized as facial expression, pulse, and breathing. Furthermore, the corresponding wearable system can be designed appropriately according to the modulus and signal strength of different deployed parts.

In addition to detecting these visible movements, some more exceptional work extends the concept of wearable motion monitoring. Previously, we developed an intelligent artificial throat (Wei et al. 2019) that can obtain subtle voice sounds in the throat, showing the potential for speech recognition and interaction. This is good news for patients with laryngeal cancer resection and other people with laryngeal

Table 10.1 Summary of healthcare indicators in wearable monitoring

Basic categories	Detection targets	Deployment locations	Applications	Ref
Vital health signs	Heart rate	Wrist, arms, chest, fingertip	Heart disease	Lin et al. (2017); Majumder et al. (2017)
	Respiratory rate	Chest, nose, waist	Respiratory diseases	Zhao et al. (2016)
	SpO_2	Fingertip	Cardiopulmonary dysfunction	Huang et al. (2014); Arakawa (2018)
	Blood pressure	Arm, chest, wrist	Hypertension	Arakawa (2018); Kim et al. (2019b); Al-Qatatsheh et al. (2020)
	Body temperature	Anywhere	Infect	Trung et al. (2018); Xiang et al. (2019); Arman Kuzubasoglu and Kursun Bahadir (2020)
Physical or physiological parameters	Intraocular pressure	Eyes	Eye diseases	Ma et al. (2021)
	Optical attenuation	Skin	Breast cancer	Heikenfeld et al. (2018)
Biochemical substances	Metabolites	Skin, eyes, nose, mouth	Diabetes, osteoporosis, gout	Gao et al. (2017); Bandodkar et al. (2019); Pataranutaporn et al. (2019)
	Heavy metals	Skin, eyes	Osteoporosis, Parkinson's	Gao et al. (2017)
	Electrolytes	Skin, eyes	Electrolyte disturbance	Gao et al. (2017)
	pH	Skin, eyes	Diabetes, poisoning	Gao et al. (2017)
	Other disease biomarkers	Skin, eyes, nose, mouth	Malnutrition, cancer	Gao et al. (2017); Pataranutaporn et al. (2019); Ma et al. (2021)
Electrical activity	EEG	Head	Epilepsy	Imani et al. (2016); Ha et al. (2018); Kang and Ha (2018)
	ECG	Chest, arm	Heart disease	
	EMG	Arm, wrist, feet, legs, chest	Myogenic damage	

dysfunction, who can have a lower learning cost and a more comfortable experience than traditional rehabilitation programs such as implanted vocal prosthesis esophageal speech. Similar ideas can be applied to breathing, heart, and gastrointestinal sounds detection (Zheng et al. 2020).

Another critical physical physiological parameter is body temperature, whose abnormal changes indicate symptoms like wound healing, cognitive status,

cardiovascular diseases, etc. Therefore, it is necessary to check body temperature regularly in healthcare management and clinical diagnosis. Flexible temperature sensors possess high-resolution sensitivity in terms of spatial distribution and numerical value, continuously measuring by attaching directly to the skin surface under different postures and motions.

Electrophysiological signals in nerve and muscle tissue provide another dimension of neurological disease, cardiovascular disease, and apparent movements. Recently, wearable electronic skin has provided a multi-functional platform for electrophysiological bio-signal monitoring. For example, electroencephalography (EEG) is a powerful tool for developing new brain–computer interfaces (HMI) and diagnosing brain function and neural conditions diseases, such as tumors, brain diseases, and sleep disorders. In the case of epidermal electrical measurement, the skin-electrode is used to extract the depolarization signal from the myocardium (called ECG). In addition, ECG evaluates the general state of the cardiovascular system. Through the properties of specific peaks of ECG signal, i.e., peak intensity, shape, and periodicity, patients can conveniently identify their heart condition and realize early diagnosis of severe heart problems such as cardiomyopathy arrhythmia and hypertension using skin-attached ECG sensors. At the same time, the HMI with electromyography (EMG) as a control signal combined with stimulation as feedback represents another essential healthcare application in a robot, artificial limb, and machine-assisted life (Pu et al. 2018).

10.2.2.3 Non-invasive Detection of Biochemical Substances

Metabolites, including polyols, uric acid, lactic acid, cholesterol, glucose, etc. can directly reflect the physiological activity of cells (Goyal et al. 2021). Excessive changes in these small molecular weight metabolites can adversely affect acid–base balance and organ function. These substances usually exist in blood, tissue fluid, lymphatic fluid, and other body fluids. In some, exudated body fluids also exist in a particular concentration, and present a strong correlation. For example, sweat contains abundant metabolite information and is often used as an ideal skin detection target (Kim et al. 2019a). Several personalized human subject studies have revealed a close pharmacokinetic association based on sweat and capillary blood samples (Moon et al. 2021). In contrast, although saliva, urine, and tears also contain rich metabolite information, the convenience of the wearable system cannot be well expressed in sample collection and poses a more significant challenge to device design. Therefore, it is usually used as an alternative for the non-invasive detection of biochemical substances.

However, traditional invasive liquid biopsy methods, especially blood sampling, result in pain, poor portability, and are time-consuming. Therefore, people have been devoted to developing a non-invasive and portable metabolites detection scheme. The flexible wearable sensor has been proved to be a new trend in on-demand healthcare monitoring, which can detect metabolite levels conveniently, in real-time, and safely. Several sensing principles, including optics and electrochemistry, have been applied to wearable biochemical sensing. A stretchable optical sweat sensor based on a thin and soft enclosed microfluidic system has been developed by

Koh et al. (Koh et al. 2016), which can collect sweat directly and quickly without producing sweat evaporation or contamination, thus solving the traditional challenge of sweat and allowing complex sweat sampling and measurement.

In addition to these micro molecules, some immunogenic disease markers can also be detected by wearable devices. The most typical example is the cancer markers such as prostate-specific functional antigen (PSA, related to prostatic cancer) (Wang et al. 2016), human epidermal growth factor receptor (HER, related to breast cancer) (Fu et al. 2017), etc. However, long-term detection is a significant challenge for immune sensors to be overcome. In Munje et al.'s work, the subjects' sweat was used for in vitro evaluation. They used room-temperature ionic liquids to compensate for the change of sweat acidity and basicity, and the stability of antibody receptors was improved up to 96 h (Munje et al. 2017). Nevertheless, since there are only trace amounts of these biomacromolecules in vitro, accurate detection depends more on the body's current state, which limited practicability in continuous monitoring applications. Thus, we prefer liquid biopsies or even in situ detection in vivo.

10.3 Ingestible Biosensors for Healthcare

10.3.1 Biologically Compatible Encapsulation Methods

Wearable sensors cannot reach some niduses inside the body, and detectable health indicators on the body surface are easily interfered with. As a complement strategy, ingestible biosensors can travel close to significant parts through the gastrointestinal (GI) tract, monitor a wide range of biomarkers and therapeutic targets, and serve as practical clinical tools for diagnostics and therapy (Steiger et al. 2019). In addition, the ingestible sensor can benefit from better space tolerance in the gastrointestinal tract and integrate multiple functions into a swallowable capsule (Fig. 10.2). Hence the main challenge of ingestible biosensors is the biocompatible approach to encapsulation. Early ingestible sensors primarily consist of inorganic materials and encapsulated with rigid non-degradable polymers, such as polydimethylsiloxane, parylene, or epoxy (Mimee et al. 2018). While this can improve device retention, it also poses significant risks. Recent research shows that edible and nutritive electronic materials are more suitable for biocompatible packaging materials. These nutritive materials can be mainly dissolved, digested, and absorbed after diagnosing or treating diseases in the gastrointestinal tract (Sohn et al. 2020), including nutritive inorganics and organics, such as edible inert metals, trace elements and their oxides, bio-pigments, and polymers (Stehle et al. 2016). This provides a powerful toolkit for the design of biologically compatible sensors.

10.3.2 Detectable Healthcare Indicators of Ingestible Biosensors

Ingestible sensors can detect a wide range of bio-signals to monitor health state, including tissue imaging, local biomarkers (such as electrolytes, metabolites, and

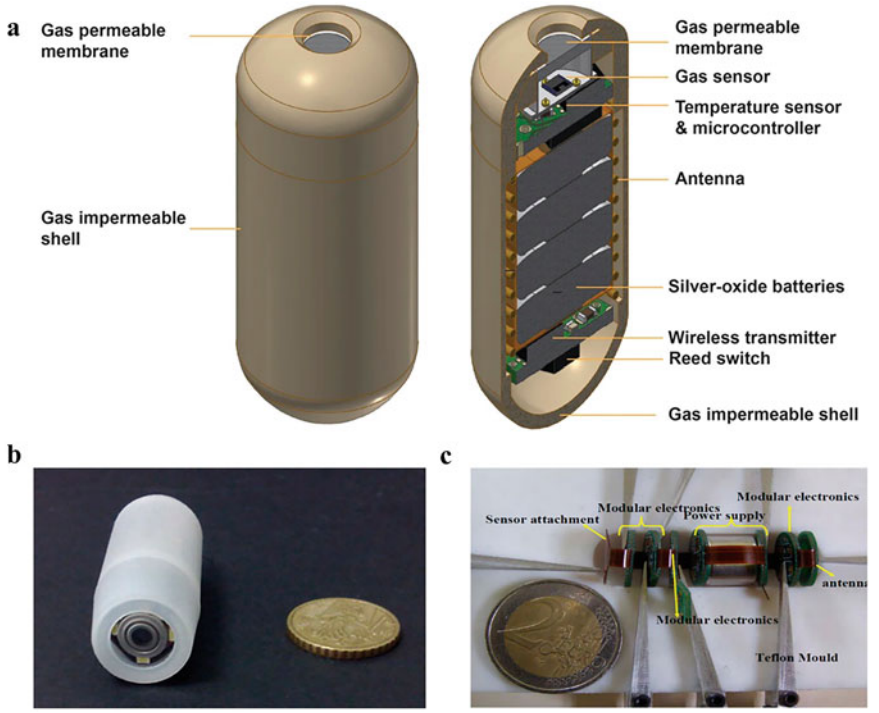

Fig. 10.2 Typical ingestible capsule sensors. (**a**) Schematic of a gastric gas profiler (a gas sensor capsule) (Ou et al. 2016). (**b**) A capsule prototype (Cavallotti et al. 2009). (**c**) The small construction of a swallowable capsule prototype (O'Mara et al. 2018)

enzymes), microbiome, etc. This enables the ingestible sensing technology to be applied in tissue imaging evaluation, monitoring of gastrointestinal inflammation, and health status (Kalantar-Zadeh et al. 2017). Besides, it can assess the drug medication compliance and the adjacent organs' functions, such as heart sounds or respiratory sounds (Traverso et al. 2015) and hemo-dynamic parameters (Phan et al. 2008). Here we focus on the relevant detectable bio-signals of the GI tract. Clinical proof-of-concept studies demonstrated the first developed generation of ingestible electronics to measure the GI tract's temperature, pressure, and pH (Frias et al. 2017). Besides, numerous biomarkers in the GI tract, such as the microbiome, metabolites, electrolytes, physiological gases, proteins, and nucleic acid, can potentially be utilized to assess health and disease states in real-time.

Advances in camera technology led to the development and widespread adoption of pill cams, eliminating the need for complex endoscopic procedures. Optical biopsy of the GI tract can provide painless and convenient clinical evidence to support the diagnosis of polyp, tumors, internal bleeding, Barrett's esophagus, and other lesions. A recent study demonstrated that X-ray-based ingestible electronics could evaluate the GI tract (Gluck et al. 2016). Patients must ingest the contrast agent before using the capsule. The capsules can map the gastrointestinal tract by emitting

and probing two-dimensional X-ray beams, while an integrated electromagnetic tracking system feedbacks the position and orientation of the capsule (Kalantar-Zadeh et al. 2018). The collected 2D data sets can generate 3D images of the gastrointestinal tract. Although the proof-of-concept studies have been conducted and the clinical safety of these medical tools has been proved by clinical case studies, more extensive clinical assessments should be made to further ensure their safety and efficacy (Kimchy et al. 2017).

10.4 Invasive Biosensors for Healthcare

10.4.1 Introductions and Advancements of Invasive Biosensors

Although in-vitro detection devices and ingestible biosensors can measure biological signals in various dimensions, in-situ sampling or detection can further enrich detection objects and improve signal quality. Therefore, invasive biosensing techniques are necessary for healthcare. For example, microneedle-based structures can significantly reduce fluid sampling volume while minimizing pain and injury for patients (Miller et al. 2012, 2014). Furthermore, the microneedles combined with wearable devices enable continuous and immediate patient monitoring and even the ability to deliver drugs (Cahill et al. 2018), which lead to integrated systems for diagnosis and treatment.

However, close contact between invasive sensors and the human body inevitably leads to biocompatibility problems. In recent years, with the developments of various device structures and advances in flexible and compatible materials such as biodegradable materials, biocompatible hydrogels, and conductive nanocomposites (Lee et al. 2016), the concept and applications of the minimally invasive and implantable biosensors are proposed and improved to realize high-quality detections and monitoring in vivo which are capable of physiological and electrical examinations in the real-time diagnosis or long-term sensing for targeted treatments. However, the complex mechanism of organs/tissue and their responses to foreign devices impede the progress and applications of the implantable biosensors (Frost and Meyerhoff 2006). Sensors in vivo should minimize the injuries to target organs/tissue, improve their performances and achieve long-term biocompatibility. Most of the design strategies introduced in the wearable and ingestible sensors are still applicable to implanted sensors, but with more stringent requirements. Thus, the rapid development of the implantable biosensors focuses on solving the essential issues and improving the key performances, such as the sensitivity, which is related to the limit of detection, selectivity, sensing reliability, tissue response and sensors' long-term compatibility and flexibility in vivo.

10.4.2 Detectable Indicators of Physical Health

10.4.2.1 Electrophysiological Signals

The implantable biosensors are used to detect neural action potentials fast and process the electrophysiological signals with high sensibility and sensing reliability, which promises the monitoring and warning of long-term diseases or diseases arising spontaneously, such as Parkinson's disease, essential tremor, and chronic electrophysiological recordings. However, on the one hand, in terms of structures, the electrode dimensions, wide spacing of the electrodes, and insufficient electrodes lead to inaccurate recording (Horton and Adams 2005). On the other hand, the rigid materials prevent the devices from fitting the curved surface of the brain. Thus, novel structures and bio-compatible materials or new innovative sensor devices have been invented. For example, Guan et al. developed an internal ion-gated organic electrochemical transistor based on a reversible redox reaction and hydrated ion reservoirs within the conducting polymer channel, which can achieve chronic intracranial encephalography from the surface of the brain, deeper neural structures in freely moving rats, and even the real-time detection of epileptic discharges (Guan et al. 2019).

Furthermore, compared with conventional structures, a neuro-tassel with ultrasmall sizes and high flexibility consisting of an array of high-aspect-ratio microelectrode filaments is proposed to record electrical signals and neural activity stably (Goyal et al. 2021). It is worth noting that deep brain stimulation is a widely used method to cure neurologic conditions. A fully implantable device with both chronic electrophysiological recording and stimulating capabilities has been proved effective. In addition, the device has a high resolution, low noise, and stimulation artifacts.

10.4.2.2 Biomarkers and Chemicals

The biomolecules and chemicals in vivo are strongly responsible for physical health and disease. At the same time, the changeable and complex micro-physiological environment containing a large number of coexisting substances with similar structures and properties challenges the sensing selectivity and reliability of the implantable biosensors. Thus, reliable concentrate and composition detections and real-time kinetics observation of the biomolecules and chemicals in vivo, such as dopamine (DA) and glucose, have received more attention.

As a significant neurotransmitter, DA widely distributes in the central and peripheral nervous system and kidney endocrine system and can regulate essential physical and cognitive functions. Therefore, research on the detection of DA shows its importance both in physiological and clinical applications. Based on the fundamental carbon fiber electrode structure (CFE), the CFE coated by PEDOT/graphene oxide is proposed to detect the DA at high speed and high sensitivity and exhibit an $880 \pm 88\%$ increase in DA sensitivity without significantly altering electrode kinetics (Zhang et al. 2018). At the same time, the carbon fiber microelectrodes (CFMEs) modified with copper(I) sulfide functionalized nanocomposites of the reduced graphene oxide (Cu_2S/RGO) possess high selectivity of DA meanwhile

avoiding the interference of other components like histidine, ascorbic acid, uric acid, and others (Pu et al. 2018). Besides, ascorbic acid (AA), similar to DA, can also be detected using the CFE.

As diabetes with its complications seriously endangers human health, there is an urgent need for continuous blood glucose monitoring method that helps realize the real-time controlling of diabetes. A flexible enzyme-electrode sensor with a cylindrical working electrode modified with a 3D nanostructure achieves glucose monitoring based on interstitial fluid analysis. Moreover, it addresses the sensing sensitivity by monitoring even under hypoglycemic conditions (Hao et al. 2016).

PH monitoring of the central nervous system in the live brain shows great clinical and pathogenic significance, which helps comprehend acid–base chemistry and responses to the brain activities and physiological methods. Odijk et al. demonstrated a potentiometric method for in vivo monitoring of pH in the central nervous system using carbon fiber–based proton-selective electrodes, which disclosed and explained that brain acidosis is induced by CO_2 inhalation and brain alkalosis is induced by bicarbonate injections, respectively (Odijk et al. 2015). Continuously monitoring PH in the brain may also promote the detection and monitoring of positive ions such as K^+, Na^+, and Ca^{2+} in vivo. A novel type of K^+ sensitive solid-state, ion-selective electrode probe based on PEDOT is fabricated to measure multiple parameters relevant to the neurological phenomenon of spreading depression (SD) which is considered necessary in brain diseases such as stroke, traumatic brain injury, and migraine with aura (He et al. 2013).

10.4.2.3 Mechanical Pressure

As a high-risk disease, intracranial hypertension is possibly caused by traumatic brain injury, tumors, hydrocephalus, stroke, and meningitis. Implantable pressure sensors can continuously and wirelessly monitor intracranial pressure (ICP). It is worth mentioning that Chen et al. report an implantable and bio-absorbable multifunctional sensor for continuously monitoring ICP, minimizing the risk of infection, and reducing pain for a patient (Boutry et al. 2019). Furthermore, accurate and continuous elevated intraocular pressure (IOP) monitoring can prevent or relieve decreased vision and blindness. Furthermore, a multi-functional contact lens sensor with a sandwich structure is reported to continuously and wirelessly monitor IOP. Also, considering the significance of blood flow detection for recovery after surgeries, a pressure sensor that consists of a bilayer coil structure for radio frequency data transmission and a fringe-field capacitive pressure sensor are reported by Boutry et al. (Fig. 10.3) (Boutry et al. 2019).

10.5 Transforming Healthcare Technologies with Biocompatible Biosensors

Over the decades, researchers have developed a variety of compelling biosensors, some of which are already commercially available. These biologically compatible sensors are making detection trickle down to the client and revolutionizing current

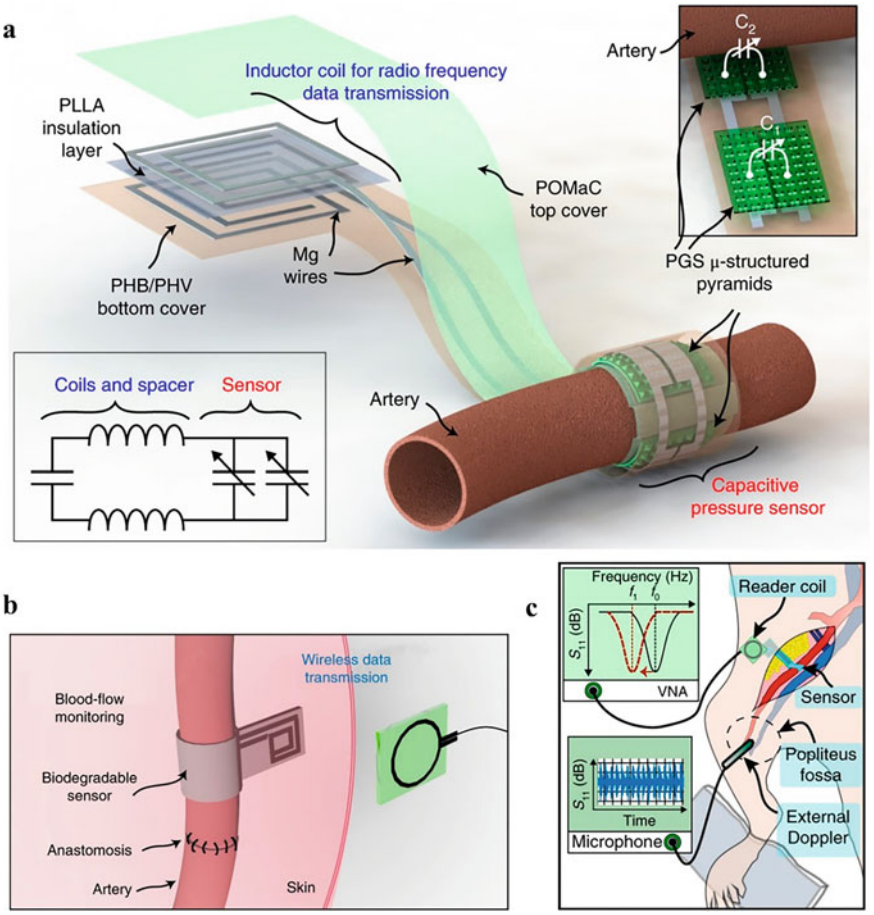

Fig. 10.3 (**a**) Schematic illustration of a cuff-type pressure sensor and a bilayer coil structure. The right inset shows the pressure-sensitive region of the sensor with the two variable capacitors C1 and C2. Figures (**b**) and (**c**) depict wireless monitoring of the arterial blood flow and the wireless measurement setup schematic diagram, respectively (Boutry et al. 2019)

healthcare technologies. From the perspective of application level, the new generation of healthcare technology presents three forms: the next generation of intelligent diagnosis, integrated diagnosis and treatment equipment, and the improvement of medical service and management level (such as competent bedside care, chronic disease management, and improvement of inpatient treatment efficiency).

10.5.1 A Prototype for the Next Generation Diagnostics

The core connotation of the subsequent generation diagnosis is personalized diagnosis and precision medicine. A variety of environmental sensors and biocompatible sensors provided multidimensional data to support the development of precision medicine. However, data is still read and judged manually and ultimately collected in electronic or paper medical records in most healthcare facilities. Moreover, specialized data analysis methods are not widely used. Therefore, to overcome the obstacles of data acquisition and analysis, the intelligent algorithm is introduced into the medical sensing system, which significantly promotes the development of personalized and accurate diagnoses. The organic combination of these novels, biologically compatible sensors, and artificial intelligence (AI) is considered the prototype of the next generation of diagnostic technologies. AI algorithms can significantly improve the accuracy of the diagnosis, making the physiological signals in situ play the leading role. For example, Veeralingam et al. reported an artificial intelligence/machine learning-enabled multi-functional sensing platform with high accuracy and speed for simultaneous and continuous monitoring of specific vital body parameters, including the hydration levels of the skin, glucose concentration, and pH levels in biofluid sweat (Veeralingam et al. 2020). Furthermore, to facilitate a human–machine interface that can analyze data from large sample sizes, the sensor was interfaced with the open-source microcontroller board (QueSSence), wherein Artificial Intelligence (AI)-based K-Nearest Neighbors (KNN) algorithm enabled precise and faster data acquisition from complex mathematical conjunctures.

Notably, AI algorithms and models build bridges for comprehensive, integrated perception, and health assessment based on environmental and biologically compatible sensors. This set of forward-looking concepts has been tested in animals. For example, Zhang et al. specially designed a wearable multi-sensor system that can continuously obtain real-time data of environmental and physiological parameters of live sheep (Zhang et al. 2020). They established a prediction model between environmental and physiological parameters and a comfort and health evaluation model. The results show that the wearable multi-sensor system had high accuracy and stability of data collection, and the power consumption and communication performance can meet the monitoring requirements. Notably, the correlation analysis showed that there was a significant correlation between environmental and physiological parameters. Therefore, obtaining environmental information could predict health conditions in the case of unknown vital health parameters. This inspires us to improve the inpatient environment and building layout and provides a temporary alternative to the wearable intelligence revolution in medical institutions that lack wearable devices, to predict the health status of patients through changes in environmental parameters. This was fully demonstrated in the COVID-19 pandemic. Despite the lack of real-time monitoring of the progress of the inflammatory storm, measures such as improving ventilation in buildings and limiting the flow of people have reduced a large number of potential infections.

10.5.2 Tiny Integrated Systems for Therapeutic Interventions

Personalized and accurate diagnosis can guide the treatment of some diseases. Small systems based on biocompatible sensors enable targeted therapeutic interventions, including but not limited to exercise rehabilitation (Yamada et al. 2011) and drug therapy (Banerjee et al. 2019). Take laryngeal function rehabilitation as an example. Neurological diseases such as stroke, epilepsy, Parkinson's disease, and esophagus cancer treatment often cause patients to suffer from swallowing difficulties. Restoring conscious laryngeal function first requires perceiving the patient's intentions in the present moment. Fortunately, some wearable devices based on EMG or mechanics can sense subtle signals in body parts when swallowing and speaking and have demonstrated feasibility in rehabilitating patients with throat disabilities. In recent years, with the development of computational science, new algorithms enable EEG signals to support the extraction of speech features, which provides an alternative option for the rehabilitation of laryngeal dysfunction.

In pharmacotherapy applications, in addition to wearable drug delivery devices, ingestible sensors have an inherent advantage in drug delivery, as patients generally prefer oral delivery over other delivery routes. Besides, some techniques to promote absorption, such as improved ultrasound-mediated drug permeation and electronically controlled delivery of active pharmaceutical ingredients, are integrated into ingestible systems. Such devices with wireless telemetry functionalities can shuttle drugs to preferred absorption sites along the GI tract and address the problems of low solubility, low stability, or poor permeability. Active sensing also allows for precise drug delivery. For example, the first electronically controlled GI drug delivery system, IntelliCap, autonomously determines the device's localization by assessing the GI tract's pH profile via the integrated pH sensor (van der Schaar et al. 2013). Other sensing technologies such as gas-based or corrosion-based changes can also be deployed to control drug delivery. This provides a potential tool for automatically releasing drugs according to personalized treatment plans.

The most exciting concept of integrated diagnosis and treatment is the unlimited prospect offered by implantable systems, and the implantable brain-computer interface (BCI) is a concrete example of such an integrated system. The implantable BCI provides a high-quality interface for information technology to interact with organic biological systems, and it can be used for disease monitoring and rehabilitation treatment of frostbite and high paraplegia. Furthermore, some implantable electrodes can be used for diagnosis as a tool for inspecting internal electrophysiology and some biomolecules, while other works demonstrate the ability to interact, thus achieving a closed-loop of diagnosis and treatment. For instance, Sohn et al. introduced the bi-directional brain–computer interfaces (BD–BCI) (Sohn et al. 2020) to restore movement and sensation. In addition, a prototype artificial sensory stimulator was integrated into the benchtop system, which incorporates an active charge balancing mechanism based on pulse-width modulation to ensure safe stimulation for chronically interfaced electrodes. Thus, it could prevent brain tissue and electrode damage and achieve concurrent operation of recording and decoding motor commands from the brain.

10.5.3 Improvement of Medical Services and Management

As the top application of healthcare technologies, medical service and management have initially realized informatization, while bedside care and medical management promote intelligence by integrating biologically compatible and mature environmental sensors. IoT technology has extensively promoted the development of clinical management and telemedicine. Although biocompatible sensors can detect more in-situ information, the biggest challenge is quickly obtaining it, particularly important for ingestible and implantable sensors. It has become necessary for biocompatible sensor terminals to communicate with cloud or relay devices represented by mobile phones via far-field radio-frequency (RF) communication, cutaneous electrodes, wireless networks, blue tooth, etc. Especially in an aging society, which often means higher rates of chronic disease and the attendant high health care costs. With the ability to quickly implement instant testing, this technology can improve people's life quality and realize telemedicine and personalized treatment while reducing stress on the healthcare system and minimizing its operating costs.

One of the salient problems in clinical treatment is drug compliance. Non-adherence to chronic drug treatment plans causes delays in treatment and a run on resources for medical services. It has also caused hundreds of billions of dollars of preventable fiscal burden on governments (Osterberg and Blaschke 2005). To address this problem, oral medicines can be tagged with radio frequency identification (RFID) chips to monitor patient compliance with the specific treatment plan (Belknap et al. 2013). After ingestion and contact with gastric fluid, the ingestible system is powered by a galvanic couple and communicates its identification code to a receiver patch, which the patient wears. Proof-of-concept studies assessing the transmitting efficacy have been performed. Therefore, ingestible electronics are ideal for conventional medication to solve medication non-adherence. Notably, although the evaluation of transmitting efficacy and improved clinical outcomes have been demonstrated in patients, the increased cost of drugs incorporating the chip in practical application promotion should also be taken into accounts.

10.6 Prospects and Challenges

In the last decade, we have seen a boom in the intersection of different technologies and biologically compatible sensors. Well-designed biosensors have been able to obtain a wide range of physiological health information. In-situ, real-time, and multi-marker simultaneous detection has become a common pursuit and technical level, which provides an essential basis for precision medicine and personalized diagnosis. Notably, some of the emerging new biomarkers are also potential detectable targets that deserve continued attention. Advances in materials science, device design, and processing methods have enabled long-term, real-time monitoring to help monitor the progression of chronic diseases, understand their course, and further identify potential biomarkers.

Making good use of these powerful healthcare technologies is also a problem worthy of attention in the future, which is reflected in the selection of sensing technology suitable for application scenarios, the processing of massive multimodal data, and the comprehensive management of multi-individual data. At present, researchers have fully explained a variety of sensing technologies in some common disease application scenarios and have also made some beneficial explorations in the accurate diagnosis assisted by artificial intelligence algorithms and the improvement of medical service management. New information and sensing technologies and clinical needs will further transform the next generation of diagnostics.

However, the current applications and research of biocompatible sensing technology in healthcare, particularly in the care of chronic diseases and invasive, implantable sensing applications, are targeted at a limited number of fields and cases. Furthermore, much of the research has focused on technology, with a lack of user-centric applications. As electronic devices become integrated with organisms, we should also recognize the challenges that lie ahead, including ethics, safety, communication (signal elicitation), dynamics, control, and organizational interactions. In addition, most of the current studies on intracellular monitoring only prove the feasibility of the principle and function. If it is to be applied in clinical practice, user experience improvement is also an important aspect. The commercialized wearable electronic products provide a good reference for the development of the next step of technology. The arrival of this moment deserves all of us to look forward to and make efforts.

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Onsite Quality Controls for Food Safety Based on Miniaturized Biosensing

11

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Abstract

Health and wellness are linked to the food we regularly consume. Although the emergence of advanced technologies, such as intelligent packaging, safety, and transportation in temperature-controlled containers, has greatly improved the quality of food, certain microbial invasions and deliberate adulterations are unavoidable when it involves long transportation and extensive commercialization. These activities eventually make the food unhealthy to consume. Onsite quality control (QC) tends to check such food items not only to control the food spoilage in commercialization but also to protect the consumers from the consumption of unhealthy food. In this context, advances in miniaturized sensing devices have paved numerous possibilities to monitor food quality in an onsite context. This chapter discusses the existing ways of ensuring quality assurances

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_11

for food safety and their associated challenges. Thereby, common indicators of quality and spoilage in different types of food items have comprehensively been described as control of the different types of food. Thereafter, the miniaturized biosensor-based devices for food quality assurance have been described, where a brief discussion on the development processes, analytical performances, and commercial potentials are discussed with various examples and reported potential products for food quality assurance and safety.

Keywords

Food quality · Food safety · Miniaturized devices · Food sensors · Onsite quality control

11.1 Introduction to Packaged Food and the Need for Onsite Quality Controls

Over the last five decades, the food industries have grown exponentially to meet the needs of the vast majority of the population. Since then, packaged, ready-to-eat food has come into our habits and lifestyle. Unfortunately, many of these ready-to-eat processed foods are compromised with nutritional values and have preservatives in them, which are critical not only for health concerns but also for socio-economic impacts (Lozano et al. 2019). While the global demand for healthy food has been fulfilled by various farms and food processing companies, food safety is still posed as one of the biggest concerns. To ensure food safety, the governing bodies and policymakers have enforced strict regulation at various steps of production and commercialization processes including farm produce, food processing, storage, transportation, and retailing. However, the frequent occurrence of food-borne pathogenic outbreaks and mass food poisoning proves the hidden nature and threats to food safety (Mahato and Chandra 2019). Several such instances have been reported across the world in the recent past. For example, the *Escherichia coli* outbreak in Germany in 2011 and the mass sickness due to contaminated infant formula in China in 2008. Such occurrences not only cause casualties but also severely impact the economic condition, which may eventually result in a crisis (Lim and Ahmed 2016).

To avoid such disasters and improve the food habits, food technologists, scientists, and industries have been collaborating to address the issues and shortcomings by introducing a variety of detection strategies and techniques for fast, responsive, reliable, and inexpensive tests for checking the food quality and contaminating/spoiling agents (Ibrišimović et al. 2015; Lozano et al. 2019). For quality evaluations at the food safety assessment center and QCs, several instruments and techniques are commonly being employed, which include chromatography, spectrophotometry, and immunoassays-based assessments. Although these techniques offer highly sensitive and reliable determinations, several limitations viz. requirement of highly trained personnel, dedicated laboratory spaces, etc. are also associated with their application in onsite settings. Also, the usage of such

high-end instruments adds the analysis costs to the commodities and thus greatly impacts the commercial values, especially in larger volumes (Mahato et al. 2017, 2018e).

In this context, the miniaturized tools capable of rapid and equally sensitive detections are of utmost need to fulfill onsite examinations of the food items. Among various such miniaturized detection devices, the biosensors-based detectors have found great attention for the detection of food quality and spoilage biomarkers to incorporate to realize onsite and efficient QCs. The biosensor-based modules detect the quality and safety biomarkers of food items by targeting the molecules, pathogenic determinants, or any chemical markers that are either responsible or arose due to the spoilage/contamination of the food. Such detections/monitoring have been done by the sensor probe, composed of the bio-receptors, which are governed by various interactions viz. receptor/ligand, antigen/antibody, enzyme/substrate, nucleic acid hybridization, and chemical interactions (Kumar et al. 2020a; Weston et al. 2021). The following section describes such processes of biosensor developments, their concerns for designing onsite food quality and safety assessment.

11.2 Food Biosensors: Design and Development

The biosensor is an analytical device composed of receptors and the transducer surface that generates the quantifiable signal upon interaction with the analyte molecule (Kashish et al. 2017; Mahato et al. 2021). The commonly used receptors for the detection of biomarkers are antibody, cell, and enzyme, which are coupled with various kinds of transducer surfaces viz. electrochemical, optical, and piezoelectrical (Mahato et al. 2016a; Prasad et al. 2016b). The transducer converts the bio-interactions of the food quality/spoilage markers with the sensor probe and produces quantifiable signals based on analyte concentrations. The choice of transducer surfaces is highly influenced by the nature of the analyte and the QC test that are to be made. For example, the qualitative estimation of the food spoilage markers is commonly done by the optical transducer, which mainly offers yes/no based detection. However, in case of quantification of the contents (that is, low sugar, high sugar, and percentage of alcohols) are mainly estimated by the sensitive electrochemical-based transducers (Mahato et al. 2016b, 2019). Estimating the nutrient levels in processed food requires high-frequency estimations and are commonly offered by electrochemical-based detections, owing to their reusable and inexpensive nature (Mahato et al. 2020a).

In the conventional QC centers, detections are mainly performed in a randomized manner, where the arbitrary sample is collected from a batch and checked in the centralized laboratory. These tests are mostly time-consuming, and resource-intensive, which has led to the development of minimalistic approaches for lessening the burden. This huge demand for onsite QCs has paved the way for exponential increment of biosensor-based research for quality assurance at onsite settings. This section describes various components of biosensors and detection techniques

focusing on the types of markers for ensuring food quality and safety from the markers originated from the food items and the markers from environmental exposure (viz. bacteria, fungus, and other associated indicators), which play a key role in food spoilage.

11.2.1 Components of Biosensors

The major components of a biosensor include the bio-receptors, transducer, and processor (Mahato et al. 2018f). Bioreceptors are the biological molecules and are primarily interacting molecules (Purohit et al. 2019a). These interactions are broadly classified under two categories: biocatalytic and bio-affinity. The biocatalytic interactions generally use enzymes, whole cells, or tissue slices, which generate the electroactive species or the bi-product in presence of the analyte (Prasad et al. 2016b). Another type of bio-receptor is mainly for affinity-based detection, which follows the receptor-ligand conjugation strategies (Kumar et al. 2019b). These comprised the antibody, aptamer, affimer, and affibody-based strategies, which are highly specific receptors (Mahato 2019). These are highly specific and coupled with the traducer surface majorly in the electrochemically active transducer (Mahato et al. 2021). The impedance-based electrochemical technique is commonly used for such affinity-based interactions. This technique shows higher sensitivity, however, are more prone to the interferences, due to the presence interfering agents co-exist in testing samples, and their time requirement for the affinity binding limits from their usage in the rapid onsite detection of food quality or spoiling markers. In some cases, these are used where there is no chance of generating the electroactive species for quantifications (such as, estimation of toxins and bacteria). In recent times, the biosensors-based strategies for checking the marker molecules have been greatly recognized, and are constantly being improvised in terms of their analytical performance (Mahato et al. 2018b, f; Kumar et al. 2019c, 2020b; Mahato 2019; Purohit et al. 2020). Figure 11.1 depicts various components of the biosensors and the classification based on the bio-receptors and the transducer. The formal definition of biosensors is coined as the device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals (Mahato et al. 2018a). The performances of biosensors have been evaluated in various parameters viz. selectivity, sensitivity, reusability, dynamic ranges, detection limit, etc.

To enhance the analytical performances, several transducer materials have been employed. Among all, nanomaterial-based transducers have widely been accepted due to their extremely powerful electron transfer capability, which eventually allows better sensitivity (Baranwal et al. 2016; Prasad et al. 2016a; Mahato et al. 2018c, 2019). In some cases, the adoptions of nanomaterials in biosensing mechanism have leveraged enhanced sensor performances in miniaturization for the detection in onsite settings with comparable performance to the gold standard techniques (Purohit et al. 2020b). In the recent past, various nanomaterials have been used for

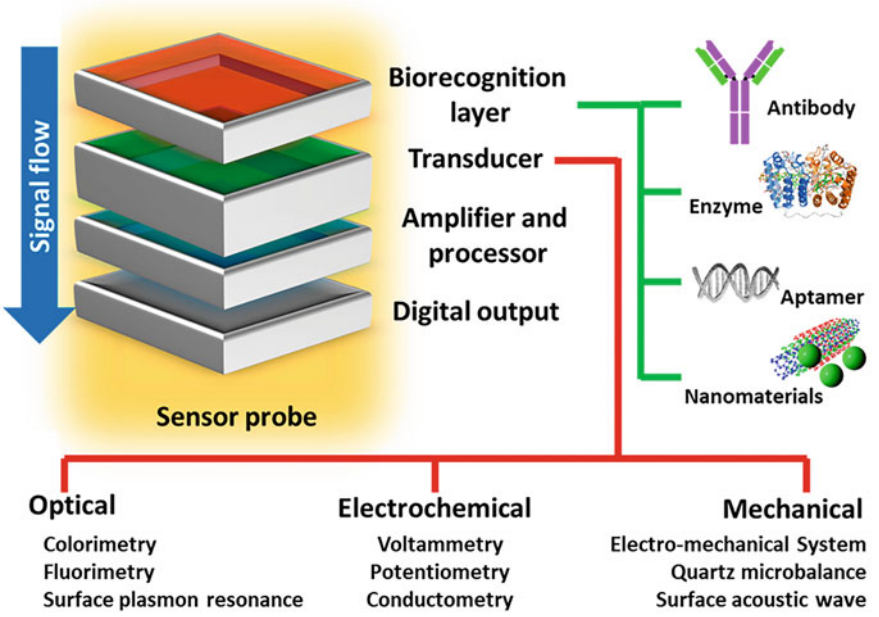

Fig. 11.1 Anatomy and the classification of the biosensors: (Left) components of the biosensor. (Right) different types of the bio-recognition layers, and (bottom) different types of biosensors based on the transducer surface

developing biosensing matrices, which include metallic nanoparticles, carbon-based nanomaterials, polymeric nanomaterials, quantum dotes, nanocomposites, etc. (Purohit et al. 2019b; Mahato et al. 2020d). Due to their size-dependent optoelectronic properties and nano-catalytic activity, these nanomaterials can be incorporated as recognition elements (Kumar et al. 2019a). The coupling of specific bio-receptors to the nanoparticle-based transducer has also been employed to introduce the selective detection of the analytes, where the covalent coupling/immobilization is preferred for better stability and reproducibility (Mahato and Chandra 2019). To fabricate the biosensors for onsite detection, various platforms have been used, among which lateral flow assay, dipsticks, electrochemical chip-based modules, and microprocessor-integrated wearable modules have found great attention for the biomedical detections and onsite- QCs (Teymourian et al. 2021; Mahato and Wang 2021). So far, different types of techniques have been used for developing biosensors-based modules for food quality assurances and their safety. Among these, optical and electrochemical-based strategies are widely accepted in food safety and quality detection due to their ease of usage and cost-effectiveness. This section describes various techniques that have been employed for detecting the biomarkers of food quality and spoilage.

11.2.1.1 Optical Biosensing Techniques

In optical biosensors, the optical properties of the transducers are commonly exploited to detect/quantify the analytes, which exhibit perceivable optical signals upon the interaction of the target analyte with the sensor probe. Due to their simple and non-destructive operation, these are the first choice for quality estimations and screening. Optical biosensing techniques are most widely used for the qualitative testing of food quality and spoilage. There are various formats adopted for the optical-based determination, which rely on color change, fluorescence change, and surface plasmon alteration (Mahato et al. 2020b), using an interferometer, resonators, garters, refractometers, etc. (Estrela et al. 2016; Kumar et al. 2020a). The nanomaterial-based colorimetric detection involves the recognition of the analyte, which upon the interaction changes the color of the working surface/area and is correlated to the concentrations (Kumar et al. 2015). The color change could be perceivable by the naked eye and aided eye for qualitative and detailed quantification, respectively. Since the colorimetric sensors offer easy handling, this format has widely been adopted among the other optical-based sensing formats (Estrela et al. 2016). The operation simplicity offers the best suitability in on-site QCs and collection centers where the batches of food items are being produced, packaged, or processed.

11.2.1.2 Electrochemical Biosensing Techniques

Although the preliminary screening saves the food items from mass spoilage, it does not confirm the prolonged shelf-life, as these are prone to get invaded by microbes during storage and transportation. Thus, the periodic evolution of the food items is necessary to achieve a prolonged shelf-life. The major limitation of most of the optical-based detections is for single-use, which limits its periodic usage to fulfill the constant/periodic tracking of the biomarkers. However, multiple sensors can be used for fulfilling the purposes, but that would certainly add more cost. Hence, electrochemical detections are being adopted for mitigating such challenges, which not only offer better sensitivity but also are capable of delivering stable detection. These are advantageous because of their easy integration to the analyzer modules, incorporating the onsite quantifiable detection with ease. The commonly used electrochemical-based techniques are voltammetry, amperometry, and impedimetric sensing as most of the bio-analytical reaction either produces the conductance or the impedance. In the format where the bioanalytical reaction produces electroactive species, voltammetry of the amperometry/conductometry is being used for the detection, while the bioanalytical reaction does not produce electroactive species, impedimetric-based approaches are commonly used, such as receptor–ligand interactions. For instance, a toxin found in milk "aflatoxin M1" has been detected employing the immune-complexation process followed by the impedance recording, which changes when the receptor and ligand bind at the sensor probe surface.

11.2.2 Indicators of Food Quality

Quality is a crucial attribute for any food processing industry, which determines the company's growth in this competitive market. The food industries can broadly be categorized under "beverages," "dairy," "meat," and others. The beverages are recreational food items and are mainly alcoholic and non-alcoholic sugar-sweetened beverages. For the quality improvement of the alcoholic beverages, mainly, ethanol, lactic acid, malic acid, polyphenols, and glycerol contents are detected, while in the sugar-sweetened beverages glucose, fructose, aspartame, and ascorbic acid have been commonly estimated for evaluating the quality. The dairy industries have a wide range of products and thus have various indicators for improving the quality of the specialized variant of a product. However, in all cases, lactose has been the major indicator of dairy food quality. Similar to beverage industries, another major sector is covered by the meat industries, where the meats (viz. seafood, fish, chicken, beef, pork, etc.) of various kinds are processed for maintaining the quality for a longer time and are sold in the market. The major challenge in quality assurance for such processed meats are their improper processing, additives and preservatives. As amines and nitrates are commonly employed for the preservatives and antioxidants at the meat curing process, the nitrate content provokes in situ nitrite formation in meat, which has reported of having carcinogenic properties. Thus, the estimation of amine and nitrate in the meat is essential for its quality improvement. Apart from this, in seafood meats, the number of heavy metals is more prominent, thus the estimations of toxic heavy metal ions are much important to ensure the quality of the food. Bakery industries are another popular food sector that uses grains, flour, and additives as common raw materials in preparing the recipes, which has prolonged the shelf life. Due to the involvement of high-temperature heating, the chances of the formation of toxicants (acrylamide which forms in the presence of aliphatic amide and asparagine) are very high, thus acrylamide detection is used as a major biomarker in bakery industries.

11.2.3 Indicators of Food Security

To sustain the food for a longer time, preservatives and antibiotics are commonly added during the processing and packaging. While there are state-of-the-art facilities of the packaging machinery, microbial invasion is inevitable in most of the packaged food, which not only degrades the food but also can elicit the pathogenic attack to the consumers. Therefore, various such markers/indicators have been targeted to check the spoilage in the QCs at the warehouses and the point of use for safer food consumption. These include pesticides, antibiotics, pathogenic microbial forms, allergens, heavy metal traces, etc. (Luong et al. 1997; Lozano et al. 2019). Pesticides like organophosphate, carbaryl, carbofuran, methomyl, Iprovalicarb, etc. are majorly used for agriculture-based food production, which eventually transfer to the crop due to their persistent accumulation in soil (Zamora-Sequeira et al. 2019). Although the antibiotics do not directly impact the health of the consumer in their prescribed

dosage, their overdose can potentially harm the health by initiating side effects. Their uncontrolled use in the food industry to check microbial invasion in processed food to meet the prolonged shelf-life could trigger resistances against these, which eventually becomes a major threat to public health (Landers et al. 2012). The common antibiotics used in the food industries are penicillin, tetracyclines, and quinolones; however, these are not limited. (Landers et al. 2012). While various sterilization processes have been employed to get rid of microbial forms, their invasions are inevitable in the food, either fresh or processed ones. This is because of the available nutrients for their growth, which is an essential requirement. Mainly the bacterial, fungal, and viral-based pathogenic elements have been reported in food. Among all, a few have become threatful to individuals and public health concerns. Thus, for their determination in food, various microbe-specific determinants/markers are targeted to achieve food safety. Similarly in agriculture-based foods, various allergens and heavy metal traces are most commonly found in food items. Moreover, the adulteration in food also degrades its quality and sometimes becomes a health hazard.

11.3 Biosensors for Food Quality and Safety

The quality preservation of the food is important. For its assurance, several biosensor modules have been reported. This section discusses various biosensors developed that have the potential to be applied in the food industries.

11.3.1 Biosensing Prototypes for Supporting QC's of the Beverage Industry

The quality of beverages is governed by their constituents and ingredients. For example, in sweetened beverages, the sugar content is crucial for its taste and the health of the consumer. Excessive of it causes disorders such as obesity and diabetes, to the consumers. Thus, the detection of the glucose becomes crucial in the sweetened beverages (eg. juices, carbonated drinks, etc.) apart from its well established biomedical applications (Majer-Baranyi et al. 2008; Cinti et al. 2020; Kostejnova et al. 2021; Zhuang et al. 2021). The most common bioreceptors used for glucose sensors are glucose oxidase and glucose dehydrogenase. The diversity in the glucose biosensor development is due to its extremely stable enzyme bioreceptor "glucose oxidase," which offers a higher specificity, turnover rate, and greater stability. Another crucial parameter for the quality of the beverage is the fructose concentrations. The excessive consumption of it can cause serious health problems (i.e., fructosuria) in the individual's deficit of the fructokinase, which is the enzyme that breaks fructose. Therefore, fructose monitoring is required not only for beverage quality but also for consumer protection.

For developing the fructose biosensors, the d-Fructose-5- dehydrogenase and hexokinase coupled with fructose-6-phosphate kinase enzymes are commonly used.

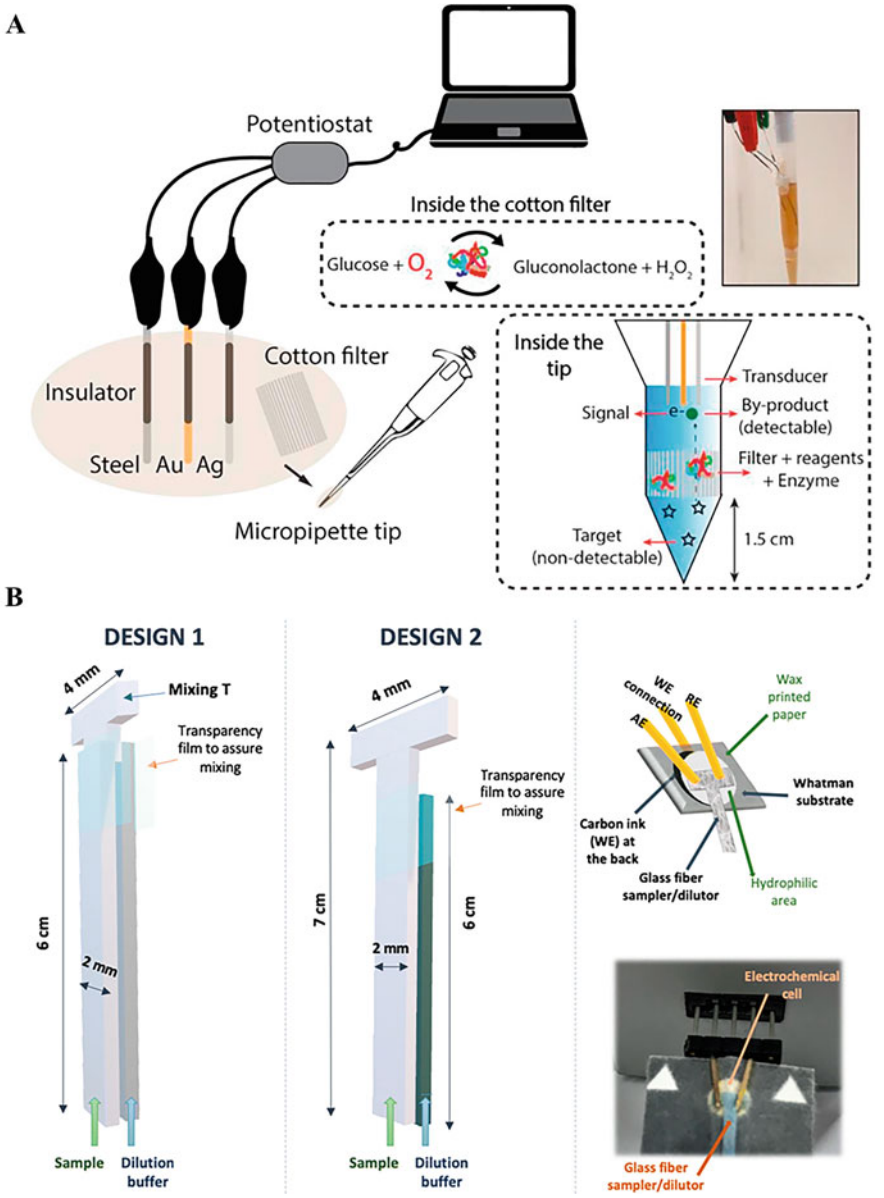

Fig. 11.2 Potential works that could serve the accurate detection of glucose contents in beverages: (**a**) working principle and the different parts of the lab-in-a-tip based approach by fusing wire electrodes into the pipette tip (Reprinted with the permission from Cinti et al. 2020; © Elsevier Inc.). (**b**) A paper assisted sampling of the beverage samples to the testing area for the detection of glucose using the wire-electrodes. (Reprinted with the permission from Amor-Gutiérrez et al. 2021; © Springer Nature)

In recent years, several biosensing prototypes for quality estimations have been developed for detecting the alcohol, sugar, vitamin, and other nutrients in alcoholic and non-alcoholic beverages. For instance, a pipette-based lab-on-a-tip electrochemical sensor (Fig. 11.2a) was reported for the rapid detection of glucose content in beverages with excellent analytical performance. The developed biosensor has shown a dynamic range of 0.5–10 mM with a detection limit of 170 μM (Cinti et al. 2020). Similarly, in another report, a glucose biosensor has been developed using a lab-on-paper approach for delivering a cheaper and more rapid detection of the sugar content in beverages (Fig. 11.2b) (Amor-Gutiérrez et al. 2021). This sensor is capable of detecting glucose in the concentration range between 0 mM and 15 mM in commercial orange juice and cola beverage samples. Using the same device, the authors have demonstrated the efficacy of the developed sensors for the detection of glucose from several samples simultaneously (Amor-Gutiérrez et al. 2019). Apart from the quality of beverages, safety and security are crucial for maintaining their quality for consumption, and thus the preservative, contaminants, and spoiling agents were also attempted to detect. Although, in alcoholic beverages, the microbe-borne spoilage is mostly unlikely, sugary non-carbonated beverages are more prone to such invasions. Thus, normal and pathogenic bacteria detection is routinely required. In addition, allergens and toxins are other more common threats found in beverages. Recent developments have targeted the detection of all such safety threats (Goud et al. 2016, 2017, 2019). For instance, Goud et al. have developed a miniaturized biosensor for the detection of aflatoxin B1 in alcoholic beverages. The sensor has shown remarkable analytical performance with the dynamic range and detection limits 0.05–6.0 ng mL^{-1} and 0.05 ng mL^{-1}, respectively (Goud et al. 2017).

11.3.2 Biosensing Prototypes for Supporting QC's of the Milk Industry

Another major segment of the food industry is based on milk and its products. The milk is enriched with various compounds including lactose, fat, citrate, nitrogen, casein, minerals, and non-proteinaceous nitrogen compounds that make milk a staple food. Lactose sugar in the milk is an indicator of its quality and the products. It also serves as an indicator of mastitis, where its level decreases upon the progression of the disease. Also, at the QCs, lactose estimation becomes more important due to its intolerance among the consumers. Several biosensing prototypes have been developed for the detection of lactose in milk. For instance, an enzymatic approach has been employed to develop the milk assessment, where β-galactosidase and glucose oxidase were co-immobilized on the sensor surface to develop the sensor probe. In the presence of lactose, the β-galactosidase enzyme converts it galactose and glucose eventually increasing the concentration of glucose. The generated glucose is subsequently cleaved by the co-immobilized glucose oxidase enzyme. This sensing prototype has shown a lower detection limit for lactose detection and was reported with 0.17 mg mL^{-1}(Jasti et al. 2014). Similarly, another device is developed by

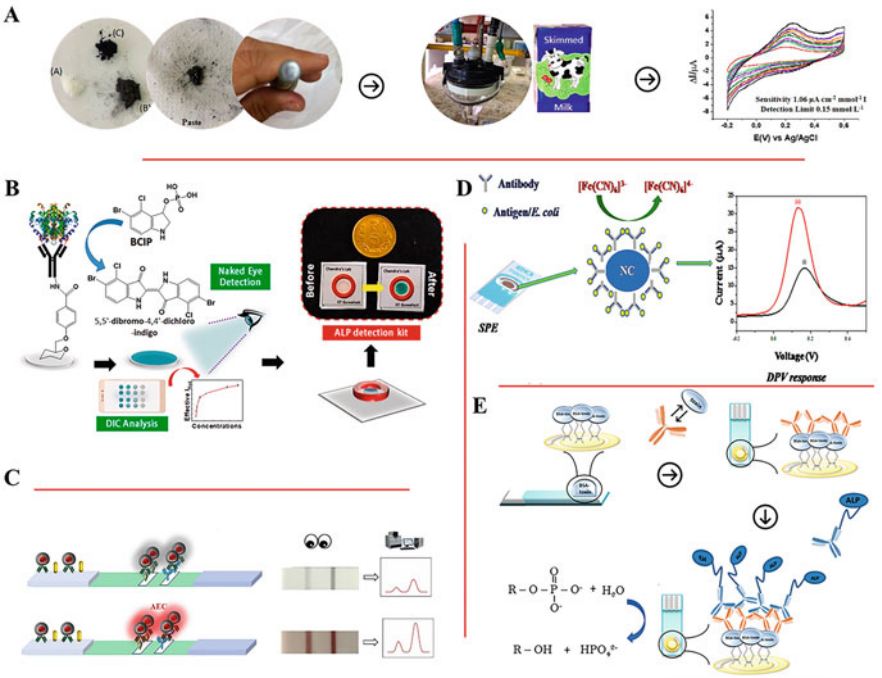

Fig. 11.3 Potential reported works that could lead to milk safety and quality improvement in dairy and dairy products: (**a**) Sensor prototypes for quality assessments based on lactose estimation in dairy products. (Reprinted with permission from de Brito et al. 2021, © Elsevier Inc.). (**b**) Portable colorimetric device for the qualitative and semi-quantitative estimation of milk pasteurization based on alkaline phosphatase content. (Reprinted with permission from Mahato et al. 2019, © Elsevier Inc.) (**c**) Lateral flow–based device for milk safety assessment based on the antibiotic (streptomycin) detection. (Reprinted with permission from Wei et al. 2020, © Elsevier Inc.) (**d**) Sensitive electrochemical device for the detection of bacterial forms *(Escherichia coli.)* in milk and milk products. (Reprinted with permission from Khan et al. 2021, © American Chemical Society). (**e**) Immunosensor-based chip for onsite detection of toxins (aflatoxin M1) in milk. (Reprinted with the permission from Karczmarczyk et al. 2017, © Elsevier Inc.)

de Brito et al. by employing electrochemical biosensors using lactase enzymes in nanomaterial-based transducer materials (Fig. 11.3a). This biosensor has shown excellent analytical performance with the detection limit of 0.15 mmol L^{-1}. The operational stability of the sensor was reported, which was found to be 12 h in consecutive usage of 10 days. Due to the nutrient richness of milk, microbial invasions are most common, which eventually lead to its spoilage, thereby, spoiling the milk by producing lactic acid. The invasions could also harbor the pathogenic bacteria, and consumption of which could cause more severe infectious pathogenic diseases. Therefore, to avoid such milk-borne contamination, a pasteurization process is employed, which kills all microbial forms. For confirming pasteurization, alkaline phosphatase is used as an indicator. Owing to the catalytic properties, alkaline phosphatase cleaves the chromogenic substrate and produces colorimetric

detection. Exploiting this behavior, Mahato et al. have developed a miniaturized device for the detection of alkaline phosphatase, thereby estimating the pasteurization status (Fig. 11.3b) (Mahato and Chandra 2019). The lower concentrations (in milli-units per liter) confirm the higher degree of pasteurization (ideally the pasteurized milk contains <250 mU of the alkaline phosphatase enzyme) as most of the molecules of it get denatured at this temperature. This device can detect alkaline phosphatase in the range of 10–1000 U/mL, which covers the entire concentration ranges of raw milk from a healthy cow and mastitis cow. This device has shown an excellent capability of sensing the target qualitatively and quantitatively when coupled to the smartphone. For achieving better productivity, antibiotics are commonly being employed for livestocks growth and commercial rearing. A study reports, approximately 63.151 (± 1.560) tons of antibiotics are being employed in livestocks yearly. The milk produced from such dosed livestock has found a significant amount of antibiotics due to their injudicious usage. Although antibiotics do not directly harm the consumers, the microbiota inside the gut may develop resistance on their constant exposure, which can be lethal if any virulency occurs inside the gut. To check the antibiotic contaminant in milk, several biosensors have been developed, using colorimetric and electrochemical formats. For instance, Wei et al., have developed lateral flow assay-based colorimetric detection of streptomycin using the gold–platinum bimetallic enzyme incorporated with a tetramethylbenzidine/hydrogen peroxide-based colorimetric system (Fig. 11.3c). This has shown a reasonably great analytic performance, where the detection limit of 1 ng mL^{-1} in the lateral flow module. Such efficient detection methodologies can be adopted for the onsite detection of various other antibiotics. The microbial invasions can also be tested by checking their populations in milk, however, the direct counting is hectic and laborious. Thus, biosensing strategies have been employed to detect the bacterial concentrations in milk. In this context, several works have been reported, which directly (label-free) or indirectly (with label) assess the presence of bacteria in milk. In this context, Khan et al. has developed a biosensing module for the detection of *Escherichia coli* from milk samples. The sensor was developed using the nano empowered miniaturized electrochemical transducing system, which has offered better sensitivity (Fig. 11.3d). The developed sensors show the detection limit of 2 CFU mL^{-1} using a redox couple. Toxins are the other contaminating agents, produced by the microbial forms. Consumption of toxins can cause severe complications in an individual's health. In contaminated/spoiled milk, several such toxins have been reported including, 5-vinyl-oxazolidine-2-thione, pyrrolizidine, swainsonine, trematode, aflatoxin M1, etc. (Liener and Liener Bsc 2002). Biosensors have been developed for the detection of such toxins to check the suitability of the milk for consumption. For instance, an immunosensor has been developed for the detection of Aflatoxin M1 (Fig. 11.3e). The sensor probe has been designed using the capture antibody and the secondary antibody conjugated alkaline phosphatase on the modified gold screen printed electrodes.

11.3.3 Biosensing Prototypes for Supporting QC's of the Meat Industry

Another major food sector is the meat industry, where various kinds of meat produced from livestock are processed, packaged, and commercialized. For producing good quality meat, the rearing of the livestock and storage conditions play an important role. Good and hygienically reared livestock produce healthy consumable meat that is devoid of pathogens, pesticides, drugs, heavy metals, and toxins, which are regulated by the regulatory bodies for commercialization to ensure good-quality meat for safer consumption. Although strict laws and consumer awareness have helped to maintain the quality of commercial meat to a greater extent, the inevitable malpractices even on small scale can spoil the product of the entire batch. In addition, the integrity of meat is also a big concern in the global society. To maintain integrity, various regulatory bodies are functioning across the world, which ensure and certify for purity by identifying the mixtures of meat. The conventional instruments for their detections are highly sensitive and are capable of detecting such contaminants in meat. However, employing these for the detection of every retailer/consumer is not a practical solution. This limits the usage of instruments to save the consumers from consuming spoiled meat even after having a great analytical performance. Thus to ensure safe consumption, onsite detection is essential.

So far, several miniaturized modules based on biosensors have been reported for the determination of meat quality. In a report by Labrador et al., a biopsy needle-based sensor has been developed, which can sense nitrate, nitrite, and NaCl contents in the meat samples, which are used as preservatives and carry carcinogenic effects. This biosensor has been tested in a real meat sample, and showed excellent profiling of nitrate and nitrite detection (Fig. 11.4a). Similarly, nitrates present in the meat have been tested using an electrochemical method. The sensor probe was developed using mesoporous carbon composite materials to obtain sensitive detection. The detection showed excellent analytical performance with the detection limit of 2.1 nM. Another major challenge is to keep the integrity of the meat quality in terms of the source of meat. In this context, Flauzino et al. have developed an electrochemical module for the detection of meat adulteration (Fig. 11.4b). The sensor detects the specific DNA sequence of porcine mitochondrial origin to find meat adulteration. The developed sensor shows 45 days of stability with the detection limit of 9% by the weight corresponding to beef and pork weight. Similarly, targeting porcine mitochondrial DNA, Ali et al. have developed a sensitive biosensor based on carbon-reduced graphene oxide electrodes using the screen-printed electrode module (Hartati et al. 2020). The excellent analytical performance dynamic range of 0–10 μg/mL and the detection limit of the 1.76 μg/mL of target DNA are capable of detecting the meat integrity in many commercial contexts.

In addition, antibiotics such as chloramphenicols, tetracyclines are being extensively used in the rearing farms from the birth of the livestock, which has become a food security hazard. Other chemicals such as ractopamine and clenbuterol are commonly used with feed to obtain increased growth and lean meat of the animals, which is a threat to the human cardiovascular and nervous systems if consumed for a

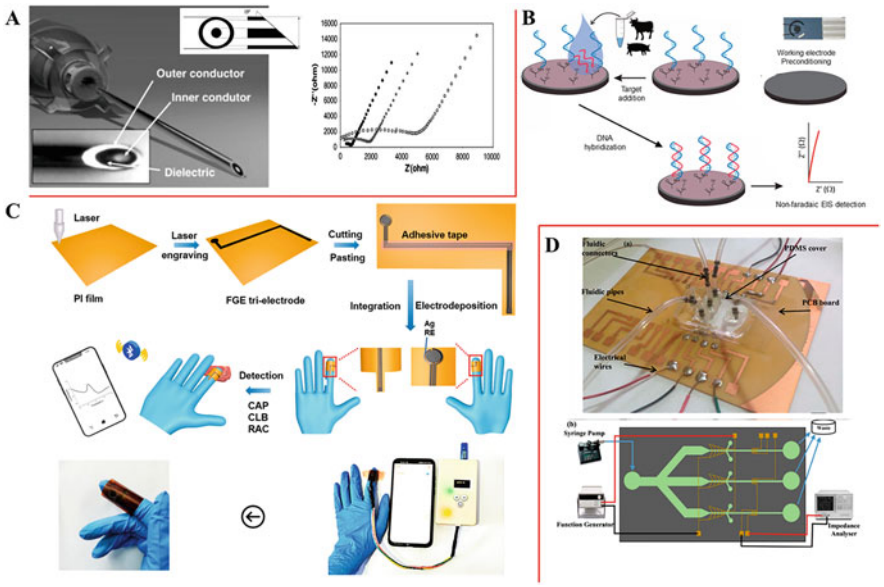

Fig. 11.4 Potential works that could lead to meat safety and quality improvement in the meat industry and products: (**a**) Portable needle-based device for nitrate, nitrite, and NaCl detection in meat samples for estimating the meat quality. (Reprinted with the permission from Labrador et al. 2010, © Elsevier Inc.) (**b**) Device for meat adulteration safety and integrity assessment based on the gene identification. (Reprinted with permission from Flauzino et al. 2022, © Elsevier Inc.) (**c**) Flexible electrochemical device for meat-based fast food security based on antibiotic profiles (chloramphenicol, clenbuterol, and ractopamine) detection. (Reprinted with permission from Li et al. 2022, © Elsevier Inc.) (**d**) Immunosensor based device for the onsite detection of *Salmonella serogroups* in meat samples. (Reprinted with the permission from Jasim et al. 2019, © Elsevier Inc.)

prolonged duration. Considering the potential health hazards, these drugs are banned in many countries across the globe. To detect such drugs in meat, Li et al. have developed a flexible globe-based electrochemical biosensor for the simultaneous detection of chloramphenicol, clenbuterol, and ractopamine in the meat sample (Fig. 11.4c). The developed sensor shows excellent analytical performance where the detection limits of 2.70, 1.29, and 7.81 μM and linear ranges of 10–200, 5–80, and 25–250 μM were obtained for chloramphenicol, clenbuterol, and ractopamine, respectively. Microbial invasions are another factor for meat spoilage and thus the detection of such contaminants and spoiling agents are of utmost need not only for the prolonged shelf-life of the meat but also to save the consumers from pathogens. In this context, an attempt has been made by Jasim et al. where they have developed microfluidic-based immunosensors for the detection of *Salmonella* serogroups of bacteria (Fig. 11.4d) using the electrochemical impedance spectroscopy. This biosensor is capable of delivering an excellent limit of detection of 7 cells/mL. The entire detection time for this sensor is approximately 40 minutes, which is a little longer for onsite detections. The developed biosensor could serve as the potential

module for bacterial detection in meat samples in onsite settings if the detection time is improved.

11.4 Commercial Biosensors for Food Quality Assessment

The important requirement of onsite QCs is the complete profiling of the food items, which has attracted simultaneous assessment of the given sample for delivering efficient controlling of the quality. These include biosensor contaminants, sugar contents, alcohols, amino acids, flavors, sweeteners, etc. Also, food allergens, toxins, pathogens, and additives are the major targets for these commercial devices. In the demand for these, the commercial instruments incorporated with multiple biosensors have become more promising to the food industries. The YSI 2700 Select food analyzer is the most prominent biosensors-based commercial instrument, which can detect essential amino acids, lactose, glucose, ethanol, and starch simultaneously. Thus, this instrument has been in demand at the QCs of several food processing industries (viz. beverages, meat, dairy, etc.). Similarly, another instrument, ABD 3000 biosensor assay system, is a multiplexed biosensor array that can detect and quantify L-lysine alcohol, L-amino acids, ascorbate, glucose, lactate, lactose/galactose, oxalate, and sucrose in the sample. These modules are employed for food safety, however, their cost-consuming nature practically limits to be used for every sample. Thus, to realize the onsite QCs for food products, a cheaper alternative is required to minimize the burden on the QC-centers for quality assessment as well as in the points of retail. The common formats of commercial devices are integrated autoanalyzer, manual benchtop, and most advanced portable biosensors. Few of them are reported in the table below (Table 11.1) (Bahadir and Sezgintürk 2015). The commercial success of these spin-offs from the industry standards' customized products indicates the competency for safeguarding the food products for consumption.

11.5 (Bio)/Sensors for Food Packaging

Several smart strategies have been adopted to protect the quality and prevent food spoilage by using biosensors. Among all, smart packaging strategies with biosensing modules have found great attention in recent times (Ghaani et al. 2016). These active packages when exposed to the environment of potential indicators, issue an alert. Although such biosensor-based active food packaging cannot detect the quality or the spoilage of the packaged food item, it can certainly be able to detect the possible exposure of contaminating and spoiling agents from the environment, which would prevent mass spoilage (Sobhan et al. 2021). In recent advancements, these are being coupled with artificially intelligent machinery and spatiotemporal geotagging facilities, which makes them commercially viable. These strategies are not only capable of avoiding mass spoilage but also offer an increased shelf-life. Mainly, these smart packaging systems follow the purpose of "something extra," which is a

Table 11.1 Various commercial food sensors have been summarized (Copyright 2015; reused with the permission of Bahadir and Sezgintürk (2015))

Company	Biosensor	Country
Oriental Electric	Fish deterioration tracking	China
Massachusetts Institute of Technology	Detection of E. coli O157:H7 in lettuce (canary)	USA
Michigan State University's electrochemical biosensor	Detection of E. coli O157:H7 and salmonella in meat products in the USA	USA
Georgia Research Tech Institute	Detection of salmonella and campylobacter in the pork industry	USA
Naval Research Laboratory	Detection of staphylococcal enterotoxin B and botulinum toxin A in tomatoes, sweet corn, beans, and mushrooms	USA
Universitat Autonoma de Barcelona in collaboration with CSIC	Detection of atrazine traces	Spain
Molecular Circuitry, Inc.	E. coli O157, salmonella, listeria, and campylobacter	USA
Research International	Proteins, toxins, virus, bacteria, spores, and fungi (simultaneous analysis)	USA
Universal Sensors	Ethanol, methanol, glucose, sucrose, lactose, l-AAs, glutamine, ascorbic acid, and oxalate	USA
Texas Instruments, Inc.	Peanut allergens, antibiotics	USA
Yellow SPRINGS Instruments	Glucose, sucrose, lactose, l-lactate, galactose, l-glutamate, ethanol, H2O2, starch, glutamine, choline	USA
Affinity sensors	Staphylococcus aureus and cholera toxin	UK
Ambri Ltd	Pathogens such as salmonella and enterococcus	USA
Biacore AB	Water-soluble vitamins, chemical veterinary residues, and mycotoxins	Sweden
BioFuture Srl	Glucose, fructose, malic acid, and lactic acid (fermentation)	Italy
Biomerieux	Microorganisms	France
Biosensor systems design	Microorganisms and toxic substances	USA
Biosensores S.L.	Toxic substances	Spain
Chemel AB	Glucose, saccharose, ethanol, methanol, and lactose	Sweden
IVA co. Ltd	Heavy metals	Russia
Motorola	Microorganisms and genetically modified organisms	Japan
Iventus Bio Tec	Ascorbic acid	Germany
Analox Instruments	Ethanol, methanol, glucose, lactate, glycerol	UK, USA
Gwent Sensors	Glucose	UK

fundamentally valued addition to the batch of products. The common parameters that are crucial for any food storage and its prolonged shelf life are the pH, temperature, and moisture contents, which are directly related to its freshness. Thus, the smart sensor–based packaging mainly contains the modules that primarily detected the above parameters. The commercially available smart packaging modules have mainly included gas indicators (Soon and Manning 2019), time–temperature indicators (Mohebi and Marquez 2014), freshness indicators (pH sensor, metabolite sensors) (Fang et al. 2017), pathogens (toxins, bacterial, and fungal indicators) biosensors (Fang et al. 2017), etc. The technological advancement in communication has supplemented the tracking features, that have been exploited in building these strategies, where the indicator mechanism has been coupled with the communication module with barcode-enabled RFID patches for easy screening.

These are various opportunities for biosensors in the packaging and handling of food. Mainly, these include food freshness, food integrity, fruit ripening, contamination, spoilage detection of food, and commercialized food items, especially where the food is preserved for a long time. The freshness of protein-based food is a common concern and most of them are prone to bacterial or fungal invasions. The spoilage of them produces nitrogen-based compounds (viz. ammonium gas, nitrogen gas, aldehydes, ketones, etc.) that can be detected by the colorimetric biosensors. For instance, an indirect biosensor (using the glucose-sensing strategy), proposed by smiddy et al. detects the L-cysteine which is a biomarker of meat freshness and its spoilage (Smiddy et al. 2002). The phenolic compounds are majorly released in ripening fruits, and thus sensing these indicators can tell the ripening stages and the spoilage by overripening. For example, malic acid has been developed for the detection of fruit ripeness (Vargas et al. 2016). Additionally, in the most ripened fruits, the sugar content increases over-ripening, which can also be exploited in the onsite QCs while packaging the batch of fruits. The growing needs for the food items have attracted the overuse of pesticides and antibiotics in crop production, which eventually get stored in the food items, and may be harmful if consumed above the permissible limit. Thus, biosensors detecting such analytes can be incorporated in smart packaging in a comprehensive examination of the packaged food at the QCs and packaging centers. The conceptualization of smart and active packaging has been since the early 2010s; however, these have been limited to get commercial attention due to their technical challenges, which include integration difficulty, miniaturization capabilities, accuracy, and the cost of fabrication. However, in recent decades, the advancement of microfabrication process and the nano empowered techniques have significantly increased the accuracy and lowered the cost of the biosensing modules, which may play a key role for realizing smart packaging for saving the food.

11.6 Conclusions and Future Directions

Healthy food is an essential need for good health. However, the malpractices have severely compromised the quality of food to a greater extent. At the commercial level, various technologies including intelligent packaging, safety, and cold storage transportation have been supported to retain the food qualities; however, microbial invasion and deliberate adulteration have attracted serious concerns. The biggest challenge in food industries is the preservatives, which provide a longer shelf-life; however, a few of them prove severely detrimental if consumed above the limit. The onsite detection of those is the best way to prevent toxic-level consumption. This chapter summarized the various attempts taken for saving food by retaining its qualities using the biosensing modules. The food biomarkers commonly targeted for ensuring the quality and detection of spoilage have also been discussed. Here, the focus was to collate the available prototypes that could be employed at existing QCs or at the point of need for facilitating the efficient screening of food for safer consumption. The future direction would be to discover customized exclusive biomarkers for a particular food and the development of low-cost biosensing modules for it to save the consumers from health deterioration and mass spoilage of food during its commercialization and storage.

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Gold Nanoparticle-Based Colorimetric Sensing of Metal Toxins

12

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Abstract

Miniaturized sensing devices have emerged as prevailing micro-scale analysis devices in the past few decades. In this context, metal nanoparticle-based sensors have proved their potential in developing highly sensitive and selective on-site detection techniques for various analytes and environmental toxins. Among various environmental pollutants, heavy metal contamination is the most severe problem worldwide because of its potential toxicity and non-biodegradable nature, even at lower exposure levels. Conventional analytical techniques for measuring metal toxins include atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), and reversed-phase high-performance liquid chromatography. These methods give accurate results but are time-consuming, require a dedicated laboratory setup, sophisticated equipment setup, and trained personnel to operate. Therefore, an alternative user-friendly and cost-effective method is required for rapid and real-time monitoring of heavy metal toxins in groundwater and industrial wastewater monitoring. Efforts are being made in developing metal nanoparticle-enabled sensors because of distinct optical and electrical properties, which renders better selectivity, sensitivity, and portability that can be readily used in developing commercial products. The sensing process is based on the aggregation of nanoparticles in the presence of specific metal ions coupled with visible color change detected by naked eyes, indicating the presence of targeted heavy metal toxins. This chapter summarizes

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_12

various synthesis processes and potential colorimetric-based sensing applications of metal nanoparticle-enabled sensors for assessing clean and safe drinking water.

Keywords

Metal nanoparticles · Gold nanoparticles · Metal toxins · Colorimetric sensing · Lab-on-phone · Machine learning

Abbreviations

AAS	Atomic adsorption spectroscopy
AgNP	Silver nanoparticle
AHMT	Amino-3-hydrazino-5-mercapto-1,2,4-triazole
AI	Artificial intelligence
ANN	Artificial neural network
CE	Counter electrode
CNN	Convolutional neural network
CTAB	Cetyl-trimethyl ammonium bromide
DLVO	Derjaguin–Landau–Verwey–Overbeck
DMSA	Dimercaptosuccinic acid
DTPA	Diethylenetriaminepentaacetic acid
EDL	Electric double layer
EPA	Environmental Protection Agency
GNP	Gold nanoparticles
GNR	Gold nanorod
GSH	Glutathione
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
LOC	Lab-on-chip
MBA	Mercaptobenzoic acid
ML	Machine learning
MLR	Multiple linear regression
MNP	Metal nanoparticles
NADH	Nicotinamide adenine dinucleotide hydrogen
PCD	Paper-based colorimetric device
PEG	Polyethyleneglycol
PPB	Parts per billion
PtNP	Platinum nanoparticle
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
RE	Reference electrode
RGB	Red, green, blue
SPR	Surface plasmon resonance
SVM	Support vector machine

TA	Thioctic acid
TDA	Thiodiacetic
TG	Thioguanine
TMB	Tetramethylbenzidine
TOAB	Tetraoctylammonium bromide
WE	Working electrode
WHO	World Health Organization
XRF	X-ray fluorimetry

12.1 Introduction

Metals are always considered important materials in manufacturing, science, engineering and technology, and commercial aspects (Santos 2017). These bulk metals, when fragmented to nanosize having dimensions 1–100 nm, are termed metal nanoparticles or metallic nanoparticles (MNP) (Venkatesh 2018). The advent of MNP marked a revolutionary change in sensing technology and biology because of distinct electronic, optical, biochemical, and physicochemical properties. Facile synthesis and surface modification of MNPs with diverse functional moieties like antibodies, enzymes, ligands, proteins, and drugs of interest facilitate target-oriented binding with analytes rendering selectivity and an efficient sensing platform. The large surface-area-to-volume ratio and spatial confinement of free electrons offer massive numbers of binding sites on the surface of metal nanoparticles (MNP). It brings an excellent scaffold to immobilize with large quantities of ligands and biomolecules, making it highly interactive with the analytes (Venkatesh 2018; Siontorou 2019). Metal nanoparticles (like gold, silver, copper, and platinum nanoparticles) have the exceptional feature of absorption and scattering of light that originates from the collective oscillation of surface electrons to unique optical properties of MNPs (Vasquez et al. 2018; Maghsoudi et al. 2021). When exposed to appropriate frequencies of electromagnetic waves (light), it induces the excitation of electrons on the surface of MNPs known as surface plasmon resonance (SPR). This property of MNPs is also responsible for their vibrant color in an aqueous solution and can be easily tuned by changing the shape and size of the metal nanoparticle. For example, gold nanospheres of ~20 nm diameters appear wine red in color. However, their color becomes purple and blue as the size increases to ~100 nm. Likewise, the silver nanoparticle of 20 nm is yellow colored in an aqueous solution, and with the increasing size of the silver nanoparticle, color changes to red (Shrivas et al. 2015; Venkatesh 2018; Willner and Vikesland 2018). This size-dependent change in color of metal nanoparticles can be exploited to develop visual colorimetric sensors (chemical sensors, biosensors, and electro-optical sensors) where small nanoparticles aggregates in the presence of analytes change in color of the solution (Chen et al. 2014b). The metal nanoparticles are synthesized by two approaches, top-down and bottom-up (Wang and Xia 2004). Top-down synthesis involves bulk

material as a precursor, broken down to nano-range particles using different physical lithography techniques such as soft lithography and electron-beam lithography. Top-down plays a vital role in the large-scale fabrication of nanostructure; it has limitations such as imperfections in resulting material, expensive, and time-consuming (Khandel et al. 2018). Bottom-up synthesis relies on the assembly of molecules or atoms to build complex nano-constructs. Some common processes used in bottom-up methods include sol-gel (Epifani et al. 2000), chemical vapor deposition (Murty et al. 2013), laser ablation (Kumari et al. 2014), and solvo-thermal method (Choi et al. 2013), but the most popular is the chemical reduction that provides the advantage of fine control over shape and size of the nanoparticle. The chemical reduction method of nano-metal synthesis involves reducing precursor metal salt in the presence of a suitable stabilizer. The quasi-spherical-shaped nanoparticles are thermodynamically most stable; therefore, the synthesis of spherical nanoparticles with different size ranges can be easily achieved by altering the concentration of precursor salt, concentration and rate of addition of reductant, and temperature. Since the optical properties of nanostructures vary with shape and size, a process to synthesize non-spherical nanoparticles has been developed to utilize these anisotropic nanostructures in different applications. Anisotropic metal nanostructures are synthesized by step-wise growth in the presence of nanoparticle seed and structure-directing agents, like cetyl-trimethyl ammonium bromide (CTAB) (Murphy et al. 2011; Chang and Murphy 2018). High surface energy and short inter-particle distances of nanoparticles make them unstable and coalesce, forming thermodynamically favored stable bulk particles. In the absence of interfering repulsive forces, metal nanoparticles attract each other resulting in larger aggregates. To maintain spatial confinement in the nano range, stabilization of metal nanoparticles is essential, which can be accomplished by steric or electrostatic stabilization using stabilizing agents like polymer, ligands, and surfactants having suitable functional groups (Olenin 2019; Sperling and Parak 2010). Stabilization of metal nanoparticles leads to the formation of an electric double layer that furnishes repulsive force to remain without aggregated in dispersed form (Venkatesh 2018; Polte 2015). Functionalization of metal nanostructures with proper capping agents not only provides stability to nanoparticles but also renders specificity toward target analytes and, thus, finds several applications in sensing (Yu and Li 2019), drug delivery (Ghosh et al. 2008), cell imaging, and photothermal therapeutics (McQuaid et al. 2016).

In the present rapid industrialization scenario, heavy metal contamination in the environment is a significant problem globally. The presence of excessive levels of heavy metal pollutants in soil and water affects the quality of surface and groundwater, resulting in a severe threat to human health and the deterioration of environmental resources. The heavy metal pollutant enters water bodies by various natural and anthropogenic sources, including mining, manufacturing, industrial, and municipal waste discharge (Poornima et al. 2016). Arsenic, chromium, lead, mercury, and cadmium are some of the most concerning heavy metals due to their high toxicity, even at low concentrations. Therefore, regulatory organizations like Environmental Protection Agency (EPA) and World Health Organization (WHO) set a standard on

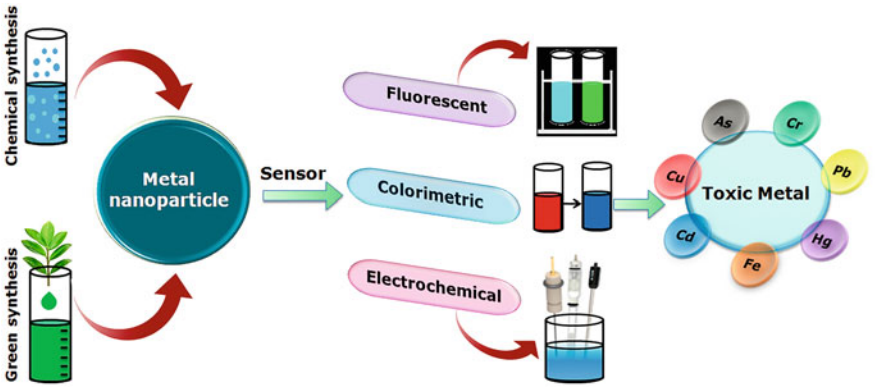

Fig. 12.1 General schematic of MNP synthesis and their use in sensing applications of toxic metal ions

permissible limits of heavy metal consumption (Varun and Kiruba Daniel 2018; Zhang et al. 2019a; Li et al. 2013; Tchounwou et al. 2012). Consumption of heavy metals above permissible limit triggers bio-toxic effects by altering cellular activities and developing severe disorders, including cancer. Since they are not quickly metabolized or excreted, they tend to accumulate in soft tissues for years, which slowly causes mental and central nervous dis-functioning, damage to the liver, kidneys, lungs, and other vital organs (Li et al. 2013). The traditional techniques for detecting these toxic metals are based on either spectroscopy or chromatography, which includes inductively coupled plasma mass spectrometry (ICP-MS), atomic adsorption spectroscopy (AAS), inductively coupled plasma optical emission-spectrometry (ICP-OES), X-ray fluorescence spectrometry (XRF), and high-performance liquid chromatography (HPLC). These techniques are highly selective, sensitive, and efficient in quantification; their large-scale implementation is still a challenge due to their complexity and sophisticated installation procedure. Moreover, they require technical expertise to operate, involve toxic chemicals, multiple sample preparation steps, time-consuming, and dedicated laboratory setup, and time-to-time maintenance of instruments makes analysis highly expensive (Zhang et al. 2019a; Lu et al. 2018; Buledi et al. 2020). Thus, there is a need to develop an inexpensive heavy metal detection technology that is rapid, easy-to-handle, user-friendly, portable, and operated as a point-of-use device. In this context, nanoparticle-enabled colorimetric sensing technology has huge potential to detect metal toxins on-site with improved performance as a device in terms of selectivity, sensitivity, and reproducibility that can be readily developed into commercial products. A general schematic of MNP synthesis and its advantages in sensing application has been shown in Fig. 12.1. This chapter reviews the colorimetric sensing strategies for heavy metals based on aggregation and dispersion of metal nanostructure, specially focusing on gold nanoparticle-based sensors. It also highlights recent advances in developing a miniaturized, point-of-use colorimetric sensor for metal toxins on paper substrate. Moreover, the integration of smartphone

camera readouts and machine learning approach with colorimetric sensors introduces a new lab-on-mobile concept, which has been discussed in the later section of this chapter.

12.2 Metal Nanoparticle-Based Sensor

Sensors are devices that convert the chemical or physical properties of a specific analyte into a measurable signal proportional to the analyte concentration (Jayabal et al. 2015a). Metal nanoparticle-based sensing devices are characterized in three units, i.e. (a) metal nanoparticle, (b) a recognition component that furnishes selectivity, and (c) a signal transduction system, which supplies information about the presence and absence of analyte (metal toxin) in a sample (Willner and Vikesland 2018; Mahato et al. 2018). The resultant signals originating from the MNP sensor can be of different types, and based on these signals, sensors can be categorized as optical, electrochemical, and piezoelectric sensors (Willner and Vikesland 2018). Optical sensor depends on the interaction of toxic metals with electromagnetic radiation (like ultraviolet, visible, or infrared light) in the form of emission or absorption, which spectroscopic techniques can monitor. Colorimetric and fluorescence are two methods commonly used as reporting signals in optical sensors. Colorimetric sensing is based on surface plasmon resonance of metal nanoparticles. SPR peak of the metal nanoparticle is highly sensitive to the inter-particle distance between nanoparticles. Plasmon coupling causes aggregation of metal nanoparticles with pronounced color change and concomitant red-shift of SPR peak. Most of the gold/silver nanoparticle-based colorimetric sensors explore the property of color change coupled with aggregation and dispersion of nanoparticles in the presence of target heavy metal (Doria et al. 2012). The fluorescence sensor consists of a fluorophore as a signal-transducing element, which exhibits the property of photoluminescence. When the fluorophore is irradiated with electromagnetic radiation, it absorbs the photon energy, and its orbital electrons are excited to a higher energy level (singlet state). Fluorescence occurs when the excited electron relaxes to a lower energy state (ground state) by emitting a photon. Upon interaction with the heavy metal toxin, the fluorescent signal changes as either "turn-off" or "turn-on." The metal nanoparticles of size 3 nm or smaller (like nanodots and nanoclusters) can be directly used as a fluorescent marker as they exhibit inherent fluorescence properties. Moreover, MNPs which lack their fluorescence can be functionalized with a fluorophore to obtain a fluorescent sensor. Quenching and restoration of fluorescence property indicate interaction of toxic metal and nanoparticle-based changes in the sensor (Xiong et al. 2019; Willner and Vikesland 2018). An electrochemical sensor is a device that transforms chemical reactions into electrical signals (Alam et al. 2020). When an electrical circuit is introduced to heavy metal, the molecular binding of toxic metal near electrode surface initiates oxidation/reduction process, which generates or modulates electrical current in the form of charge (electron) transfer between the electrode and toxic metal ion. This charge transfer may lead to the completion of an incomplete circuit or alteration in current, potential,

or resistance measured by instruments like potentiostat or galvanostat (Willner and Vikesland 2018; Doria et al. 2012). Electrochemical sensors may exist in a two-electrode system or three-electrode system (Andrea Scozzari 2008). In the two-electrode arrangement, a working electrode (WE) is coupled with a counter electrode (Auxiliary, CE), and the difference of electric potential is measured between WE and the potential of CE. Examples of the two-electrode system are amperometric sensor (measures electric current between CE and WE in the presence of a constant electric potential) (Sahin and Kaya 2019) and potentiometric sensor (measures the potential difference between two electrodes, i.e., WE and CE in the absence of current flow) (Isildak and Özbek 2020). Three-electrode consists of a reference electrode (RE) along with WE and CE. The best example for a three-electrode system is a voltammetric sensor, which measures current response as a function of applied potential. Current is linearly dependent on the concentration of electro-active species (toxic metal ion) (Power and Morrin 2013). Among these sensors, colorimetric sensors have several advantages: simplicity, unmatched sensitivity, and inexpensive and fast detection time. Moreover, it operates in a visible range of the electromagnetic spectrum. The resultant signals can be detected by naked eyes, making it possible for wide-scale use by the common people. Therefore, it gained huge attention for quick detection of metal toxins in solutions (Kim et al. 2012; Jayabal et al. 2015b).

12.3 MNP-Based Colorimetric Detection Strategies

Metal nanoparticles are highly flexible because of precise control on size, shape, composition, assembly, and optical properties during the synthetic process. Thus, it has been extensively investigated for colorimetric sensing of toxic metal ions. WHO has standardized the consumption limit for these toxic metal ions; for instance, the permissible limit for arsenic is 10 ppb, chromium 50 ppb, lead 15 ppb, mercury 2 ppb, copper 15 ppb, and cadmium 5 ppb. Thus, their trace level monitoring is essential, and for which researchers are coming up with new techniques based on metal nanoparticles. This section deals with MNPs-based colorimetric sensors for the determination of toxic metals. Zhou et al. reported 4-mercaptobenzoic acid (4-MBA) modified silver nanoparticles (AgNP) for colorimetric sensing of Cu^{II} ion in water. 4-MBA consists of –SH group that binds with AgNP and –COOH groups exposed on the surface that chelates Cu^{II} forming carboxylate–Cu^{II}–carboxylate bridges. Chelation of Cu^{II} causes aggregation of AgNP and color changes from yellow to purple (Fig. 12.2a) (Zhou et al. 2011). Chen and coworkers demonstrated a paper-based colorimetric device (PCD) for Hg^{II} detection using citrate stabilized PtNP. 3,3,5,5-tetramethylbenzidine (TMB) and H_2O_2 produce blue color in the presence of PtNP that mimics the peroxidase activity by catalyzing the reaction. However, the introduction of Hg^{II} in the reaction system inhibits the catalytic activity of PtNP resulting in a color change from blue to colorless. (Fig. 12.2b) (Chen et al. 2016). Shrivas et al. reported colorimetric sensing of Pb^{II} using polyvinyl alcohol (PVA) functionalized AgNP and paper-based analytical devices.

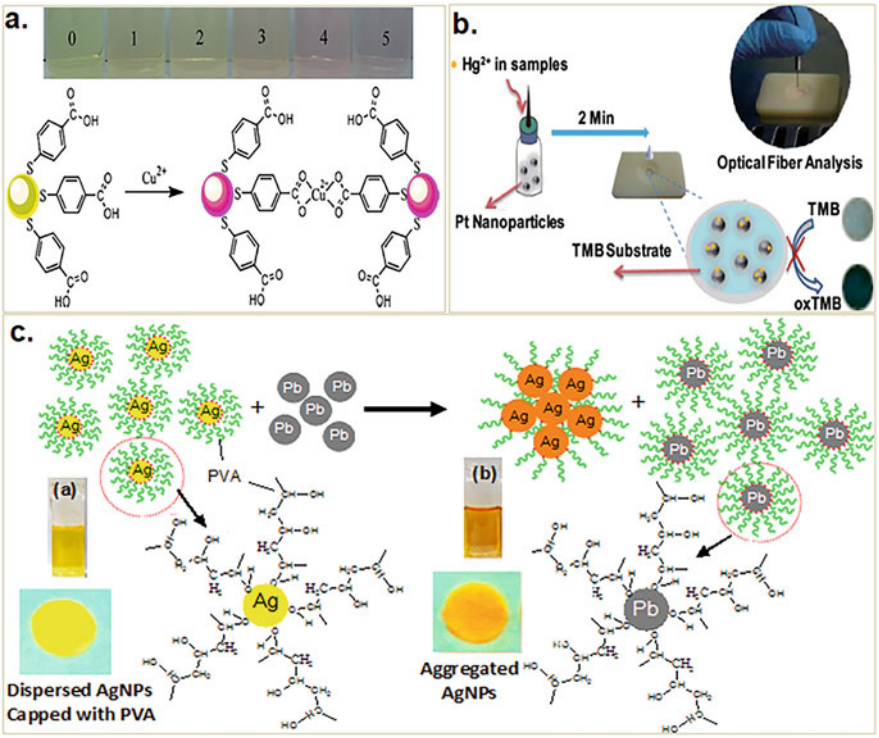

Fig. 12.2 Metal nanoparticle-based colorimetric sensor for heavy metal. (**a**) 4-MBA-AgNP-based colorimetric detection of Cu^{II} with color change from yellow to purple. (**b**) Paper-based analytical device for detection of Hg^{II} using PtNP in the presence of TMB and H_2O_2. (**c**) Colorimetric sensing of Pb^{II} using PVA-AgNP coated paper substrate showing color change from yellow to red. Reproduced from Ref. Zhou et al. (2011) (**a**). Open access with proper citation (**b**). Reproduced from Ref. Shrivas et al. (2019) (**c**)

After interaction of Pb^{II} with AgNP-PVA, the color changes from yellow to red and color intensity were recorded on a smartphone followed by processing in ImageJ software (Fig. 12.2c) (Shrivas et al. 2019). Similar color-based sensing methods have been reported for toxic metals like As^{III}, $Cr^{III/VI}$, Cd^{II}, Pb^{II}, Cu^{II}, and Hg^{II} using modified metal nanoparticles including Au, Ag, Pt, and Pd nanoparticles, which have been listed in Table 12.1. Silver and gold nanoparticles exhibit prominent SPR-based properties associated with a color and have been widely explored for the same. Though Pt-like nanoparticles exhibit SPR features, they are examined mainly for their catalytic activity and enzyme mimetic behavior for inducing an indirect color change in sensor application. Among all these metallic nanoparticles, gold nanoparticles (GNP) received much attention in colorimetric sensing applications (Singla et al. 2016). GNP can be prepared by simple methods with high stability and provides a suitable platform for multi-functionalization with various biological and organic ligands for selective binding of target toxins. They have a high surface-to-

Table 12.1 MNP-based colorimetric sensing of toxic metal in solution and paper-based analytical devices

MNP	Synthesis process	Modification	Target metal	LOD (ppb)	Dynamic range (ppb)	Method of detection	Ref
AgNP	Chemical reduction by $NaBH_4$ in presence of polyvinylpyrolidone (PVP)	Aptamer	As^{III}	6 0.0	50–700	Aggregation	Divsar et al. (2015)_
	Chemical reduction by trisodium citrate	Polyethyleneglycol (PEG)	As^{III}	1.0	1.0–15	Yellow→blue (aggregation)	Boruah et al. (2019)
	Chemical reduction by $NaBH_4$	Trisodium citrate	Cr^{VI}	0.075	$0.05–5 \times 10^4$	Yellow→purple (aggregation)	(Ravindran et al. 2012)
	Chemical reduction by $NaBH_4$	Tartrate	Cr^{III}	3.1	5.19–60.7	Yellow→red (aggregation)	Xu et al. (2013)
	NaOH	3,4-dihydroxyphenylalanine (L-dopa)	Cr^{VI}	10	$10–1 \times 10^4$	Yellow→brown (aggregation)	(Joshi et al. 2016)
	Chemical reduction by $NaBH_4$	Tartrate	Cr^{III} Cr^{VI}	2.0 3 0.0	5 – 100 10 – 100	Yellow→red (aggregation)	Shrivas et al. (2016)
	Green synthesis by fruit extract of *Durantaerecta*	Phytochemicals	Cr^{VI}	100	1×10^4– 1×10^5	Yellow→colorless (aggregation)	Ismail et al. (2018)
	Chemical reduction by $NaBH_4$	Polyvinylpyrolidone (PVP)	Cr^{VI}	1.7	5.19–124.5	Yellow→red (aggregation)	He et al. (2019)
	Chemical reduction by $NaBH_4$	Trisodium citrate	Cr^{VI}	26	10–700	Point-of-care device; yellow→red	Kumar et al. (2020)
	Green synthesis using L-tyrosine reduction	L-tyrosine	Hg^{II}, Mn^{II}	0.8	3.2–132	Yellow→colorless (etching of AgNP) Yellow→brown (aggregation)	Annadhasan et al. (2014)
	Green synthesis by fruit extract of water apple (*Syzygium aqueum*)	Phytochemicals	Hg^{II}	170	1002–20,050	Yellow→colorless (aggregation)	Firdaus et al. (2017)

(continued)

Table 12.1 (continued)

MNP	Synthesis process	Modification	Target metal	LOD (ppb)	Dynamic range (ppb)	Method of detection	Ref
	Green synthesis (leaf extract)	2-aminopyrimidine-4,6-diol	Hg^{II}	0.35	0–13,033	Brown→yellow (aggregation)	Prasad et al. (2018)
	Chemical reduction by $NaBH_4$ in the presence of polyvinylpyrolidone (PVP)	Methionine	Hg^{II}	0.2	4–20	Yellow→colorless (aggregation)	Balasurya et al. (2020)
	Chemical reduction by $NaBH_4$	Polyvinyl alcohol (PVA)	Pb^{II}	20	50–1000	Paper-based analytical device (yellow→red)	Shrivas et al. (2019)
	Green synthesis by 3,4-dihydroxy-l-phenylalanine (DOPA) in the presence of NaOH	DOPA (mussel-inspired protein)	Pb^{II} Cu^{II}	0.019 0.005	0.012–31	Yellow→red Yellow→brown	Cheon and Park (2016)
	Chemical reduction by dopamine in the presence of NaOH	Dopamine	Cu^{II}	3.2	3.2–512	Yellow→brown	Ma et al. (2011)
	Chemical reduction by $NaBH_4$	4-mercaptobenzoic acid	Cu^{II}	1.5	6.35–6350	Yellow→purple	Zhou et al. (2011)
	Chemical reduction by $NaBH_4$	Gelatin hydrogels	Cu^{II}	0.63	0.63–6350	Yellow→green	Jeevika and Ravi Shankaran (2014)
	Chemical reduction by $NaBH_4$ in the presence of KOH and ethanol	N-acetyl-L-cysteine	Fe^{III}	4.4	4.4–4464	Brown→colorless	Gao et al. (2015)
	Chemical reduction by $NaBH_4$	5-sulfosalicylic acid	Cd^{II}	0.33	0–123.6	Yellow→red	Jin et al. (2015)
	Chemical reduction by trisodium citrate	1-amino-2-naphthol-4-sulfonic acid	Cd^{II}	9.7	0–1124	Yellow→brown	Huang et al. (2016)

Pt NP	Chemical reduction by $NaBH_4$ in the presence of trisodium citrate	Trisodium citrate	Ag^{I}	8.4×10^{-4}	0.001–0.32	Blue→colorless (peroxidase mimetic activity of PtNP inhibited by Ag^{I})	Wang et al. (2017)
	Chemical reduction by trisodium citrate	Trisodium citrate	Hg^{II}	16.04	–	Blue→colorless (peroxidase mimetic activity of PtNP inhibited by Hg^{II})	Bertolacci et al. (2020)
	Chemical reduction by $NaBH_4$	Trisodium citrate	Hg^{II}	2.0	0–20,050	Paper-based colorimetric Device (PCD) (blue→colorless Peroxidase mimetic activity of PtNP inhibited by Hg^{II})	Chen et al. (2016)
Pt-se NP	Chemical reduction by ascorbic acid	Polyvinylpyrolidone (PVP)	Hg^{II}	14.0	0–501	Blue→colorless (peroxidase mimetic activity of PtNP inhibited by Hg^{II})	Guo et al. (2017))
Ag-Cu	Chemical reduction by $NaBH_4$	Trisodium citrate	Hg^{II}	0.1	0.2–2.0	Yellow→light orange	Li et al. (2018b)
Au-Ag	Trisodium citrate	Poly(diallyl dimethylammonium chloride)	Hg^{II}	1	0.5–80	Brownish–orange→purple	Mathaweesansurn et al. (2020)
Au-Ag	Chemical reduction by $NaBH_4$	Chitosan	Hg^{II}	0.18	0.18–18,045	Brown→purple	Zhang et al. (2019b)
Au-Ag	Chemical reduction by $NaBH_4$	Trisodium citrate	Pb^{II}	5.28	2–20	Reddish orange→purple	Sahu et al. (2020)

volume ratio and exhibit unique optoelectrical and catalytic properties imparting useful surface plasmon behavior that generates a detectable response in the presence of metal ions. These distinctive properties make them "star" among the other nanoparticles providing researchers a broad spectrum for sensor application (Jans and Huo 2012).

12.4 Synthesis, Functionalization, Properties, and Sensing Strategy of GNP

12.4.1 Synthesis of GNP

The sensing application of gold nanostructures is dependent mainly on their shape, size, and surface functionality, and thus selection of suitable synthetic procedures is crucial in designing a sensor (Yeh et al. 2012; Yu and Li 2019). In the last few decades, numerous chemical and physical procedures are adopted to precisely control the shape, size, and mono-dispersity of nanoscale gold. However, green synthesis technologies involving biological entities are gaining much attention nowadays due to the environmental-friendly and biologically safe approach to synthesizing gold nanoparticles (Zhang et al. 2020). The details of some commonly used synthesis procedures for nanoscale gold are summarized here.

12.4.1.1 Turkevich–Frens Method

Turkevich first reported the synthesis of gold nanoparticles in 1951 (Turkevich et al. 1951). In this method, gold chloride salt ($HAuCl_4$) is heated (~90 °C) in the presence of a reducing agent like sodium citrate resulting in monodispersed spherical gold nanoparticle suspended in the water of around 20–40 nM in diameter (Fig. 12.3a). Here, citrate plays the role of both stabilizing and reducing agents (Saha et al. 2012). Later, in 1973, Frens improved this protocol and prepared gold nanoparticles of different sizes ranging between 16 and 147 nm. This method provides precise control over the size of the gold nanoparticle by adjusting the proportion of chloroauric acid salt to sodium citrate solution (Razzaque et al. 2016). The citrate reduction provides a negative surface charge to gold nanoparticles and prevents aggregation by imposing repulsion induced by Coulombic force (Chen et al. 2014a). Citrate forms a weak coordination layer with gold nanoparticles that adds easy replacement of citrate with functionalizing agents like thiols (Zhu et al. 2003), polymers, and biomolecules (Nghiem et al. 2010).

12.4.1.2 Brust–Schiffrin Method

In 1994, Brust and Schriffin introduced another protocol for synthesizing gold nanoparticles in an organic medium that results in water-immiscible gold nanoparticles (Brust et al. 1994). This synthetic strategy involves bi-phasic reduction of gold salt to produce thiol-protected gold nanoparticles (Li et al. 2011; Perala and Kumar 2013). The gold chloride salt is transferred from the aqueous phase to the organic phase (toluene) using the surfactant tetraoctylammonium bromide (TOAB)

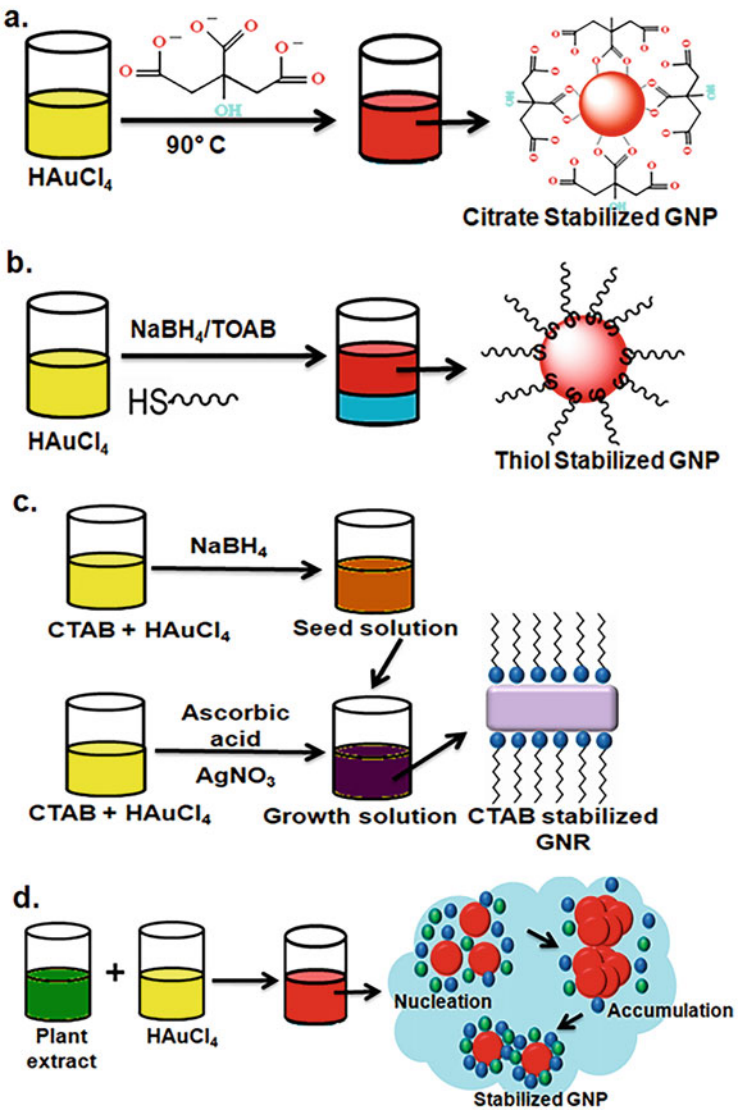

Fig. 12.3 Various synthesis procedures of gold nanoparticles. (**a**) Turkevich–Frens method. (**b**) Brust–Schiffrin method. (**c**) Seed-mediated growth method for anisotropic gold (GNR). (**d**) Green synthesis of GNP using plant extract

followed by the addition of dodecanethiol as a stabilizing agent. Next, sodium borohydride (a strong reducing agent) is added that reduces gold salt to thiol-stabilized gold nanoparticles producing deep brown color in toluene (Fig. 12.3b). This method results in 1.5–5 nm gold nanoparticles having low dispersity.

Alkanethiol forms a monolayer on GNP surface enabling easy modification with various functional groups (Saha et al. 2012).

12.4.1.3 Seed-Mediated Growth

The seed-mediated growth is the most reputed procedure used in the preparation of anisotropic gold nanoparticles like nanorod (GNR), introduced by Murphy and coworkers (Jana et al. 2001) and El-Sayed group (Nikoobakht and El-Sayed 2003). Cetyltrimethyl ammonium bromide (CTAB), a surface-active molecule, is used as the template for directed growth of anisotropic nanostructures (Meng et al. 2019). The synthesis of gold nanorod is achieved in two steps (Fig. 12.3c). Step 1 involves the seed solution synthesis where a golden brown–colored gold seed is prepared by reducing gold chloride salt ($HAuCl_4$) with freshly prepared ice-cold borohydride ($NaBH_4$) sodium in the presence of CTAB. Step 2 involves the growth of nanorods in the presence of CTAB and silver nitrate ($AgNO_3$). Gold salt is reduced with a mild reductant ascorbic acid followed by seed solution. The growth solution is left undisturbed for 12 hrs to grow the seed crystals into gold nanorod under the action of surfactant (CTAB). The nanorods of desired aspect ratio (length/width) are achieved by altering the concentration of gold chloride salt and seed (Murphy et al. 2011; Li et al. 2018a).

12.4.1.4 Green Synthesis of Gold Nanoparticles

Green synthesis of nanoparticles emerged as an attractive substitute for conventional chemical synthesis procedures. This method involves the use of unicellular and multicellular biological entities like plant extracts, bacteria, actinomycetes, fungus, yeast, and viruses. The biological entities act as a factory for nanoparticle synthesis that offers non-toxic, inexpensive, and environmental-friendly approaches both extra and intra-cellularly without using toxic chemicals during synthesis, thus also termed as "Green chemistry" (Fig. 12.3d) (Baranwal et al. 2016; Zhang et al. 2020; Salem and Fouda 2021; Sengani et al. 2017) Das and coworkers synthesized 20 nm spherical gold nanoparticles by using flower extracts from *Nyctanthes arbor-tristis* (night jasmine) (Das et al. 2011). Narayanan and Sakthivel used leaf extracts of *Coriandrum sativum* to synthesize gold nanoparticles of size 7–58 nm (Narayanan and Sakthivel 2008). Husseiny group reported the extracellular preparation of GNP using a bacterial strain Pseudomonas aeruginosa. The mechanism involves the transfer of an electron from nicotinamide adenine dinucleotide hydrogen (NADH)-dependent reductase enzyme resulting in the reduction of Au^{3+} to Au^0 and itself oxidized to NAD^+ (Husseiny et al. 2007). Synthesis of nanoparticles using plant extracts is comparatively easier, faster, and cost-effective than bacterial synthesis as it does not require complex and multiple-step processes like isolation, culturing, and maintenance of bacterial strain (Iravani 2011).

12.4.2 Colloidal Stability of Gold Nanoparticles and Functionalization for Sensor Application

Colloidal stability of gold nanostructure depends largely on the surface energy, surface composition, and the inter-particle behavior arising from the surface and intermolecular forces. Nanoparticles exhibit high surface energy and short inter-particle distance, which make them unstable and result in aggregation. The chemistry behind the aggregation of the nanoparticle is complicated due to the involvement of different kinds of forces like electrostatic repulsion, van der Waals, and magnetic forces (Rance et al. 2010). However, the van der Waals force of attraction dominates due to short inter-particle distance, which compels them to form aggregates. Therefore, to avoid the agglomeration of nanoparticles, repulsive force is introduced by adding a capping agent during the synthesis of nanoparticles. The capping agents bind on the surface of the nanoparticle, providing two types of stabilization, electrostatic and steric. In an aqueous environment, most of the nanoparticles carry some surface charge due to ionization of the surface group or adsorption of charged molecules or ions. To balance the surface charge, a cloud of opposite charges is created. This charged cloud consists of the inner stern layer and outer diffuse layer forming an electric double layer (EDL), creating an electrostatic repulsive force between particles. In the case of steric stabilization, a physical barrier is created by the adsorption of ligands on the particle surface that prevents particles from aggregation (Amina and Guo 2020; Moore et al. 2015). The stabilization of gold particles was described by DLVO (Derjaguin–Landau–Verwey–Overbeck) theory of balance between the repulsive (electrostatic interactions) and the attractive force (van der Waal). According to DLVO theory, the sum of electrostatic and van der Waals force between two nanoparticles represents the total force acting on the colloidal solution (Zhou et al. 2009; Aldewachi et al. 2018).

Due to the nanometer size, gold nanoparticles have a high surface to volume ratio making them extremely active, and therefore, surface capping is required to lower the surface energy and increase stability. Surface functionalization of gold nanoparticles also renders specificity for the target analyte during the sensing procedure. Therefore, surface modification of GNP accomplishes two objectives: (1) chemical stability and (2) target-specificity (Zhang 2013). Gold nanoparticles can be functionalized by thiol-containing ligands, biomolecules, and polymers using different strategies like covalent coupling (Au-S bonding), specific recognition (e.g., antibody-antigen, DNA, aptamer), and electrostatic interaction. Sulfur-containing molecules are highly effective functionalizing agents, since, Au-thiol bonds are strong, resulting in highly stable gold nanoparticles. Many thiol-containing compounds like thioctic acid, glutathione, thioguanine, cystamine, thiolated–diethylenetriaminepentaacetic acid (SH-DTPA), and thiolated–PEG (SH-PEG) are used to modify gold nanoparticles and implicate in sensing of toxic metal ions (Mahato et al. 2019). Chai and coworkers reported glutathione functionalized gold nanoparticle (GSH-GNP) for Pb^{II} detection based on aggregation and red to blue color change of GSH-GNP (Chai et al. 2010). Xue and group demonstrated 6-mercaptonicotinic acid and L-Cysteine co-functionalized GNP as Cd^{II} sensor

(Xue et al. 2011). Wang and coworkers reported 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT) functionalized gold nanoparticles for colorimetric Cd^{II} detection. AHMT consists of thio, amino, and triazole groups which can form bonding with GNP surface. However, among these groups, thiol preferentially binds with GNP forming (Au–S) bond while other groups are involved in the chelation of Cd^{II} ion (Wang et al. 2013). Compared to other molecules, thiol-ligands bind easily with the gold nanoparticle surface, which can be attributed to the mechanism of ligand exchange that replaces the already bound ligand with the thiol compound without altering the structural integrity of GNP. Liu and Lu fabricated a lead biosensor using DNAzyme-directed aggregation of DNA modified gold nanoparticles. DNAzyme consists of an enzyme specific to Pb^{II} ions. DNA functionalized GNP forms a blue-colored assembly in the presence of DNAzyme. The introduction of Pb^{II} in reaction activates DNAzyme to cleave DNA stand and change the color of GNP from blue to red (Liu and Lu 2003). Lee and Mirkin developed a highly selective Hg^{II} detection assay based on thymine-Hg-thymine base paring. The GNP surface was functionalized with a thiol modified DNA probe (probe 1 and probe 2). Though the thiol modified-GNP is stable, it loses stability in the presence of Hg^{II}, forming a bridge of thymine-Hg-thymine, leading to mismatch in T–T base pairs. This causes red-colored GNP to turn blue leading to aggregation (Lee and Mirkin 2008).

12.4.3 Optical Properties

The refinement of bulk gold to nanoscale dimension allows them to interact with light, causing strong absorption at specific wavelengths. When gold nanoparticles are exposed to light, the electromagnetic field of light causes polarization of free electrons present on GNP's surface, resulting in their collective oscillation. When the frequency of incident light coincides with the frequency of collective oscillation of surface electrons, it absorbs the radiation of that particular wavelength giving an absorption band known as surface plasmon resonance (SPR). The SPR of gold nanostructures ranges from the visible to the near-infrared region of the electromagnetic spectrum depending on the size and shape of the gold nanoparticles (Fig. 12.4a) (Wang and Yu 2013). A spherical gold nanoparticle of 20 nm possesses SPR peak at 520 nm in the visible region responsible for the red color of the colloidal solution. As the size of the GNP increases, the SPR band gradually shifts to a higher wavelength with a concomitant color change of colloidal solution. Thus, the colored appearance (red, orange, brown, purple, and blue) of the colloidal gold solution is dependent on the size of gold nanoparticles (Amina and Guo 2020). Moreover, as the symmetry changes from spherical to nanorod, the SPR splits into two Plasmon bands known as transverse bands arising due to electron oscillation along the short axis (width) and longitudinal band due to electron oscillation along the long axis (length) (Fig. 12.4a) (Yasun et al. 2013). Richard Gans, in 1912, explained that the change in the shape of nanoparticles leads to alteration in position and number of SPR band and thus depends on the aspect ratio (length/width) of nanoparticles, not absolute dimension.

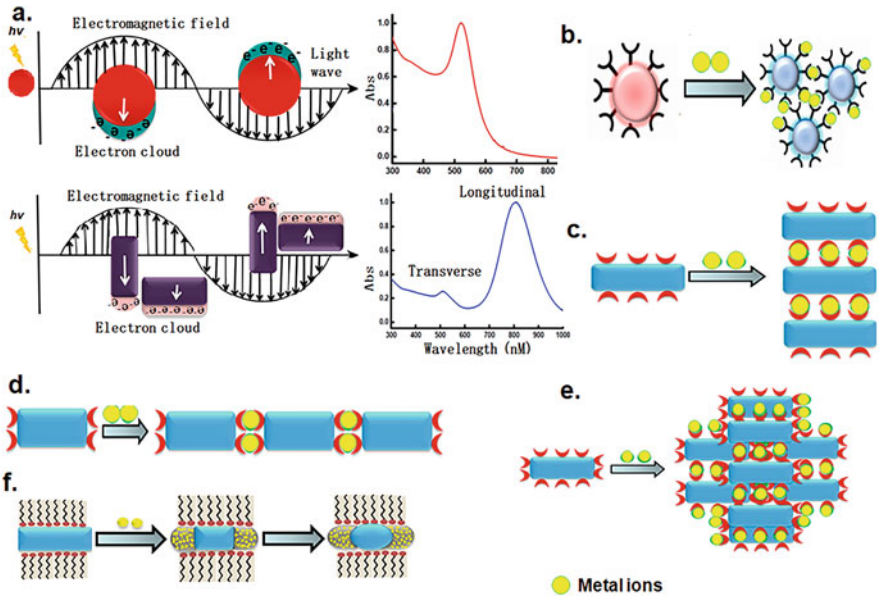

Fig. 12.4 Schematic illustration of surface plasmon excitation of GNP and GNR (**a**), metal ion-mediated aggregation of GNP (**b**); metal ion-induced side by side (**c**), end to end interaction (**d**), both side by side and end to end interaction forming total aggregate of GNR (**e**), and metal ion-mediated etching of GNR to spherical-shaped nanoparticle (**f**) responsible for the change in optical properties of gold nanostructures

Thus, a change in SPR band can be used to track the interaction of analytes with gold nanoparticles (Nath et al. 2018b).

The detection mechanism of colorimetric sensors is dependent on the change in color and absorption band associated with the aggregation and disaggregation of nanoparticles. Interaction of heavy metals with spherical gold nanoparticles forms aggregates with a shift in SPR and red to blue color change (Fig. 12.4b). However, gold nanorod aggregates in different ways, namely end to end, side by side, aggregation, and etching of nanorod (Vigderman et al. 2012). End to end interaction occurs when metal toxins bind at the edge of nanorod forming chain/wire-like structure resulting in bathochromic shift of longitudinal band. Metals toxins when bound on the longer edge of the nanorod result in side by side interaction with a blue shift in longitudinal SPR. Aggregation-based assembly first initiates with end to end and ends side by side, forming a total-aggregate nanorod with decreased absorption band intensity. Some metal toxins etch nanorod at longitudinal edge forming spherical shape coupled with red shift and change in color from purple to pink. The change in optical properties with toxic ion-mediated aggregation has been shown in Fig. 12.4c, d, e, f.

12.5 GNP as a Colorimetric Sensor for Heavy Metals

As discussed in Sect. 12.4.3, gold nanoparticles are extremely responsive to the local dielectric, which results in aggregation with change in SPR and the color of colloidal solution indicating the presence of test analyte. Researchers have extensively investigated this feature to develop a color-based sensor for metal ions, which has been discussed in this section. Chen et al. reported DMSA functionalized GNP for trace level detection of $Cr^{III/VI}$. Chromium exists as an aqueous complex with six water molecules coordinated with the Cr^{III} ion. Cr(III) has empty orbitals that accept one pair of electrons from the oxygen of DMSA, forming a metal–O coordinate covalent bond by replacing water molecules from aqueous chromium. However, in the case of $Cr_2O_7^{2-}$, the Cr^{VI} ion lacks coordination sites as they are already occupied by oxygen atoms. Therefore, chromium binding occurs through hydrogen bonding OH----O involving carboxyl –OH of DMSA and –O of $Cr_2O_7^{2-}$ (Fig. 12.5a). (Chen et al. 2015) Nath et al. reported a red to blue color change sensor for arsenic (III and V) using 2-mercapto-4-methyl-5-thiazoleacetic acid (MMT) and

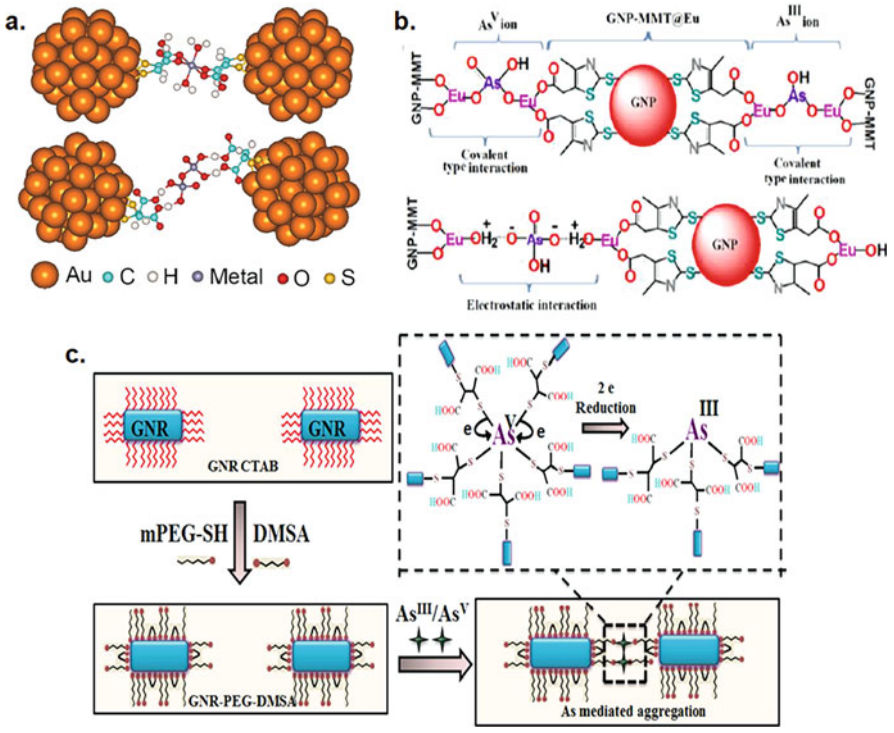

Fig. 12.5 (**a**) Interactions between DMSA-GNP and Cr^{III} (top) and Cr^{VI} (down). (**b**) $As^{III/V}$ mediated aggregation of GNP-MMT@Eu. (**c**) $As^{III/V}$ induced aggregation of DMSA functionalized gold nanorod. Reproduced from Ref. Chen et al. (2015)) (**a**). Reproduced from Ref. Nath et al. (2018a)) (**b**). Reproduced from Ref. Priyadarshni et al. (2018) (**c**)

europium chloride ($EuCl_3$) functionalized gold nanoparticle (GNP-MMT@Eu). The SPR peak intensity of GNP-MMT@Eu at 525 nm decreased while a new peak at 650 nm appeared due to arsenic-mediated aggregation of nanosensor. GNP-MMT@Eu exhibits Eu-OH group exposed on GNP surface which are the sole binding sites for arsenic ion. The As-OH/As-O^- groups of arsenic and –OH groups of Eu(III) bind forming an inner-sphere arsenic complex between GNP with the release of H_2O and OH^- moieties. The response of GNP-MMT@Eu for As (V) was quick compared to that of As(III). The nanosensor surface attains a partial positive charge at pH ~6-7 as the Eu-OH converts to Eu-OH_2^+ which initiates the binding between $H_2AsO_4^-$/$HAsO_4^{2-}$ and GNP-MMT@Eu through electrostatic interaction. Thus, both covalent and electrostatic modes of binding prevail between arsenic and the nanosensor, which are accountable for rapid response to As^V (Fig. 12.5b) (Nath et al. 2018a). Priyadarshni et al. demonstrated aggregation-based detection of $As^{III/V}$ using gold nanorod (GNR) modified with mPEG-SH and DMSA. After interaction with $As^{III/V}$, the bluish-purple color of the GNR sensor turns colorless with a small shift (778 to ~802-820 nm) and decrease in SPR peak suggesting side to side and end to end binding forming total aggregate. DMSA contains two thiols (–SH) groups; one binds with GNR and other complexes with $As^{III/V}$. At pH ~7, As^{III} and As^V exist as H_3AsO_3 and H_3AsO_4/$H_2AsO_4^-$ while thiol remains protonated. As−OH groups participate in As−S bond formation and release H_2O due to removal of hydrogen from –SH and displacement of $–OH^-$ to form a complex between $As^{III/V}$ and GNR, inducing arsenic-mediated aggregation of nanorods (Fig. 12.5c) (Priyadarshni et al. 2018).

12.5.1 Detection on Paper Substrate

Conventional detection methods for metal toxins have constantly improvised with new-age technological developments to miniaturize the setup and provide a decentralized approach. The emergence of microfluidic techniques resulted in the notion of the "lab-on-chip (LOC)" concept back in the 1990s (Guan and Sun 2020; Sackmann et al. 2014). In recent years, paper-based microfluidics have emerged as promising LOC sensing devices (Li et al. 2012; Yetisen et al. 2013; Kumar et al. 2015). Microfluidics that couple paper-based devices with colorimetric analysis are particularly attractive, attributed to easy fabrication, portability, and inexpensive, i.e., provide a cheaper alternative for point-of-use testing. Moreover, the paper has a porous matrix that offers self-pumping and capillary flow to the solution (Mahato et al. 2020; Xiong et al. 2020; Mahato et al. 2017). This section discusses some paper-based colorimetric methods for sensing toxic metal ions in water. Nath et al. reported trace-level determination of As^{III} on Y-shaped microfluidic paper device using thioctic acid and thioguanine conjugated gold nanoparticle (Au-TA-TG). The two arms of the device were used as the inlet, each for Au-TA-TG and As^{III}, and the reaction occurs on the paper surface resulting in red to blue color change, suggesting the existence of As^{III} (Fig. 12.6a) (Nath et al. 2014). Zhang and coworkers demonstrated the detection of Cu^{II} by etching of nanorod in the presence of

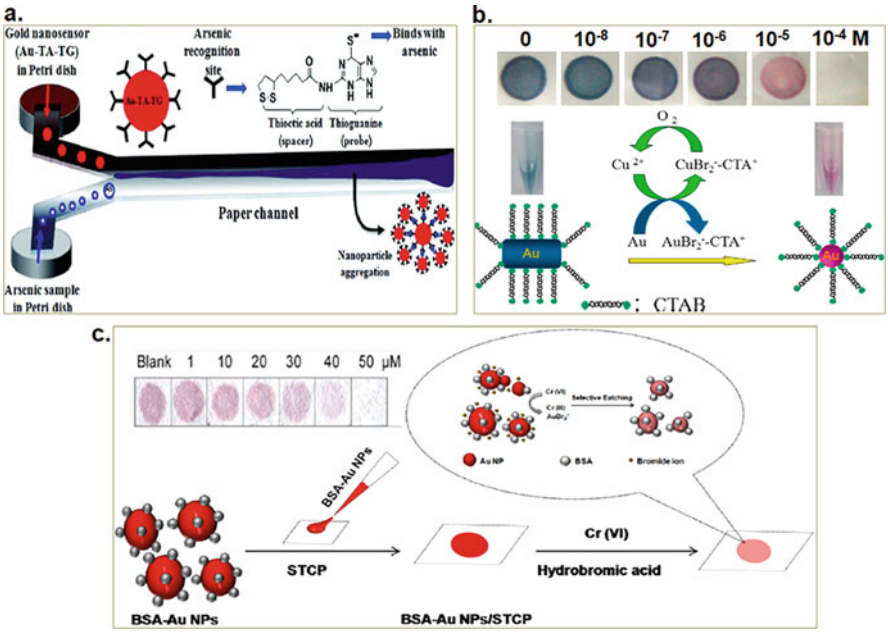

Fig. 12.6 (**a**) Au-TA-TG on Y-shaped paper strip for colorimetric detection of As^{III}. (**b**) Paper-based colorimetric detection of Cu^{II} by etching of nanorod in the presence of HBr turns blue-to-red. (**c**) Paper-based colorimetric detection of Cr^{VI} by aggregation of BSA-AuNP/STCP. Reproduced from Ref. Nath et al. (2014)) (**a**). Reproduced from Ref. Zhang et al. (2014) (**b**). Reproduced from Ref. Guo et al. (2016) (**c**)

hexadecyltrimethylammonium bromide (HBr) on a paper substrate. When Cu^{II} in combination with HBr is added to nanorod, HBr induces transformation of Au(0)-to-Au(I) and Cu^{II} catalytically etches the longitudinal edge of GNR accompanied with color change from blue to red (Fig. 12.6b) (Zhang et al. 2014). Paper-based colorimetric metal ion detection using gold nanoparticles has been shown in Fig. 12.6 and listed in Table 12.2.

12.6 Smartphone and Machine Learning (Color Readout)-Based Quantification of Heavy Metals

The enhanced technical capabilities of smartphones, especially wireless connectivity and high definition cameras, enable various innovative ideas for detecting environmental toxins like heavy metals (Mutlu et al. 2017). The addition of a simple colorimetric sensing apparatus to a smartphone makes it lab-on-phone, cost-effective, portable, and accurate (Sajed et al. 2020; Wang et al. 2019). Most of the integrated smartphone detection systems introduced so far rely on RGB (red, green, blue) intensities of the colorimetric sensor. Chen et al. developed a smartphone integrated colorimetric sensor using meso-2,3-dimercaptosuccinic acid

Table 12.2 GNP- and GNR-mediated colorimetric detection of metal toxins in solution, paper substrate, and smartphone

Target metal toxin	Modification	LOD (ppb)	Aggregation-based color change of GNP/GNR	Ref
Arsenic				
GNP	Glutathione(GSH), Dithiothreitol (DTT) cysteine (Cys)	1.0	Red→blue	Kalluri et al. (2009)
	Aptamer	1.26	Red → blue	Yu (2014)
	GSH-DTT-Cys-PDCA	2.5	Red →blue	Domínguez-González et al. (2014)
	Cationic polymer and aptamer	5.3	Red → blue	Wu et al. (2012)
	Citrate	1.8	Red → blue	Gong et al. (2017)
	Polyethyleneglycol (PEG)	5.0	Red→ blue	Boruah and Biswas (2018)
	Thioctic acid-thioguanine (TA-TG)	1.0	Paper-based; red→blue	Nath et al. (2014)
	2-mercapto-4-methyl-5-thiazoleacetic acid (MMT)- europium	1.0	Paper-based; red→blue	Nath et al. (2018a)
	Sucrose	4.0	Smartphone-based color intensity extraction using ImageJ software; red→blue	Shrivas et al. (2020)
	Glutathione (GSH)	0.12	Smartphone-based RGB extraction; red→blue	Zheng et al. (2021)
GNR	Meso 2,3-Dimercaptosuccinic acid (DMSA)	1.0	Paper-based; purple→colorless	Priyadarshni et al. (2018)
Chromium				
GNP	5,5′-dithiobis (2-nitrobenzoic acid) (DTNBA	93.6	Red →blue	Dang et al. (2009)
	Triazole	72.6	Red →blue	Chen et al. (2013)
	Citrate	15.5	Red →blue	Liu and Wang (2013)
	Citrate	5.3	Paper-based; red →blue	Elavarasi et al. (2013)
	Tween 20	Cr^{III}: 0.83 Cr^{VI}: 0.46	Red →blue	Wang et al. (2015)
	4-amino hippuric acid.	60.7	Red →blue	Jin and Huang (2017)

(continued)

Table 12.2 (continued)

Target metal toxin	Modification	LOD (ppb)	Aggregation-based color change of GNP/GNR	Ref
	Gallic acid	78	Red →blue	Dong et al. (2016)
	O-phospho-l-serine dithiocarbamic acid (PSDTC)	218	Red →blue	Lo et al. (2015)
	1,5-diphenylcarbazide (DPC)	15.5	Red →blue	Liu et al. (2016)
	Cysteamine-pyridoxal (CAPY)	596.9	Red →blue	Bothra et al. (2017)
	Ribavirin	1.55	Red →blue	Salimi et al. (2018)
	Thiol modified nanodiamonds (ND-thiol)	0.019	Red →blue	Shellaiah et al. (2018)
	Bovine serum albumin (BSA)	14.5	Paper-based; red →blue	Guo et al. (2016)
	Meso-2,3-dimercaptosuccinic Acid (DMSA)	0.51	Smartphone readout; red→blue	Chen et al. (2015)
GNR	Bovine serum albumin (BSA)	16.6	Purple→red (etching)	Alex et al. (2018)
Lead				
GNP	Glutathione	0.002	Red →blue	Chai et al. (2010)
	L-glutathione	0.1 umol/L	Red →blue	Mao et al. (2011)
	Maleic acid	0.5	Red →blue	Ratnarathorn et al. (2015)
	Thioctic acid (TA--Dansyl hydrazine (DNS)	1.0	Paper-based; red →blue	Nath et al. (2015)
	Oligonucleotide	0.5	Smartphone-based RGB extraction and machine learning; red→blue	Sajed et al. (2020)
GNR	Cysteine	0.02	Absorption Side-by-side assembly	Cai et al. (2014)
	Unmodified GNR	0.62	Etching of GNR and Pb-au alloy formation	Chen et al. (2012)
	Sodium thiosulfate	20.7	Blue→red (etching of GNR)	Zhu et al. (2016)
Mercury				
GNP	Papain	0.2	Red →blue	Guo et al. (2011)
	3-mercaptopropionate acid and adenosine monophosphate	0.5	Red →blue	Yu and Tseng (2008)

(continued)

Table 12.2 (continued)

Target metal toxin	Modification	LOD (ppb)	Aggregation-based color change of GNP/GNR	Ref
	2-[3-(2-aminoethylsulfanyl)-Propylsulfanyl]- ethylamine (AEPE)	0.035	Red →blue	Chansuvarn and Imyim (2012)
	Thioctic acid (TA)	0.01	Red →blue	Su et al. (2013)
	Label-free oligonucleotide	0.05	Red →blue	Lee and Mirkin (2008)
	ss-DNA	10	μPAD integrated with smartphone (RGB values); red→blue	Chen et al. (2014a)
	Aptamer	0.2	Smartphone-based RGB extraction and machine learning; red→blue	Sajed et al. (2019)
GNR	Pyrazole	0.002	Absorption and colorimetric (end to end assembly)	Placido et al. (2013)
	Dithiothretol (DTT)	0.08	Absorption and colorimetric (aggregation)	Bi et al. (2012)
	Silica-CN	1.08	Colorimetric (au-hg amalgamation)	Anand et al. (2013)
	Mesoporous silica	0.15	Redox mediated inner particle interaction	
Copper				
GNP	Dopamine dithiocarbamate decorated gold nanoparticles (DDTC-au NPs)	946.2	Red →blue	Mehta et al. (2013)
	Polyvinylpyrrolidone (PVP) aggregated with 2-Mercaptobenzimidazole (MBI)	317.5	Purple→red (dis-aggregation)	Ye et al. (2015)
	10-mercaptodecyl-1-iminodiacetic acid (MDIA)	508	Red →blue	Chai et al. (2017)
	Amyloid-like peptides (arginine, phenylalanine, proline)	7.62	Red →blue	Pelin et al. (2020)
	Thioctic acid (TA--Dansyl hydrazine (DNS)	1.0	Paper-based; red →blue	Nath et al. (2015)
GNR	Cysteine	0.02	Blue-green→dark gray (aggregation)	Liu et al. (2011)
	Hexadecyltrimethylammonium bromide	0.03	Paper-based; blue→red (etching)	Zhang et al. (2014)

(continued)

Table 12.2 (continued)

Target metal toxin	Modification	LOD (ppb)	Aggregation-based color change of GNP/GNR	Ref
Cadmium				
GNP	6-mercaptonicotinic acid (MNA) and L-cysteine (LCys)	11.2	Red →blue	Xue et al. (2011)
	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT)	3.37	Red →blue	Wang et al. (2013)
	2,6-dimercaptopurine	3.67	Red →blue	Gan et al. (2020)

(DMSA)-capped GNP to detect chromium ions (III and VI). The detection system was dependent on RGB extraction using an application software "color scan" and the ratio of green to red was calculated to obtain a calibration curve and determine the amount of chromium in the real water sample (Fig. 12.7a) (Chen et al. 2015). Faham and coworkers showed a paper-based colorimetric sensor integrated with smartphone readout for chromium (III) detection in solution. 2,2′-thiodiacetic acid-modified gold nanoparticle (TDA-GNP) was spotted on a paper disc and treated with various concentrations of Cr^{III} followed by image capturing using a Samsung E5 smartphone. Color intensities of the paper disc were obtained using adobe photoshop CS5 and applied to obtain Cr^{III} concentration in real samples (Faham et al. 2018). Smartphone-based detection provides real-time on-site application of colorimetric sensors with the help of simple software available by third-party service providers. However, to obtain analytical values from color, the images are highly processed and compressed, leading to alterations in final analytical data and cannot be trusted completely. Moreover, simple analytical models fail to detect the number of independent variables like in the multi-analyte sensor. The drawbacks of illumination and smartphone detection systems can be overcome by applying artificial intelligent systems like machine learning (Mutlu et al. 2017).

Machine learning (ML) is a subset of artificial intelligence (AI) that acquires information from raw data by extracting its features and uses it to tackle problems without human intervention. It is a computer program or algorithm that makes machines more intelligent in behavior and decision by enabling them to learn from past experiences and develop their own program (Cui et al. 2020; Lussier et al. 2020). In the field of sensing, ML is employed as a tool for data processing to extract features like color intensity and utilize these features to predict the concentration of analyte and toxic ions directly (Cui et al. 2020). A general workflow of machine learning and its advantages in colorimetric sensing is presented in Fig. 12.7b. ML is grouped into two classes: supervised and unsupervised learning (Ayodele 2010). In unsupervised learning, the algorithm is not trained with the input data (training data);

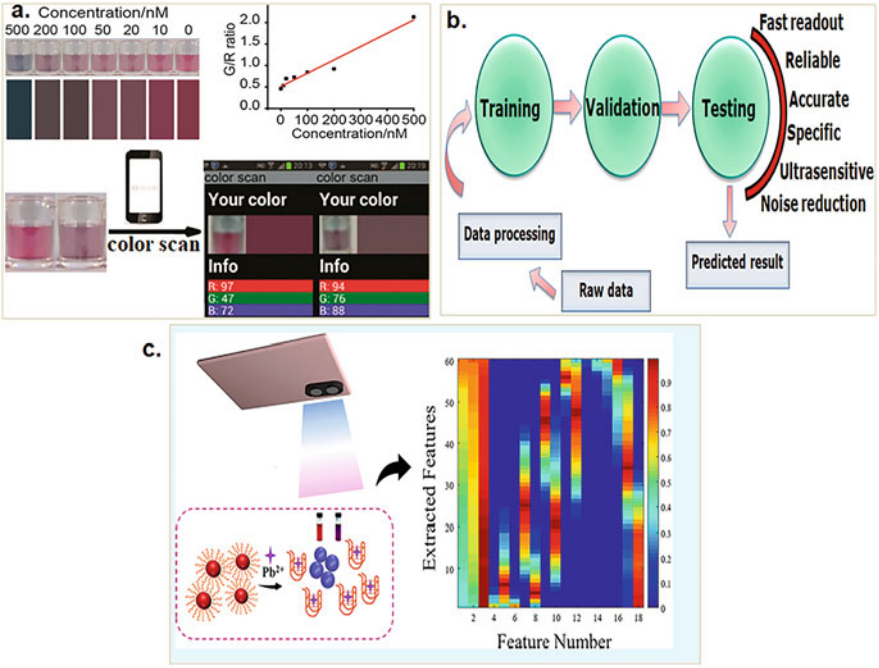

Fig. 12.7 (**a**) RGB extraction and smartphone readout-based detection of $Cr^{III/VI}$ using DMSA-GNP. (**b**) General work flow of machine learning and its advantages in colorimetric sensing. (**c**) Smartphone readout and regression-based model to process RGB features extracted from color change on interaction of Pb^{II} with oligonucleotide-GNP. Reproduced from Ref. Chen et al. (2015) (**a**). Reproduced from Ref. Sajed et al. (2020) (**c**)

instead, it learns from the pattern of untagged data, builds a concept, and predicts the output. k-Means clustering is the most used unsupervised learning algorithm.

On the contrary, supervised learning involves training of ML algorithms with input data called training data. Based on training data, the algorithm predicts the output for the unknown input data called testing data. Convolutional neural network (CNN), artificial neural network (ANN), support vector machine (SVM), and multiple linear regression (MLR) are some supervised ML algorithms, gaining attention in chemical and biological colorimetric sensors development (Cui et al. 2020). Sajed group reported a novel detection method for Hg^{II} ions in water utilizing smartphone-based machine learning regression. Aptamer modified gold nanoparticle changes color from red to purple in Hg^{II}, and the corresponding color change was captured on a smartphone camera. The obtained color cards trained the machine learning regression model to interpret mercury ion concentration based on RGB values. The smartphone was fabricated with an optoelectronic component using three-dimensional printing technology, an attachment to any smartphone. The optoelectronic device comprises three compartments: LCD board and camera's depth focus chamber, and cuvette holder. This setup provides an advantage of

blocking ambient light interference and results in reproducible LCD illumination (Sajed et al. 2019). In another work, Sajed et al. reported similar detection apparatus for Pb^{II} ion using oligonucleotide modified gold nanoparticle and machine learning regression on the mobile platform (Fig. 12.7c) (Sajed et al. 2020). Machine learning combined with a smartphone provides high sensitivity, accuracy, and easy operation with ubiquitous detection using Lab-on-phone apparatus.

12.7 Conclusion

Metal nanoparticle-based colorimetric sensing has gained special attention in the detection of environmental toxins because of distinct chemical and optical characteristics, including catalytic behavior, easy synthesis, and a broad array of surface functionalization molecules. SPR-based color change associated with the aggregation of nanoparticles is the primary mechanism to develop a colorimetric sensor. Gold and silver nanoparticles exhibit distinct SPR features. On the other hand, the platinum-type nanoparticle is explored mainly for its catalytic activity and enzyme mimetic behavior to induce an indirect color change in sensing application. Among all these metallic nanoparticles, gold nanoparticles (GNP) have emerged as a versatile platform for detecting toxic metal ions because of their stability, ease of synthesis, and functionalization with biomolecules and unique optical properties. Compared to traditional methods, GNP-based colorimetric sensor provides rapid and inexpensive detection techniques specifically for the metal toxins with a detection limit of micro-to-pico molar level. Recently, a paper-based analytical device coupled with colorimetric assay emerged as a cheaper alternative to conventional methods to develop the point-of-use testing system. This technique involves immobilization of modified gold nanoparticles on paper, and metal toxins are allowed to flow through it, exploring the self-wicking property of paper substrate toward metal ion detection. The paper-based colorimetric devices provide a field-deployable miniaturized sensing platform. The developments in sensing strategies have given a new lab-on-phone concept, which includes the addition of a simple colorimetric sensing apparatus to a smartphone. This smartphone-based sensing apparatus relies on RGB (red, green, blue) features extracted from the color intensities of samples. However, the images become highly processed and compressed during feature extraction, and thus results from the smartphone-based devices cannot be trusted completely. The drawbacks of smartphone detection systems can be overcome by applying artificial intelligence systems like machine learning algorithms. ML is employed as a tool for data processing to extract features like color intensity and utilize these features to predict the concentration of toxic metal ions directly in the field of sensing.

Acknowledgments The authors acknowledge Dr. Harish Hirani, Director, CSIR-CMERI, and Dr. Nilrudra Mondal, Head, Material processing and microsystem laboratory, CSIR-CMERI, for their support and encouragement. The author (NP) thanks CSIR-HRDG, New Delhi, for the fellowship grant CSIR-SRF (2018).

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Miniaturized Sensing Strategies for Next-Generation Nitrogen Monitoring

13

Jing Fang Tan, Joel B. Johnson, Mani Naiker, and Shaneel Chandra

Abstract

This chapter examines the applications of nanomaterials onto electrochemical sensors for nutrient-N species in the literature over the last 5 years. The chapter starts by discussing the two central *bottom-to-top* and *top-to-bottom* tenets to nanostructure synthesis. It then examines the use of nanomaterials in general chemical analyses, followed by reaction kinetics. In chemical catalysis, nanomaterials that have been used in several forms (e.g., as metals, metal oxides, and nanocomposites) are discussed, and this is where electrochemical sensing is covered as an application of surface catalysis. The chapter also includes reviewing recent works based on nano-polymers and nano-sized biomaterials. An analysis of the reviewed works is included as a general discussion, followed by an appraisal of current challenges. The chapter concludes with a projection of the future based on current nanotechnology trends where we focus on the likely benefits that are yet to be realized in nanotechnology, particularly in the novel area of consumer and citizen science.

Keywords

Miniaturized sensors · Biodevices · Nanomaterials · Analytical performance

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_13

13.1 Introduction

13.1.1 Nitrogen Detection and Its Challenges

Excess nitrogen in aquatic systems poses problems such as eutrophication, which has flow-on effects such as anoxia (Sánchez-España et al. 2017) and coral bleaching (Zhao et al. 2021). In the environment, nitrogen exists in several forms, but the naturally occurring nitrate and nitrite species are quite common as nutrients (Choodum et al. 2020). The levels of these species are also disrupted when there is high nitrogen loading into waterways causing both eutrophication and acidification (Fang et al. 2021). Therefore, it is important to be able to monitor nitrate and nitrite levels (rather than nitrogen itself) in both nutrient pollution cases or as an index of water quality.

Current methods of nitrogen detection are principally based on chromatography or spectrophotometry (Ryu et al. 2020). Spectrophotometry based on UV–vis is cost-effective, simple to operate, and becoming very common in most laboratories (Soylak et al. 2020). However, it still requires a dedicated sample collection and transport stage which could be a source of analyte loss, as well as facing spectral interferences requiring external data processing and mining (Roberts et al. 2018). Chromatography also requires sample collection off-site and uses organic solvents that are harder to dispose of (Napolitano-Tabares et al. 2021).

To address these limitations, the use of alternative detection platforms such as electrochemical sensing has started to enjoy increasing popularity in recent times. The analysis itself requires little reagent use, tends to be simple, inexpensive, accurate, reliable, and rapid (Power et al. 2018).

Due to the considerable development of nano-sized materials, their use as signal amplification components in electrochemical sensors has led to even more improved sensitivity and selectivity for analytes in complex sample types (Zhu et al. 2015).

Accordingly, in the following sections, we discuss these nanomaterials and review the creative forms that they have been crafted in and their applications toward chemical analysis. We will also explore the use of these materials in electrochemical sensors, especially applied toward the nitrate and nitrite forms of nitrogen.

13.1.2 Nanotechnology and Nanoscience

In the terminology of the twenty-first century, the new “small” is any entity that has an aspect in the nano range. As Almeida et al. explain, this is due to the growing prominence of nanotechnology, which is the use of nanoparticles bearing a critical dimension up to 100 nanometers (nm). The small size aspect confers unique and improved qualities to the particles, making their application possibilities almost endless (Almeida et al. 2020). Furthermore, the wide range of techniques available for the synthesis of nanoparticles makes it easy to create nanoparticles with tailored or customized properties.

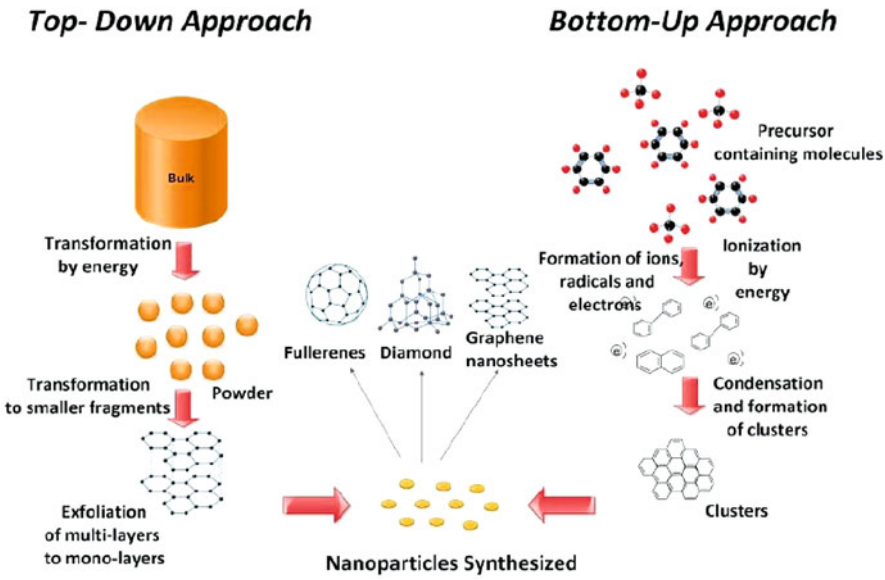

Fig. 13.1 A comparison of the top-down and bottom-up approaches to nanofabrication, using carbon-based nanomaterials as an example. Reproduced from Habiba et al. (2014) under Creative Commons 4.0 license

The process of manufacturing products with one or two dimensions in the nanometer dimension is referred to as *nanofabrication*. Nanofabrication methods are categorized into two groups. The top-down or top-to-bottom patterning route involves the deliberate downsizing of macro-sized bulk materials to the nanoscale (Fig. 13.1), and can include methods such as etching, ball milling, homogenization at extreme conditions like high pressure, and ultrasound emulsification (Iqbal et al. 2012; Sahani and Sharma 2020).

Although top-down approaches are typically easier to implement compared to bottom-up alternatives (Jamkhande et al. 2019), unfortunately, there are several limitations to these fabrication methods. Due to the often near-atomic precision involved in most nanotechnological syntheses, the small scale of craft required can lead to unavoidable imperfections, the high cost of specialized equipment required for processing, the need for high surface finished materials, and longer etching times associated with carefully controlled synthesis (Kumar et al. 2018). These are of course in addition to potentially undesired changes in the physiochemical properties of the nanoparticles themselves (Nadagouda et al. 2011).

In the contrasting approach of bottom-up assembly, nanostructures are crafted in an atom-by-atom or molecule-by-molecule fashion to produce the final product (Iqbal et al. 2012; Hassani et al. 2020). Due to the extremely high detail in the crafting of such materials, requiring what is often referred to as "atomic precision," the scope of imperfections associated with bottom-up approaches can be considerably minimized, or at the very least, controlled. In addition, this greater degree of

manufacturing control minimizes or eliminates waste or unused products. According to Zobir et al. (2019), the bottom-up approach also achieves high purity of yields.

Some of the common methods associated with the bottom-up approach include plasma arcing, deposition under high purity, low-pressure processes such as chemical vapor deposition, metal-organic decomposition, pyrolysis using lasers, classical wet-chemistry methods, and self-assembly processes (Biswas et al. 2012).

Recently, the convergence of both approaches has also evolved, whereby top-down control is used to guide bottom-up processes (Isaacoff and Brown 2017). This is known as the multiscale approach; however, it will not be the focus of this discussion.

13.2 Nanostructures in Chemical Analyses

Historically, the first published work on nano-sized entities aiding in chemical reactions was that by Rampino and Nord (1941). Using hydrogen gas to reduce the metal salts of palladium and platinum, the authors prepared poly(vinyl) alcohol-protected palladium and platinum nanoparticle catalysts. Despite this early foray, it was not until nearly five decades later that the next reported work on nanotechnology appeared in the literature. In this work, Nilsson reported the preparation of nanoparticles conjugated with enzyme and antibody for use in heterogeneous enzyme immunoassays (Nilsson 1989). The application of nanotechnology from this work was subsequently cited from 1996 onwards by others, for example, Daubresse et al. (1996) and Labhasetwar et al. (1997). Earlier, in 1990, Osseo-Asare and Arrigada reported the development of SiO_2 nanoparticles in a non-ionic reverse micellar system (Osseo-Asare and Arriagada 1990). Therefore, the period from the early- to mid-1990s may represent the emergence of the term "nanotechnology" term and subsequently, its wider applications. A more detailed examination of the history of nanocatalysts can be found in Astruc (2020).

An examination of the literature reveals an ever-expanding variety of nanocatalysts that continue to be reported and applied for several applications. Bhadouria et al. have classified these under seven categories, namely metal-based nanocatalysts, core-shell nanocatalysts, silica nanomaterials, layered double hydroxides, nanocarbon catalysts, quantum dots, and polymeric nanoparticles (Bhadouria et al. 2020). In this review, we follow this excellent classification and refer the reader to the original source for detailed reading on each type.

Metals, particularly transition metal chalcogenides, make excellent catalysts due to their diverse electrical, chemical, and physical properties. They tend to have 2-D layered structures, often down to atomic thickness or multilayers. This ultrathin aspect of the material provides a high surface area for chemical catalytic behavior. Moreover, the distinct edge and base planes allow for extra versatility in electrocatalysis applications (Li et al. 2018).

Core–shell nanostructures comprise active metal cores that are protected by shells. The core forms the catalytic component of the material, with the semi-porous shell functioning to protect the core while still permitting reactants to access

the catalytic surface (Sun et al. 2017). The composition of the core and shell can be dependent on the catalytic pathway or the reaction. For example, where the core–shell is comprised of silica, and the encapsulated core contains magnetic nanoparticles, a more powerful union is achieved by exploiting the material's superparamagnetic core that can help minimize the agglomeration of catalyst particles (by using an external magnet). The encapsulation effect of the shell also helps increase the durability of the synthesized catalyst by preventing the core from agglomerating, sintering, or being exposed to chemical conversion (Dutta et al. 2017).

Silica nanoparticles are the most abundantly engineered nanomaterials in the world and are used extensively in various applications such as construction and microelectronics (Yang et al. 2019). In chemical applications, silica is usually found in the hybridized form in order to act as a catalyst. These forms include mesoporous silica which possesses a large surface area, uniformly large pore-size aspect, making it widely used in catalysis (Zhang et al. 2018).

Layered double hydroxides (LDHs) are two-dimensional layered double hydroxides that are currently a topic of significant interest due to their extraordinary physiochemical properties, including anisotropic behavior. They are especially useful in the environmental, energy, and catalysis areas of research and application as well as in the development of biomaterials (Laipan et al. 2020). In addition to their unique intrinsic properties, LDHs can be further modified by doping these hydroxide sheets with trivalent cations which introduce a residual positive charge within the nanosheets. As a result, applications of LDHs may be further extended to controlled drug delivery, the oxidation of water, and chemical catalysis, amongst others (Hobbs et al. 2018).

While carbon can act as a catalyst, the classical carbon materials (e.g. activated carbon) themselves do not demonstrate the optimum properties for use as suitable catalysts, such as high surface activity, stability, and the ability to withstand oxidation. However, with the discovery of nanocarbon forms, a renewed appeal has emerged for carbon-based catalysts (Zhang et al. 2020). These nanocarbon forms include carbon nanotubes, graphene (Thrower and Cheng 2017), fullerenes (Itami and Maekawa 2020), nanodiamond, and their doped variants.

Quantum dots are zero-dimensional nano-sized structures that possess unique properties stemming from their ultra-small size, quantum confinement effect, excellent chemical and physical properties, and biocompatibility with many other materials (Zhang et al. 2020). Due to these attributes, they find a wealth of applications, mainly in optical sensing (Chen and Bai 2020). Quantum dots made of carbon-based materials such as graphene or polymers are commonly termed "carbon dots."

Polymeric nanoparticles can range in size from 1 to 1000 nm (Zielińska et al. 2020). Their surface properties can be amended using diverse polymers as the terminal ends or by coupling particular polymers (Madkour 2019). These modifications enable polymeric nanoparticles to become carriers for other active compounds. These active compounds may either be enclosed within or be surface-borne onto the polymeric core (Zielińska et al. 2020).

13.3 Nanomaterials and Chemical Kinetics

Catalysts are typically in the nanometer size range and hence can be acknowledged as one of the first common uses of nanomaterials in consumer applications. This is due to two factors. Firstly, the small sizes of nanomaterials tend to maximize the surface area exposed to the reactants (Alves et al. 2011). In addition, because nanomaterials mostly tend to exist as 0-D to 2-D solids, they can effectively act as heterogeneous catalysts in otherwise aqueous and liquid reactants, where the solid surfaces can serve as the active sites for catalysis (Ong et al. 2017).

The defined nanoporous crystalline structure of nanomaterials provides them with features such as high shape selectivity and chemical activity. Moreover, the defects on the nanomaterial structure can accommodate acid–base centers, which also makes them suitable for use in organochemical reactions (Sharma et al. 2015). More specifically, in such heterogeneous catalytic processes, a sequence of elementary processes characterizes the overall process. These include surface diffusion and adsorption of the reactants onto the catalyst surface, chemical rearrangement within the reaction intermediates that may be adsorbed onto the catalyst sites, and subsequent desorption of the formed products (Zhang and Yin 2014). If the reactants are unable to be retained onto the catalyst surface, it is unlikely that effective collisions between their respective molecules would arise in the first place. Then, even with collisions occurring, the catalysts can influence the selectivity of the specific catalytic reactions rather differently depending on their local bonding geometries (Zhang and Yin 2014). Therefore, selecting the optimum catalyst depends significantly on the nature of the chemical reaction being expedited, followed by the right surface attributes to facilitate the reaction. For example, palladium is seen as a versatile catalytic material due to the ease with which its nanoparticle sizes, shapes, and composition can be tuned. All of these are crucial parameters for catalytic reactivity (Astruc 2020).

Recently, a considerable amount of attention has been focused on electrochemical sensor design and improvement using nanomaterials. An electrochemical sensor is a device that measures the amount of current or potential transferred across a solid surface. In electrochemical detection, this measurement is accomplished by the use of a three-electrode system (see Fig. 13.2), of which the working electrode is the workhorse. The surface of the electrode is the outer end of the setup, which is in direct contact with the analyte, and hence is where the physical interactions of the detection process occur. As a result, this surface must be optimized to allow the particular analyte to attach for a sufficient period of time to permit electron transfer. Thus, the more receptive the electrode surface is for an analyte, the greater the chances of the analyte being analyzed.

The optimum electrode surface should ideally exhibit exclusive selectivity for an analyte. In practice, this is not feasible, so discrimination against known interferents is the next best alternative.

Traditionally, unmodified surfaces often have insufficient selectivity and sensitivity for the detection of challenging analytes in complex matrices such as aquatic samples. Nanomaterials have helped address this challenge through their

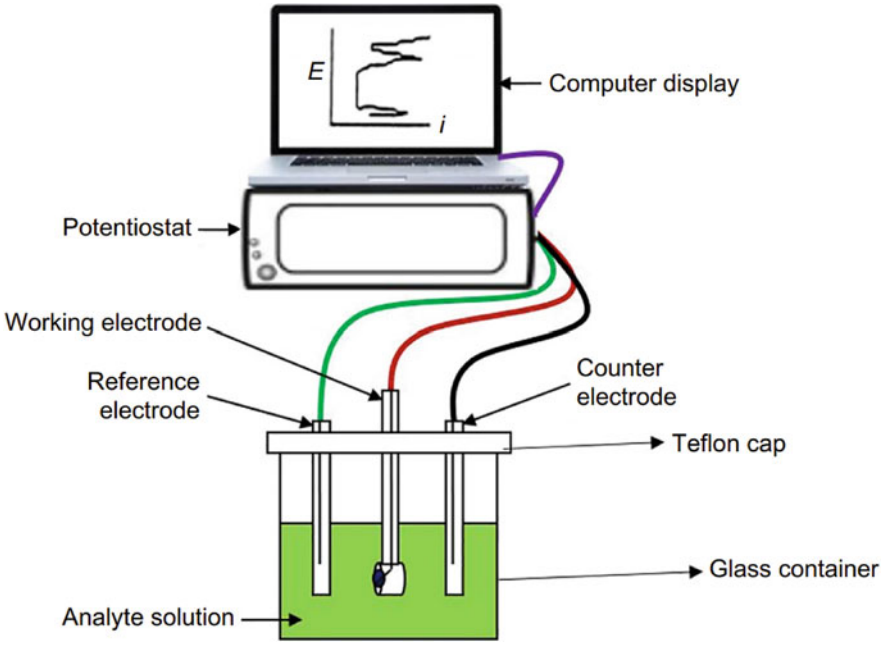

Fig. 13.2 A typical simple setup for cyclic voltammetry, showing the three-electrode system. Note the working electrode, where detection of the analyte occurs. Reproduced from Nnamchi and Obayi (2018) with permission

incorporation within the electrode surfaces (Ferrag and Kerman 2020). There is a myriad of ways in which nanomaterials can feature in this role, including preferentially targeting analyte molecules, expediting their interactions at the electrode surface, withstanding interference from non-analyte molecules, or a combination of these (Fig. 13.3). These include expediting electron transfer across the working electrode surface thus making the reaction more kinetically feasible. The first principle method of achieving this is to increase surface conductivity by its transformation, for example, by converting plain gold film surfaces to gold nanoparticles. A second pathway is the replacement of electrode surface assembly components with materials exhibiting superior electron conductivity, for example, replacing polymeric catalyst binders like Nafion® with metallic nanoparticles to increase electrode kinetics.

Another nano-based approach for improving electrochemical kinetics is to use nanomaterials with high selectivity or sensitivity for the analyte. While these are often dependent on the analyte itself, certain materials such as nanostructures of graphene have intrinsically favorable properties, which allows them to be widely used on electrochemical sensor surfaces. Attractive properties of graphene and its variants include high surface area (which facilitates catalysis at the electrode surface), nanoporosity (which can allow permselectivity of the analyte toward the polarized electrode surface), and inherently excellent conductivity. Individually, or

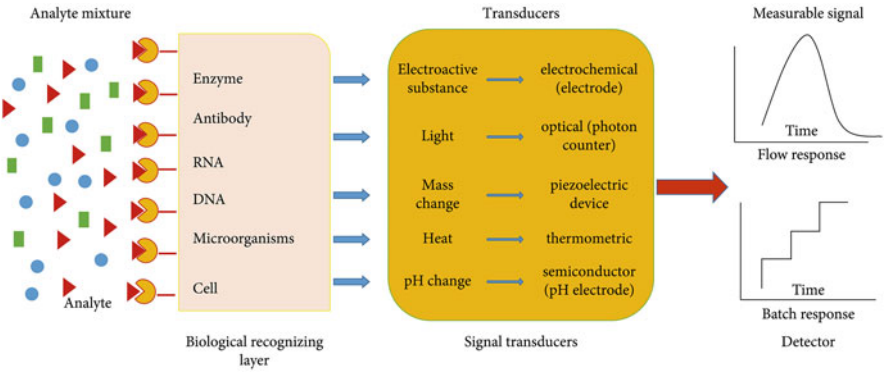

Fig. 13.3 A schematic diagram showing ways in which electrochemical sensor selectivity can be achieved through modification of the recognition layer and signal transducers. Reproduced from Noah and Ndangili (2019) under Creative Commons 4.0 License

in combination, these favorable properties make graphene and its variants a powerful material for electrochemical sensor design.

There are three basic steps involved in the electrochemical detection of any molecule. First, the analyte must diffuse through the solution to reach the surface of the electrode, followed by the transfer of electrons across the analyte–electrode interface. Finally, the products must diffuse away from the electrode, allowing new analyte molecules to access the electrode surface. Without a catalyst, this process is quite slow, particularly the electron transfer step between the electrode and the adsorbed species. Hence efficient electrocatalysts—often nanomaterials—are used to expedite the detection process (Jensen et al. 2017).

In the next section, the applications of nanomaterials in electrochemical transduction are discussed in more detail.

13.3.1 The Application of Nanomaterials in Transduction

In electrochemical detection, the working electrode surface is the location where the amount of chemical work gets done. The chemical work is converted to an electrochemical measurement using Faraday's constant (96,485 C/mol), which relates the charge to a mole of electron transfer. This conversion is at the core of electrochemical transduction. Thus, the use of nanomaterials to improve chemical reaction speed and output translates directly into a quick and meaningful signal.

Several nanomaterial types find applications as transducer components in electrochemical detection. We have previously discussed the various nanomaterial forms of carbon applied to electrochemical sensors (Power et al. 2018). Therefore, this discussion will not cover carbon. Other nanomaterial types are discussed below.

13.3.1.1 Metal-Based Nanomaterials

Very recently, Amini and co-workers synthesized Cu@TiO_2 core–shells that were immobilized with conductive polymers (e.g. Nafion) on glassy carbon electrodes (Amini et al. 2021). The sensor was used to detect nitrate and the authors reported electrode stability of up to 21 days, a linear operating range of 90 to 10,000 μM, sensitivity of 0.0039 μA/μM, and limit of detection (LOD) at 26 μM (Amini et al. 2021). Similarly, Annalakshmi et al. used strontium ferrite ($SrFe_{12}O_{19}$) nanochains as electrocatalysts for a nitrite sensor (Annalakshmi et al. 2020). The electrodes displayed a linear range of 20 nM − 3.6 mM and LOD of 6.3 nM for nitrite (Annalakshmi et al. 2020). Typical results obtained using these nanochain-functionalized electrodes are presented in Fig. 13.4, including the electrochemical responses obtained at different nitrite concentrations, and the linear calibration plot.

Metallic, bimetallic, and core-shell nanoparticles of the noble metals such as gold (Au), silver (Ag), platinum (Pt), and palladium (Pd) are predominantly sought after for numerous environmental measurements. This is because their unique size- and shape-dependent physical, chemical, and electrochemical properties (Maduraiveeran and Jin 2017). For example, Zhang et al. reported a nitrite sensor based on MoS_2 microspheres coated with gold nanoparticles with nanosheets (Zhang et al. 2019b). Operating at pH 6.0, the sensor had a linear range spanning 5.0 μM to 27.8 mM, high sensitivity of 117.0 μA mM^{-1} cm^{-2},and a low limit of detection (LOD) of 1.67 μM. Sheng et al. have loaded $Ni(OH)_2$/multi-walled carbon nanotubes loaded with Pt nanoparticles for a nitrite sensor, with a sensitivity of 145 μA mM^{-1} cm^{-2} and a low detection limit of 0.13 μM (Sheng et al. 2017). Spherical Ag nanoparticles decorated on graphene oxide were reported as electrode modifications by Zhao and co-workers (Zhao et al. 2019). They reported a LOD for nitrite of 0.24 μM as well as selectivity to nitrite in presences of common interfering species such as NaH_2PO_4, Na_2SO_4, NaCl, $NaNO_3$, and Na_2CO_3 (Zhao et al. 2019). Elsewhere, dumbbell-style AuPd and dendrite-like AuPt nanocomposites were used as modifications for

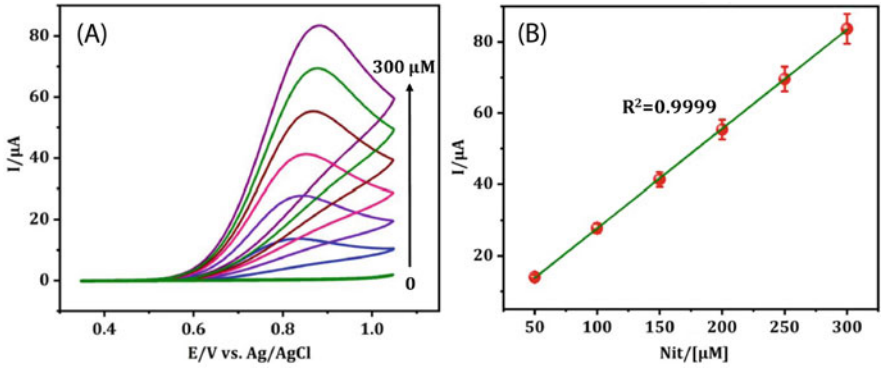

Fig. 13.4 Typical electrochemical responses obtained using a strontium ferrite nanochain-functionalized electrode for the detection of nitrite. (**a**) Cyclic voltammograms produced at increasing nitrite concentrations. (**b**) Linear calibration plot of anodic peak current at different nitrite concentrations. Adapted from Annalakshmi et al. (2020) with permission from the publisher

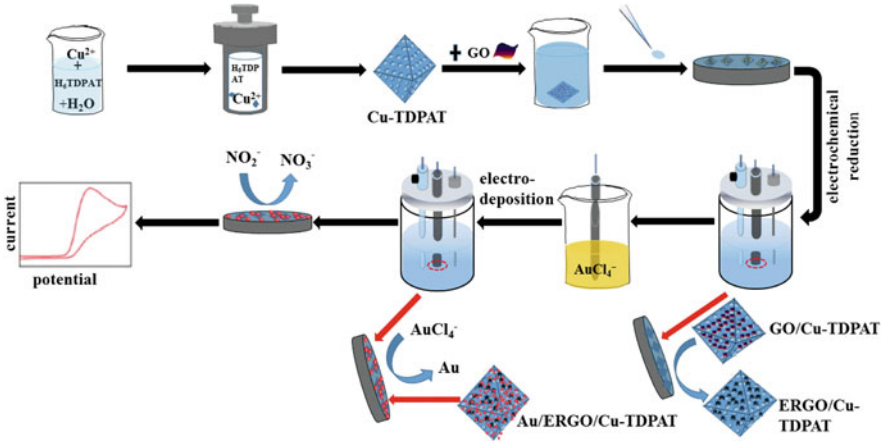

Fig. 13.5 Synthesis and application of an AuNP/graphene oxide electrode for nitrite detection. Reproduced from He and Yan (2019) with permission from the publisher

electrodes in nitrite detection (Chen et al. 2019). The authors reported the linear response concentrations for the Au-Pd sensors to range from 2 μM to 4200 μM and a 0.67 μM limit of detection (Chen et al. 2019).

He and Yan (2019) created an electrode for nitrite detection in food substrates, comprising of Au nanoparticles/reduced graphene oxide modification on a recombined Cu-based metal-organic framework. This novel combination of recognition and transducer entities allowed for in situ nanoparticles synthesis on the electrode, through a process of electrochemical reduction (Fig. 13.5).

Lei et al. reported multi-walled carbon nanotube substrates into which Co-based metal-organic framework and highly dispersed Au nanoparticles were structured. The sensor demonstrated a linear range from 1 to 1000 μM, LOD of 0.4 μM (s/n = 3), and a response time of approximately 3.5 s (Lei et al. 2021). Abdel Hameed and Medany (2019) fabricated core–shell structured nickel-platinum nanoparticles on graphene. The electrode was able to show an enhanced response toward nitrite compared to plain platinum on graphene, with a linear nitrite concentration range of 10 μM–15 mM. In addition, the electrode sensitivity was determined to be 85.18 μA mM^{-1} cm^{-2} (Abdel Hameed and Medany 2019). Han et al. (2019) fabricated a nanoflowers composite of rose-like Au nanoparticles with molybdenum sulfide and graphene. A linear range of 5.0 μM–5.0 mM with a limit of detection of 1.0 μM for nitrite was reported for this sensor (Han et al. 2019).

An electrochemical sensor based on Pd–Cu nanospheres atop molybdenum carbide was used to modify a glassy carbon electrode for the detection of nitrite by Ezhil Vilian et al. (2021). The linear response was in the 5–165 nM concentration range, with a LOD of 0.35 nM and sensitivity of 3.308 μA nM^{-1} cm^{-2}.

Another interesting metal nanoparticle application has been through their use as nanochains. Shi et al. (2017) used lab-synthesized gold and platinum nanochains for electrochemical nitrite detection. The modified electrode showed optimum

performance for nitrite determination, with a linear range from 0.5 to 366.4 μM and a limit of detection at 0.03 μM (S/N = 3). The authors also noted that the AuPt nanochains demonstrated superior performance to individual Pt and Au nanoparticles (Shi et al. 2017).

13.3.1.2 Metal Oxide Nanoparticles and Nanocomposites

Nanostructured metal oxides provide various advantages such as cost-effectiveness and improved selectivity when used with biorecognition molecules. Due to their size, stability, and high surface area, improvements include greater robustness of the working electrode interface, more rapid electron kinetics, and extending the electrical contact of protein redox centers with the transducer surface (George et al. 2018). Manjari et al. have synthesized Ag and Cu oxides nanocomposites for the electrochemical sensing of nitrite ions with a LOD of 17 μM (Manjari et al. 2020). Elsewhere, Asiri et al. modified glassy carbon electrodes with La_2CuO_4 nanoparticles for improved nitrite detection at the electrode surface. Using differential pulse voltammetry, the modified electrodes had a limit of detection at 0.04 μM (s/n = 3) (Asiri et al. 2020).

Oxides of iron have also been employed in electrochemical sensors. Nithyayini et al. integrated $NiFe_2O_4$ nanoparticles into carbon paste for nitrite detection (Nithyayini et al. 2019). The authors reported a limit of detection of 0.1236 μM (s/n = 3), a linear range between 0.1 and 1000 μM and a sensitivity of 7.9617 μA μM^{-1} cm^{-2} (Nithyayini et al. 2019).

Metal oxide nanorods have also been applied toward electrochemical nitrite detection. Sha et al. have reported a novel nanocomposite comprising of α-MnO_2 nanorods and hierarchical MoS_2 microspheres as electrode modifications in nitrite detection in potable water (Sha et al. 2019). Performance parameters included a limit of detection of 16 μM, response time <5 s, and sensitivity of 515.84 μA mM^{-1} cm^{-2} with a linear response range between 100 and 800 μM. The heterogeneous interface comprised intertwined MoS_2 nanosheets and 1D α-MnO_2 nanorods, with the edge and defect sites in MoS_2 identified as being the primary contributors to optimum detection performance (Sha et al. 2019).

Recently, Shivakumar et al. have used $NiCo_2O_4$ nanotapes to kinetically enhance the surface of glassy carbon electrodes applied toward nitrite detection in 0.1 M pH 7.0 phosphate buffer solution (Shivakumar et al. 2021). The sensor operated in a detection range between 10 and 300 μM with a LOD of 1.04 μM and a sensitivity of 1.03 μA μM^{-1} cm^{-2} (Shivakumar et al. 2021). Elsewhere, a silver/halloysite nanotube/molybdenum disulfide nanocomposite was used to modify carbon paste electrodes for the electrocatalytic detection of nitrite in aqueous solutions (Ghanei-Motlagh and Taher 2018). Linearity of 2–425 μM and a limit of detection of 0.7 μM (s/n = 3) characterized the performance of the modified sensor.

13.3.1.3 Biosensors

In recent decades, biosensors have become more prevalent for enhanced specificity and relative ease of design (Nguyen et al. 2019). Broadly speaking, an electrochemical biosensor incorporates a biologically derived recognition element (such as

enzymes or antibodies) to provide specificity and sensitivity for a given analyte (Bunney et al. 2017; Johnson et al. 2019; Rezaei and Irannejad 2019).

Within the scope of nitrogen sensing, the enzyme nitrate reductase—which reduces nitrate to nitrite—can be used for the selective detection and quantification of nitrate. Ali et al. (2017) created an impedimetric sensor for nitrate using graphene oxide nanosheets and poly(3,4-ethylenedioxythiophene) nanofibers. The LOD was 2.2 μM, with a linear response range between 7 and 7400 μM.

Another recent study using nitrate reductase and ZnO nanorods on a silver electrode found a low LOD of 1 μM, with a linear range between 1 and 3400 μM (Ahmad et al. 2017). Notably, the electrode was exceptionally stable, with 97% of the initial response found after one month of storage.

However, despite their increased selectivity and specificity, nitrate reductases can suffer from low electron transfer efficiency (Zhang et al. 2019a), short lifespans, oxygen interferences (Plumeré 2013), high production costs, and requirements for low storage temperatures (Jiang et al. 2020). A study by Massah et al. managed to extend a nitrate biosensor lifetime by using a machine learning method alongside the device to predict and correct the rate of decline in the enzymes' activity (Massah and Asefpour Vakilian 2019).

Nitrite is well known for its toxicity, which results from its action of oxidizing hemoglobin to methemoglobin, resulting in methemoglobinemia and potentially cellular hypoxia (lack of oxygen) as a consequence (Kim-Shapiro et al. 2005). Based on this principle, Raja Jamaluddin et al. (2018) created a novel biosensor using hemoglobin covalently immobilized on polyacrylic-graphene nanosheets. This sensor showed a low LOD (0.65 μM) and wide range of linearity (1–100 μM), with no significant interference from other commonly interfering ions (Ca^{2+}, Na^{+}, K^{+}, NH_4^{+}, Mg^{2+}, NO_3^{-}).

Similar to nitrate biosensors, the common enzyme utilized for nitrite detection is nitrite reductase (NiR). Monteiro et al. fabricated an electrode for use in urine comprising of nitrite reductase drop coated on carbon SPEs along with glucose oxidase and catalase to remove the interference of dissolved oxygen (Monteiro et al. 2019). The electrode reached a LOD of 4.7 μM and sensitivity of 26 mA M^{-1} cm^{-2} at the potential of −0.8 V.

13.3.1.4 Genosensors

Currently, the scope of genosensor application has been largely in physiological analyses, although Campuzano et al. (2017) have also alluded to applications in environmental and food processing industries among others. There have been no applications to date of genosensors in the monitoring of simple molecular forms of nitrogen (nitrate, nitrite, ammonia), given that these compounds do not contain any proteins or genetic material that could be targeted with a polynucleotide probe. However, genosensors have been used for the detection of harmful algal blooms (Orozco and Medlin 2013), which result from eutrophication of waterways with excess ammonia, nitrate, or phosphorus (Zohdi and Abbaspour 2019), thus potentially providing indirect information on trends in nitrogen content. Orozco et al. (2011) used a sandwich hybridization format genosensor for the concurrent

detection of the algal species *Prymnesium parvum*, *Pseudo-nitzschia australis*, and *Gymnodinium catenatum*, both of which are implicated in harmful algal blooms. Similarly, Morais et al. (2021) created a genosensor with a 70 bp DNA probe for the toxic dinoflagellate *Alexandrium minutum*.

13.3.1.5 Polymers and Biomaterials

In this section, we include polymeric forms of carbon. Rostami et al. have used magnetic nanoparticles/graphene oxide decorated with acetic acid moieties on a sensor for the determination of nitrite in aqueous solutions (Rostami et al. 2019). The results from differential pulse voltammetry experiments showed a linear response in 1–85 μM and 90–600 μM. A LOD of 0.37 μM was also reported. Rashed et al. have used reduced graphene oxide/mesoporous zinc oxide nanocomposite (Rashed et al. 2020) for nitrite and reported a detection range between 200 and 4000 μM using linear sweep voltammetry (LSV) and 20–520 μM for amperometry. The authors reported the sensitivity and LOD to be 0.3156 μA μM^{-1} cm^{-2} and 1.18 μM for LSV, and 0.2754 μA μM^{-1} cm^{-2} and 1.36 μM using the amperometric technique, respectively (s/n = 3).

Nasraoui and co-workers patterned graphene onto a flexible poly(imide) substrate, which was further modified by COOH-functionalized multiwalled carbon nanotubes and gold nanoparticle films (Nasraoui et al. 2021). The sensor demonstrated a linear operating range from 10 μM to 140 μM and a LOD of 0.9 μM, and a relative standard deviation less than 10% (Nasraoui et al. 2021).

In another study also utilizing nanoparticle-functionalized films, Pang and co-workers reported a silver-nanoparticle-functionalized poly (3, 4-ethylenedioxythiophene): polystyrene film on the common glass as an electrode substrate for nitrite detection (Pang et al. 2019). A linear response range of 0.5–3400 μM and LOD of 0.34 μM (s/n = 3) in phosphate buffer solutions were reported for the modified electrodes.

13.4 General Discussion

This review has highlighted a significant volume of research effort directed toward nanomaterials-based nitrite electrochemical sensors. A summary of the works quoted in this review is presented in Table 13.1. The most widely applied nanomaterials for sensor modification were those based on gold. Metal nanocomposites, including those comprised of the respective oxide of the metal, were also used in several sensor modification reports. Similarly, the most common electrode type used for subsequent modification were glassy carbon electrodes. There are several reasons for the popularity of glassy carbon electrodes, including robustness, the versatility of the polished electrode surface, and their relatively low cost. As Sharma (2018) outlines, the decoration of glassy carbon surfaces with other functional materials in the nano-realm represents an indirect application of glassy carbon in nanoscale devices of the type examined in this review.

Table 13.1 Summary of electrochemical sensors for nitrate and nitrite detection with their analytical features (nanomaterial, LOD, Linear range, and sensitivity)

Sensor type	Analyte	Nanomaterial	Performance parameters			Reference
			LOD (μM)	Linear range (μM)	Sensitivity (as reported)	
Metal-based nanomaterials	Nitrate	Cu and TiO_2 nanocomposite on polymers as modification on glassy carbon electrodes	26	90–10,000	0.0039 μA $μM^{-1}$	Amini et al. (2021)
	Nitrite	Strontium ferrite (SF) on glassy carbon electrodes	0.0063	0.02–3600	N.S.	Annalakshmi et al. (2020)
		Au nanoparticles on MoS_2 microspheres on glassy carbon electrodes	1.67	5–27,800	117 μA mM^{-1} cm^{-2}	Zhang et al. (2019b)
		Pt nanoparticles loaded $Ni(OH)_2$/multi-walled carbon nanotubes on glassy carbon electrodes	0.13	0.4–5670	145 μA mM^{-1} cm^{-2}	Sheng et al. (2017)
		Ag and graphene oxide on glassy carbon electrodes	0.24	1–1000	N.S.	Zhao et al. (2019)
		Dumbbell-style Au_3Pd_4 on glassy carbon electrodes	0.67	2–4200	N.S.	Chen et al. (2019)
		Au nanoparticles, co-based metal-organic framework, and multi-walled carbon nanotube on glassy carbon electrode	0.4	1–1000	0.223 μA mM^{-1}	Lei et al. (2021).
		Ni and Pt nanoparticles on graphene sheets on glassy carbon electrodes	0.49	10–15,000	85.18 μA mM^{-1} cm^{-2}	Abdel Hameed and Medany (2019)
		Au nanoparticles with MoS_2 and graphene on glassy carbon electrodes	1	5–5000	N.S.	Han et al. (2019)
		Pd-cu nanospheres and Mo_2C on glassy carbon electrodes	0.00035	0.005–0.165	3.308 μA nM^{-1} cm^{-2}	Vilian et al. (2021)

		Au and Pt nanochains on glassy carbon electrodes	0.03	0.5–366.4	N.S.	Shi et al. (2017)
Metal-oxide based nanomaterials		Ag-cu decorated ZnO nanocomposite on glassy carbon electrodes	17	100–1500	N.S.	Manjari et al. (2020)
		La_2CuO_4 nanoparticles on glassy carbon electrodes	0.04	0.05–25, 25–1000*	0.317 μA μM^{-1} cm^{-2}	Asiri et al. (2020)
		$NiFe_2O_4$ nanoparticles in carbon paste electrodes	0.1236	0.1–1000	7.9617 μA μM^{-1} cm^{-2}	Nithyayini et al. (2019)
		α-MnO_2 nanorods and MoS_2 microspheres on glassy carbon electrodes	16	100–800	515.84 μA mM^{-1} cm^{-2}	Sha et al. (2019)
		$NiCo_2O_4$ nanotapes on glassy carbon electrodes	1.04	10–300	1.03 μA μM^{-1} cm^{-2}	Shivakumar et al. (2021)
		Ag/Halloysite nanotube/MoS_2 modified carbon paste electrodes	0.7	2–425	2.9 μA μM^{-1} cm^{-2}	Ghanei-Motlagh and Taher (2018)
Polymer and biomaterials		Iron oxide magnetic nanoparticles/graphene oxide decorated with CH_3COOH moieties on glassy carbon electrodes	0.37	1–85, 90–600[a]	0.1915 μA μM^{-1}	Rostami et al. (2019)
		Reduced graphene oxide/ mesoporous zinc oxide/Nafion on glassy carbon electrodes	1.18	200–4000	0.3156 μA μM^{-1} cm^{-2}	Rashed et al. (2020)
		Laser-induced graphene functionalized by carbon nanotubes decorated by au nanoparticles	0.9	10–140	N.S.	Nasraoui et al. (2021)
		Ag-nanoparticle-functionalized poly (3, 4-ethylenedioxythiophene): Polystyrene film on glass electrode	0.34	0.5–3400	0.03639 μA μM^{-1} cm^{-2}	Pang et al. (2019)
Biosensors	Nitrate	Nitrate reductase/poly (3,4-ethylenedioxythiophene) nanofibers/ graphene oxide nanosheets	2.2	7–7400	6.15 ohms mg/L^{-1} cm^{-2}	Ali et al. (2017)
		Nitrate reductase/ZnO nanorods on ag/glass electrodes	1	1–3400	450.5 μA mM^{-1} cm^{-2}	(Ahmad et al. 2017)

(continued)

Table 13.1 (continued)

Sensor type	Analyte	Nanomaterial	Performance parameters			Reference
			LOD (μM)	Linear range (μM)	Sensitivity (as reported)	
	Nitrite	Hemoglobin on poly(n-butyl acrylate)-graphene/screen-printed electrodes	0.65	1–100	N.S.	Raja Jamaluddin et al. (2018)
		Nitrite reductase/screen printed electrodes with glucose oxidase and catalase	4.7	2–200	326 mA M^{-1} cm^{-2}	(Monteiro et al. 2019)

[a] Two linear response ranges.

The nitrite sensors reviewed in this work represent a range of performance values. The limit of detection reported in the quoted studies ranged from nanomolar to micromolar range. That this critical value ranged in three orders of magnitude represents an accurate capture of the diversity in performances of electrochemical sensors in detecting nitrite.

The sensitivity values that have been reported in the literature covered include several units. A comparison can be facilitated between those values that report the sensitivity in units of current per change in concentration per area of the working electrode (e.g. $\mu A\ \mu M^{-1}\ cm^{-2}$). All such units were normalized to those of current, length, and amount ($A\ cm\ mol^{-1}$). Based on this approach, the greatest sensitivity ($0.117\ MA\ cm\ mol^{-1}$) was reported by the glassy carbon electrodes modified using Au NPs on MoS_2 microspheres by Zhang et al. (2019b). This was followed closely by the sensitivity ($0.0331\ MA\ cm\ mol^{-1}$) of glassy carbon electrodes modified with Pd-Cu nanospheres and Mo_2C as reported by Vilian et al. (2021). The commonality of Mo-based nanomaterials onto glassy carbon electrodes is quite interesting in these.

13.5 Current Challenges and Future Directions

Despite the recent surge of work in this area, there remain several barriers to the development of sensitive, accurate, and robust sensors for nitrogen species. These may be broadly broken down into two key areas: (1) development and production of the sensors and (2) validation/translation of the sensors in real-life matrices.

13.5.1 Development and Production

Developing nanoparticle-functionalized electrodes with sufficient stability and selectivity remains one of the major challenges in this area. Due to their high surface area, nanoparticles are also more reactive and hence prone to non-specific interactions. With the recent availability of new nano-building blocks and better analytical methods for chemical, mechanical, and surficial examination of nanoparticle surfaces, more work is required to exploit this knowledge for developing more stable nanoparticle-based sensors. Similarly, entirely new sensor materials based on nanocomposites are now possible (e.g. mixed metal–metal oxides) and interfacial processes at new types of NP–NP interfaces are more accessible with the aforementioned developments. The application of these materials in environmental sensing should enable more advanced electrochemical sensor systems to be devised in the near future (Rassaei et al. 2011).

However, while many of the contemporary electrochemical nanosensors reported in the literature show exceptional sensitivity and selectivity, the associated production costs of these sensors would make mass production efforts unfeasible. In this regard, paper-based electrochemical sensors such as those used in biomedical fields are likely to afford much lower production costs (Gutiérrez-Capitán et al. 2020). This

is expected to be driven by lower procurement costs for the sensor material (substrate) onto which the respective electrodes are imprinted and subsequently modified. It is likely that with cost-effective substrates, greater economies of scale will push the cost of sensors further. In addition, paper substrates are more sustainable and minimize the environmental footprint, which would lend greater appeal.

Part of this disconnect between academic progress and real-life applications results from the relative infancy of nanoparticle applications in electrochemical environmental monitoring compared to the applications of nanoparticles in other fields such as medicine and bionanotechnology, often combined with a lack of funding for translational projects in environmental science. Consequently, much of the research in the nitrogen-sensing space has focused on the development of new types of sensors rather than optimizing their performance. We discuss this in more detail below.

In this review, the reported limit of detection has been within the micromolar range for both nitrate and nitrite. Relative to other technologies that are already reporting the same analyses at nanomolar ranges using comparable technologies like miniaturized spectrophotometry (Patey et al. 2008), micromolar ranges are quite high. Thus, it is expected that the pursuit of nitrate and nitrite using electrochemical sensors would remain as a clear pursuit in the near future.

13.5.2 Validation

Given that most electrochemical sensors developed for nitrogen species have a strong focus on academic novelty rather than real-life performance parameters, many of these sensors are not tested in real-life matrices such as freshwater, seawater, or wastewater samples (Jiang et al. 2020). These matrices show considerable analytical complexity compared to the distilled water or buffer solutions commonly used in laboratory performance testing. In such settings, nanoparticle-functionalized sensors are expected to demonstrate a further advantage over non-functionalized sensors, due to their enhanced selectivity. Similarly, field-trialed electrochemical sensors for nitrogen species are still quite rare, hence forming an area of interest for future researchers.

Another important but often overlooked facet of validation is electrode durability and stability. As previously mentioned, nitrate biosensors based on nitrate reductase provide higher selectivity but suffer from their high production costs and requirements for low storage temperatures to avoid degradation of these enzymes (Jiang et al. 2020). They are also likely to be less durable than metal or metal-oxide nanoparticle-based electrodes. However, many studies either do not report the durability of newly developed electrochemical sensors or only test for very short periods (e.g. 100 measurements, 7 days) (Jiang et al. 2020). For the deployment of sensors for the real-time monitoring of nitrogen species in waterways or estuaries, electrode durability in the order of months to years would be highly desirable. In conjunction with field-based validation, success with real-time telemetry of the measured information would result in a powerful remote monitoring system that

could assist with identifying trigger events and time and flow patterns in nitrogen discharge into the aquatic channels in the environment. This would represent a significant inroad in transitioning from a reactive, measurement, and containment approach in most present-day studies to a preventative or, at least, a targeted intervention strategy.

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Recent Trends Toward the Development of Biosensors for Biosafety and Biohazards

14

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Abstract

Biohazardous materials are a class of natural or bioengineered components of microorganisms/infectious substances that cause fatality, disease, biological complications, deterioration of food/water, damaging equipment, supplies, and detrimental reformation of the environment. Biohazards comprise biological agents and toxins such as parasites, viruses, bacteria, fungi, prions, and bioactive materials such as toxins, allergens, and venoms infectious to humans, flora, and fauna. Besides the specific issues, biohazards can cause substantial harm to the environment. A significant amount of research is required to better understand the infectious agents and toxins related to biosafety and biohazards. This chapter postulates an overview of the research framework, opportunities, challenges, and the future outlook of biosensors to diagnose, treat, and prevent a wide range of diseases and disorders caused by biohazards and the lack of biosafety. Possible precautionary approaches to take care of an array of contributing agents are also reviewed. In this respect, the researchers and the scientific community may profit from past teachings to predict potential tribulations. Implementation of the understanding of biosafety and biohazards with the use of appropriate techniques will enable the research and scientific community to avert personal, laboratory, and environmental exposure to potentially infectious agents or biohazards.

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_14

Keywords

Biosafety · Biosecurity · Biohazards · Biosensors · Biomaterials

14.1 Introduction

Biohazards and biosafety have become very important due to the extensive impact of the biohazards, such as biological agents and toxins, on humans, animals, and plants, affecting the environment and livelihood. Biohazards include the following agents: parasites, viruses, bacteria, fungi, prions, rickettsia toxins, allergens, venoms, flora, and fauna (USDA 2016). Biohazardous substance groups are listed in Table 14.1 as category A and category B respectively. As illustrated, the category A substances cause infection and health hazards to humans through some diseases. Category B will cause infection and disease to humans and animals and most of these causes are due to the transported medical waste without following any proper safety protocols. Different biohazards are classified based on the seriousness toward humans, animals, and environment affecting life, as described in Fig. 14.1 (Bayot and King 2020). The increasing level of hazard starts from level 1 to level 4, whereas the level 4 biohazard is most dangerous to human life. Numerous biological agents and toxins affect flora, fauna, and the environment due to their hazardous nature. These biohazards allow micro-organisms to grow and affect the livelihood of the environment affecting the complete ecosystem. Micro-organisms include the following (Stave and Wald 2016): viruses, rickettsia, Coxiella, Ehrlichia, anaplasma, bacteria, fungi, and parasites.

Biosafety involves the protocols, policies, and procedures adopted by industries, government, and civil societies to have a safe living environment by minimizing and avoiding the potential risks involved due to biohazards (Secretariat of the Convention on Biological Diversity 2005). There are different levels of biosafety adopted to make sure that the living organisms are safe. Table 14.2 shows the biosafety levels and the associated safety equipment (World Health Organization 2006).

Table 14.2 depicts the biosafety levels and the need for the respective safety equipment when a person involves in the works exposed to biohazards due to the

Table 14.1 Classification of biohazard agents

Category	Definition
Category A (UN2814)	Substances causing infection to humans through some diseases
Category A (UN2900)	Substances causing disease and infection to animals
Category B (UN3373)	Substances transported from one place to another place that can cause infection
Regulated medical waste (UN 3291)	Substances made from the medical treatment on humans and animals which are intended for re-use

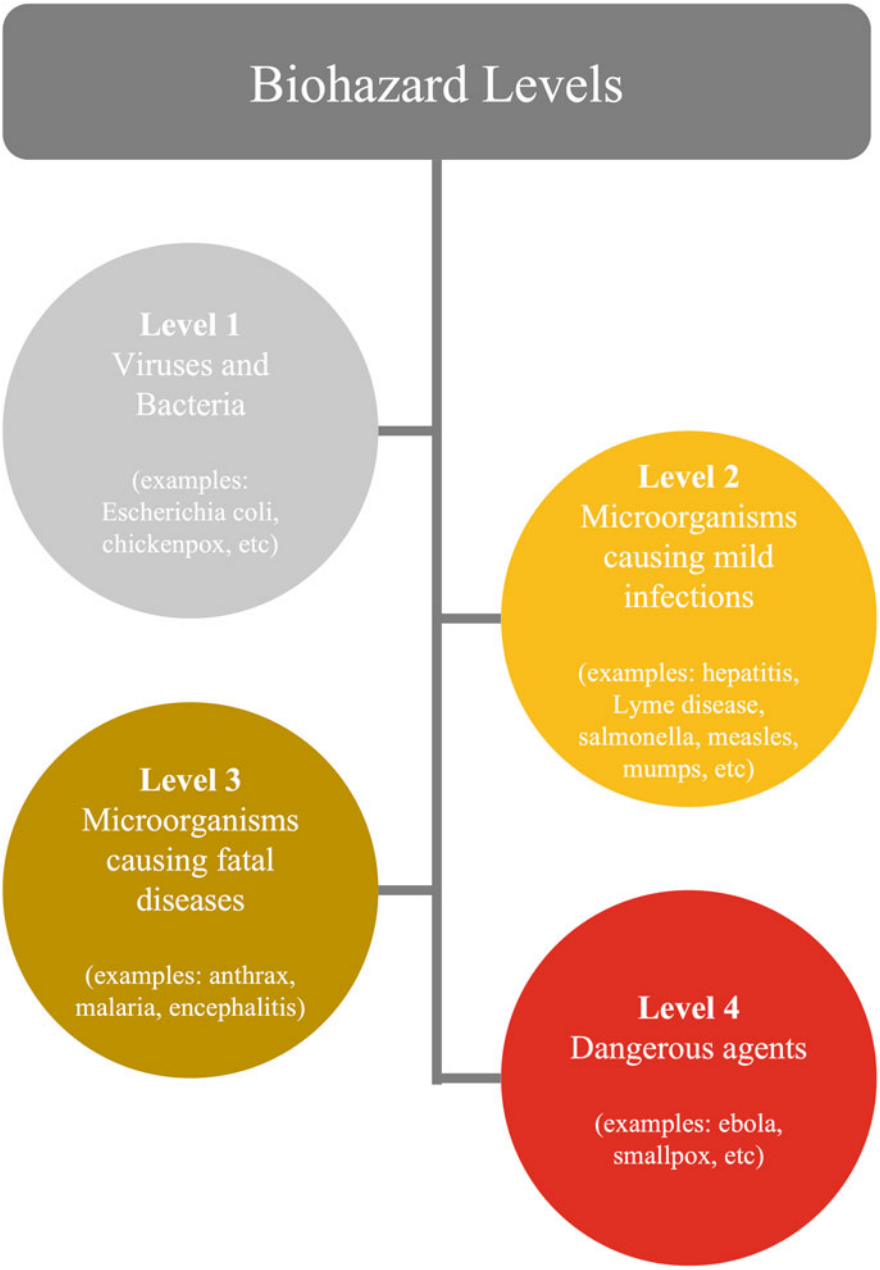

Fig. 14.1 Biohazard levels

Table 14.2 Biosafety level and safety equipment

Biosafety Level	Safety Equipment (Special Requirements)	
Basic biosafety level 1	Open bench work (no special requirements)	
Basic biosafety level 2	Open bench with biological safety cabinet Controlled ventilation system Autoclave	
Containment biosafety level 3	Biological safety cabinet and primary devices Controlled ventilation system Autoclave	
Maximum containment biosafety level 4	Class III biological safety cabinets Pressure suits Double-ended autoclave Filtered air system	Controlled ventilation system Double door entry Airlock Anteroom Effluent shower

nature of the profession or job. Level 1 would not need any special requirement, which can be carried out in an open bench environment. When the work involves level 2, then the safety of controlled ventilation, biological safety cabinet, and autoclave is mandatory. Similar to level 2, the work involved in level 3, which is the possible contaminant level, will also need the safety measures and standards adopted at level 2. Whereas level 4 would be of high-risk nature, which needs a more safer environment when compared to level 1, level 2, and level 3. Level 4 is of maximum contaminant level, which needs pressure suits, safety cabinets, filtered air system, controlled ventilation, double door entry, airlock, anteroom, and effluent shower. Generally, these types of protection and safety measures are followed in the research labs handling high-risk associated works such as research carried out on some viruses to study their nature and mutations. Since any minor accidental release of these viruses during the work progress will have a greater impact and high risk on the common public and the people working on the research. Hence, it's very important to follow the safety protocols at level 4.

Biohazards cause drastic effects on the environment, marine life, and food, due to which infectious diseases will be spread, affecting living beings and organisms. Intentional hazards have been caused due to chemical warfare by the agencies. Environmental biohazards cause a considerable threat to the environment, such as groundwater contamination, radioactive pollutants, and airborne pollutants due to various causes such as medical waste disposal, electronic waste, plastic waste, and genetically modified plants. Medical waste disposal system implementation with adequately trained personnel will avoid the reuse of already used entities that could be hazardous. Healthcare establishments should create different subgroups to dispose of the medical wastes effectively (Masum et al. 2011). Another significant environmental biohazard is the improper disposal of electronic waste to the environment. These will have a horrible effect on the health of humans and animals due to the harsh chemical exposure such as toxic phosphor to the environment. Discharge of the glass powder and other chemicals (tin, lead, brominated dioxin, beryllium,

cadmium, and mercury) into the rivers will affect the sea species and animals (Wath et al. 2011). To resolve this environmental problem due to electronic waste, there should be a system to dispose of the electronic waste properly by developing dedicated methods to handle biohazard waste with the following steps (Ma Erlyn et al. 2016).

- Input (consists of the waste bin setup).
- Process (determining the waste bin setup).
- Output (electronic biohazard waste disposal setup).

A possible biohazard to the environment through soil contamination is the construction biotechnology which involves bio cementation, bio grouting, bio desaturation, bio aggregation, and bio coating, causing the precipitation of calcium bicarbonate with reaction causing the release of chemicals such as ammonia/ammonium to the environment. This can be controlled using enzymes or non-living bacteria to ensure the biosafety (Ivanov et al. 2019). The use of plastics and improper disposal of plastics is another leading cause of biohazards for the environment. Nano and microplastics and their physical change through the microbial communities make the soil habitable (Pathan et al. 2020). Animal food biosecurity is the most important since it can slowly have a biohazard impact on the environment. Various diseases of the animals and their impact is categorized for better understanding the cause of the disease due to biohazard (Brioudes et al. 2015).

Food biosecurity is of primary importance since the possibility of a bioterrorist attack will directly impact humans (Bruemmer 2003). Biohazards can cause damage to the human lives intentionally and unintentionally. Food biohazard is possible food industries allowing the spread of HPAI H5N1 flu, making the birds more susceptible to the disease. Biosafety from these hazards is very important to safeguard food from biohazards (Marion et al. 2015). Bovine tuberculosis in cattle and its severe impacts is a more significant concern that can be significantly reduced by the attempts of farmers toward the biosecurity initiatives by standardizing biosecurity and disease (Enticott et al. 2012). Similar to the bovine disease in cattle, another disease is foot and mouth disease in animals, caused due to the food products given to them. This disease has a more significant impact in developed and developing countries, causing a severe downfall in the meat industry (Kompas et al. 2015). Most causes of animal diseases are because of the food and agricultural products being consumed by the animals. To overcome these issues, the biosecurity of agriculture plays a significant role. Integrating plant and animal biosecurity is an approach adopted to keep the animal food biosecured, which will result in a safe food product for human lives (Waage et al. 2008). Most of the biohazard causes are interconnected with the ecosystem, for example, agricultural biohazard impacts on food resulting in food biohazard, food biohazards will, in turn, impact the animals and humans. Marine biohazard will have a direct or indirect impact on all of the above. Hence the biosafety of marine is very important to avoid the biohazard caused in the marine.

Actions were taken to manage and avoid the risks caused by pets and other marine species by providing an inquisitive environment and several pathways for the new

species. Most of the marine biohazard is due to the accidental release of waste. When anchors and chains are used to hook the ship, there is a possibility of biohazard. Aquatic cargo also causes marine biohazard allowing ballast water and sediments in the marine waters. Another cause of the biohazard is the discarding of nets and packing materials. When discharge of feeds in the form of live, fresh, or frozen is done, it results in biohazard. In some cases, the biohazard happens due to the driving gear, field gear, experimental gear, and fishing gear movements in the marine waters. The above-listed factors are few that cause marine biodegradation (Chad et al. 2004; ICES 2005). Microplastics and related sediments have made the soil prone to an unsuitable plantation on the mangrove soils. Prevention and control of these pollutants are required for marine biosecurity, and for creating better ecosystems. This can also be achieved by protecting the soil against microplastic biohazards. Most important approach is to make the people nearby those areas understand the possible pollution effects. Further control can be achieved by control over the discharge of pollutants into land and marine environments (Garcés-Ordóñez et al. 2019).

Biosecurity can be achieved by different approaches to keep the biohazardous elements away from humans, animals, and the environment (Chad et al. 2017). The most effective approach used is the utilization of DNA/RNA molecules to detect the rare and invasive species at different stages to manage the biosecurity (Zaiko et al. 2018). Infectious diseases are caused to humans due to biohazards and biohazardous agents such as parasites, viruses, bacteria, fungi, prions, rickettsia toxins, allergens, venoms, flora, and fauna. The cause of the infection is mainly in the workplace due to the nature of the work carried out, such as for animal breeders, farmers, scientists, the animal lab in charge, ranchers, biologists, farmworker dental workers, health care workers, construction workers, demolition workers, archaeologists, and veterinarians. Possible diseases caused due to biohazardous agents in the workplace are listed in Table 14.3 (Rim et al. 2014; Rom 2007; LaDou et al. 2014; Marks et al. 1997).

The diseases listed above due to biohazard are not only limited to the discussed profession. In contrast, the possibility of the infection's susceptible spread will be related to other professions and other factors.

14.2 Biosensor for Biosafety and Biohazards—Techniques and Applications

A sensor detects some physical quantity and outputs the equivalent electrical output based on the input. Usually, physical quantity can be light, heat, temperature, mass, stress, strain, etc. Typically, MEMS and microfluidic devices also fall into the category of sensors. When the same principle is applied to a biological system, it is termed a biosensor. Here we are going to discuss the detailed applications of biosensors for detecting biohazards. Biosensors are used for the detection of

Table 14.3 Diseases related to biohazards

Adenovirus	Ebola Marburg virus	Marburg virus	Rubella
Aids	Congo	Measles	Sars
Arenaviral infection	Ehrlichiosis	Meningococcus	Scrub typhus
Arenavirus infection	Hantavirus infection	Monkeypox	Southern tick
Associated rash	Hemorrhagic fever	Mumps	Spotted fever
Babesiosis	Hendra	Murine typhus	Tuberculosis
Brucellosis B-virus infection	Hepatitis B	Mycoplasma infection	Tularemia
Campylobacter enteritis	Hepatitis C	Newcastle disease	Varicella
Capnocytophaga infection	Illness (Stari)	Nipah virus	Virus encephalitis
Cat scratch fever	Influenza	Omsk	West Nile virus infection
Colorado tick fever	Lassa fever	Paralysis parvovirus infection	
Crimean	Lcm	Pasteurellosis	
Crimean-Congo	Leptospirosis	Pertussis	
Diphtheria	Lyme disease	Plague	
		Powassan	
		Psittacosis	

different types of biohazards, such as warfare agents detection, food biohazard detection, infectious disease detection, marine contamination detection, and environmental toxins detection. Technology progress has made the scientists from science and engineering work together on new technology such as nanotechnology to develop novel sensors with higher sensitivity for biohazard monitoring. Various biosensors for biosecurity applications are fabricated as thin film-based, nanostructure based, CNT-based, graphene-based, quantum dot-based, and nanomaterial-based (Banerjee et al. 2021). These are generally used as sensing materials for biosafety, biosecurity, and biohazard monitoring.

Biosecurity has a significant role in detecting chemical and biological warfare agents to identify their presence in the environment. Biological agents such as nucleic acid-based and surface detection-based sensors are used to detect warfare agents (Justin Gooding 2006). Wearable biosensors based on microneedle array and paper-based origami functionalized biosensors are used to detect the organophosphorus nerve agents and mustard agents (Arduini 2021). Detecting the chemical and biological biohazards in the air to detect the presence of the warfare agents is very important to safeguard the public. A sensing device made with Au nanoparticles is used to detect the minute concentration of biohazards in the air using surface plasmon resonance and microfluidic technic to detect the nerve gases, toxic proteins, and pathogens such as anthrax (Saito. et al. 2017). In another approach, a planar waveguide array biosensor is used for finding probable biohazards (Rowe-Taitt et al. 2000). Similar to the biohazards detection from warfare agents and the related pollution in the environment, detection of biohazards in food is very important since the intake of food by every living being, including humans, is going to have a more significant impact. Immunoassay-based sensors are used to detect the presence of the staphylococcal enterotoxin B and botulinum toxoid A in canned food

beverages (Sapsford et al. 2005). Salmonella detection in food is carried out by different biosensors such as optical electrochemical and piezoelectric with the advanced nanotechnology-based approach using nanomaterials and microfluidics (Shen et al. 2020).

An infectious disease caused by biohazards is the most critical area of concern since it will cause severe damage to a country's growth due to the spread. Different biosensors are used to identify the infectious diseases caused by biohazards. Broadly classified biosensors are electrochemical, optical, amperometric, potentiometric, light-addressable, conductometric, and piezoelectric (Pejcic et al. 2006). Based on the specific functionality, the biosensors are used with enzyme as a label for biodetection. Similar other types of immunoassay biosensors include gold-label immunoassay sensor and Fe2+/3+ probe, and label-free immunoassay sensor. DNA sensing is carried out by methylene blue probe and osmium complex probe DNA sensors. Immunoassay and DNA sensors are used for the detection of diseases such as bovine viral diarrhea, dengue, foot and mouth disease, forest–spring encephalitis, hepatitis B, Japanese B encephalitis, Newcastle disease, severe acute respiratory syndrome, and Venezuelan equine (Pejcic et al. 2006).

14.2.1 Foodborne Pathogen Sensing

Foodborne pathogens are the agents such as bacteria, viruses, parasites, chemicals, and other foreign particles causing health issues to humans through food products. This is a global problem which may be caused due to the extended sales of the products beyond the expiry date and due to other reasons, such as chemical and biological reactions happening, such as external temperature, packing of the foods, and improper hygiene standard maintained (Justino et al. 2015). Most of the organisms such as Campylobacter spp., Escherichia coli, hepatitis E virus, Listeria monocytogenes, Norovirus, Salmonella spp., and Staphylococcus aureus are responsible for foodborne illnesses. To avoid foodborne illnesses in humans, it's mandatory to detect the pathogens in food with a sensor, which is termed as foodborne pathogen sensor (Velusamy et al. 2010). Various research groups have been working on different types of foodborne pathogen detection sensors to detect the microorganisms present in foodstuffs. Figure 14.2 represents the pathogen detection scheme and the different types of biosensors based on the principle of operation. Broadly, the biosensors for pathogen detection are classified into three types such as antibody, aptamer, and enzyme. Aptamer-type biosensor is classified based on the transduction mechanism such as optical, electrochemical, and mechanical based. Further optical transduction biosensors are surface plasmon resonance type and surface-enhanced Raman spectroscopy. Similarly, electrochemical biosensors are amperometry, conductometric, and impedimetric. Mechanical-based transduction biosensor is mostly cantilever based, where the detection will be based on the change in mass.

Array-based detection schemes made of low dimensional structures such as quantum dots and nanorods are adopted in the detection of foodborne pathogens

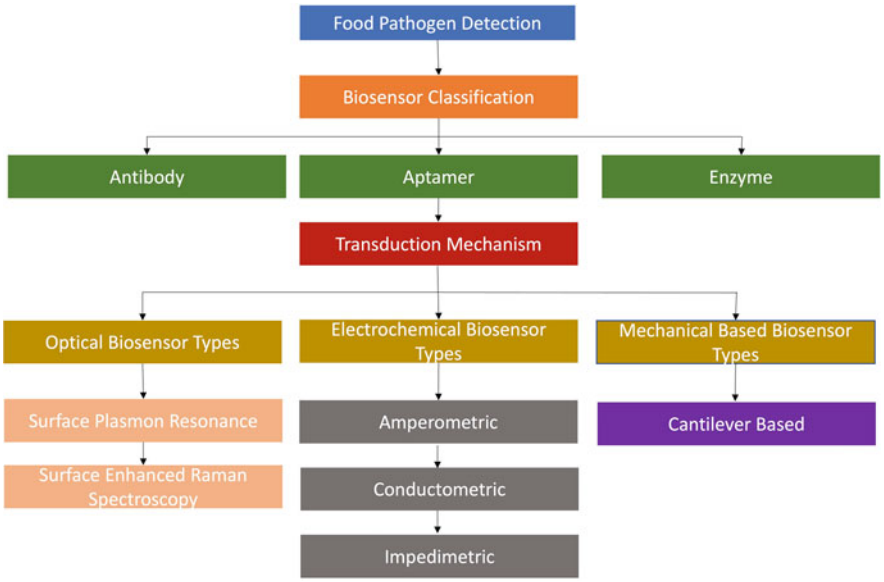

Fig. 14.2 Food pathogen detection sensors classification

due to their high sensitivity, speed of detection, and cost-effectiveness (Foddai 2020; Senturk et al. 2018). Fig. 14.2 shows the detection principle of a biosensor categorize its type as optical, mechanical and electrochemical. Optical principle is applied when the binding particle allows the changes in surface property by change in illumination or color. Mechanical principle aids detection based on the mass of the particle. Similarly, when the particle's chemical reaction allows the electrical output then the pathogens can be detected by the electrochemical sensors (Senturk et al. 2018; Zhang et al. 2019a; Narsaiah et al. 2012; Massad-Ivanir et al. 2013). Salmonella typhimurium foodborne pathogen detection was done with the piezoelectric biosensor having a limited response time for fast detection with very high sensitivity depending on the protein level in the antibody solution. Higher protein allows increased binding and sensitivity when compared to the low protein rate (Pohanka 2018). Foodborne pathogen detection using electrochemical biosensor is used due to the cost-effective and faster operation, which normally have bio-detection through physical and chemical methods. The biomolecule to be detected will be identified by the electrodes and the electrodes convert the detected signal from the bio analyte into electrical output to analyze the parameters of the analyte. Foodborne pathogen Salmonella is detected using an electrochemical sensor with the modified graphene electrode and electrolyte to detect the changes in the property of the salmonella by observing the changes in the electrical parameters. Sensitivity and the response time of the foodborne pathogen sensor are recorded to be good with the modified graphene electrode (Ye et al. 1997; Amiri et al. 2018; Ma et al. 2014a). Foodborne

pathogen detection is based on the bio analytes, such as antibody based, nucleic acid based, phage based and mammalian cell based (Luping et al. 2021):

- Biosensors based on antibody interactions
- Nucleic acid-based biosensors
- Phage-based biosensors
- Mammalian cell-based Sensor

Most of the analytes tend to get attached to the nanoparticles or nanostructures in the fabricated sensor. The attachment of the bio analyte will be based on the adhesion nature, which depends on the bonding. Hence with the above approaches, various biosensors are used for the detection of foodborne pathogens. Based on the detection scheme and the sensing principle, the biosensor is classified as a particular type of foodborne pathogen biosensor.

14.3 Biosensors for Chemical Warfare

Chemical and biological warfare is the most recent destruction method followed by most of the countries without a need for massive military weapons to get involved in the war field. Chemical warfare is more hazardous than the existing weapons causing extremely treacherous health hazards such as organ damage (causing slow death)

Table 14.4 Chemical warfare agents

Agents	Chemical used	Health effects
Blister agents	Sulfur mustards	• Damage skin, lungs, and eyes • Immediate effects of blistering agents involve burning sensations in skin and eyes
	Nitrogen mustards	
	Lewisite (L)	
Nerve agents	Organophosphorus compounds	• Sweating and muscle twitching. • Loss of consciousness.
Asphyxiants/blood agents	Cyanogen chloride (CK)	• Shortness of breath • Sudden death
	Hydrogen cyanide (HCN)	
	Arsine	
Choking/pulmonary damaging agents	Chlorine (cl)	• Permanent damage of lungs and heart damage
	Chloropicrin (PS)	
	Phosgene (CG)	
Behavioral agents/ incapacitating agents	Lysergic acid diethylamide (LSD-25)	• Temporary disability
	Ketamine	
	Carfentanil Glycolate anticholinergics	

and sudden deaths to the people on a whole, resulting in mass population ill effects and death. Different types of agents are used in chemical warfare as illustrated in Table 14.4. Toxic gases have been used as agents in wars previously by various countries as weapons for mass destruction (Ma et al. 2014b). The properties of each of the agents used in chemical warfare vary based on the nature and composition of different elements in it.

The nervous system could be affected by nerve agents. This could have a severe impact on the nerves and internal organs depending on the intensity of the chemical. Similarly, the blistering agents will have a severe effect on the body surface such as skin causing irritation and burning sensation, which may lead to very severe lung and respiratory illness when inhaled. Whereas the choking agents will have adverse effects on the lungs, throat, and nose resulting in death due to the low oxygen level. Blood agents affect the oxygen levels in blood, resulting in unconsciousness, and eventually leading to death due to oxygen deficiency. Some agents also cause psychological problems resulting in mood swings and changes in thoughts when they get into the human body through the nose or mouth, causing the change in the nervous system. Most of the toxins available in nature are produced by microorganisms such as bacteria, fungi, and other microorganisms, which are classified by action, molecular weight, and chemical composition (Diauudin et al. 2019; Chauhan et al. 2008). Agents not only damage the living beings but will also have ill effects on the environment causing serious damages to the ecosystem and the people and cattle living in the proximity (Karalliedde et al. 2000). Most of the damage not only limits itself to the humans but also disturbs the livelihood of the place by affecting the water resources which indirectly affects the pets, animals, and cattle. A few examples of the chemical warfare agents and associated health effects are listed in Table 14.4. Henceforth the need for biosensors to detect the warfare agents are more important and plays a crucial role.

Recent research progress based on the multidisciplinary approach has resulted in novel biosensors, which can detect these nerve agents. Efficacy of some biosensors for detecting nerve agents are highly sensitive and cost-effective (Ganesan et al. 2010; Arduini et al. 2016). Broad classification of the biosensors for detection of warfare agents includes the principles such as optical, electrochemical, and piezoelectric (Pohanka 2019). Photosynthetic fluorescence induction method based chemical warfare detection biosensor detects the warfare agents such as mustard, sarin, tabun tributylamine, and dibutyl sulfide, which were used as test agents for biosensor detection (Sanders et al. 2001). Multiplexed biosensor systems to detect multiple agents, which includes nerve gases at the same time, were carried out with electrochemical-based detection techniques with the detection time of 15 minutes in real-time (Pitschmann et al. 2019). Chromogenic chemosensor based on paper with cellulose saturated dithienobenzotropone detects the blister agents with the change in pH value (Saito et al. 2018). Based on the detection principle and structure, various biosensors are used for the detection of warfare agents as listed in Table 14.5 (Khanna 2008).

Table 14.5 Biosensor warfare agents detection based on sensing principle

S. no	Principle of detection	Biosensor type
1	Optical	Surface plasmon-absorption-based colour-change sensors
		Gold nanoparticle-enhanced surface-plasmon resonance sensor
		Fluorescence-quenched sensors
		Fluorescent polymer-coated carbon dot sensors
		Immunoassays using CdSe-ZnS core-shell quantum dot fluorescent biosensors
		Nanoparticle-modified fluorescence of enzyme inhibitor
		Nanoparticles of different shapes and nanoprism sensors
		Raman integrated tunable sensor coupled with surface enhanced Raman scattering substrates
2	Magnetic	Magnetic nanoparticle-based sensor
3	Electro-chemical	Zirconia nanoparticle-based sensor
4	Electrical	Nanowire- or nanotube-based FETs
5	Chem resistor	Metal oxide nanoparticle-based chemiresistor sensors
		Metal nanoparticle-based chemiresistor sensors

Each of the principles of detection listed in the above table has sub-classification of sensors to be used in the warfare agent's detection. Optical sensors work on the principle of optical detection by a color change and intensity change. Magnetic nanoparticle-based sensors detect the magnetic-based interactions on the agents, which will be based on different magnetic nanoparticles. Similarly, other types such as electrical, electrochemical, and chem resistor sensors detect warfare agents based on their operating principles.

14.3.1 Infectious Diseases Sensing

The present Corona virus disease, COVID pandemic, has been a greater challenge for the communities around the world which has a greater impact on the economy and life of the people. Since this infection has affected every individual on the planet, detection of the disease allows control over the further spread of the virus. Hence a biosensor would be much in need to detect the virus, as we are aware that the Reverse transcription polymerase chain reaction (RT-PCR) test was carried out all around the world to detect COVID. Beyond RT-PCR, there has been a lot of sensors proposed by various research groups for the detection of the virus. Multiplexed biosensors detect the exhaled breath with attached hybrid nanomaterials to sense the presence of the virus (Shan et al. 2020). Field Effect Transistor-based biosensor is used to detect the virus from the human nasopharyngeal swab (Seo et al. 2020). The respiration monitoring system was developed to identify the presence of the virus by monitoring the change in the respiration and comparing the recorded respiration with the normal respiration (Chen et al. 2021). Similar to the reported sensors to detect

COVID, there have been various sensors proposed by different research groups around the world to have control and monitor the disease (Rodovalho et al. 2015). Numerous infectious diseases and their detection has been very important to have a safe life; hence different types of sensors were fabricated and tested for various infectious disease detection through a straightforward approach (Sampath and Ecker 2004). Effective methods to detect the pathogens include ELISA and PCR due to their sensitivity and effectiveness. Transduction elements give the quantitative and semi-quantitative with the usage of extra processing steps (Cesewski and Johnson 2020).

Graphene quantum dot and gold polyaniline nanowire-based electrochemical biosensor was used for the detection of hepatitis E virus (Chowdhury et al. 2019). Screen printed microelectrodes and functionalized glucose oxide polymer nanocomposite were used for the detection of E. coli. The sensitivity and specificity of the fabricated sensor were very good (Xu et al. 2016). Pathogenic bacteria in air were detected with the Au nanoparticle-based piezoresistive biosensor to check the infectious disease spread through the environment (Zheng et al. 2019). Tuberculosis detection was carried out with the piezoelectric principle using Au nanoparticles. The sensitivity of the fabricated sensor was very good to detect the pathogenic microorganism from 5 pg limit (Kaewphinit et al. 2012). There have been various sensing methods adopted for the detection of infectious diseases based on the sensing principle and the materials used for the fabrication of the sensor. Better dynamic range should be considered when fabricating a sensor for disease detection (Liu et al. 2018). The dynamic range of a biosensor for disease detection varies based on its type (Rogers et al. 2016). The dynamic range of the sensor will also vary based on the type of sensing principle such as electrochemical, piezoelectric, and optical, respectively (Zhang et al. 2019b; Saylan et al. 2019; Senveli and Tigli 2013). Based on the sensing principle and the device structure, the sensor types used for the infectious disease detection is listed as follows:

- Electrical Impedance Spectroscopy (EIS)
- Nanowire FET
- Mechanical
- Surface Plasmon Resonance
- PC
- μNMR
- Nanopore
- SAW

Types of biosensor for infectious disease detection includes optical, mechanical, electrochemical and electrical. When low dimensional structures with a size of 1–100 nm such as nanorods, nanowires, quantum dots, nanotubes, nanocubes, and nanospheres are used for the sensing application, then those type of sensors are termed as nano biosensors.

14.4 Conclusion

We are living in an era of uncertainty and change because of the emergence of new biohazardous materials. These materials are a class of naturally occurring, bioengineered, or synthesized components of any microorganism or infectious substance, capable of causing fatality, disease, or other biological issues; deterioration of food, water, equipment, supplies, or material of any kind; and detrimental reformation of the environment. Research on new materials associated with biosafety, biohazards, and biosecurity has expanded. Biosafety and biosecurity architectures illustrate practices, training, and safety equipment to safeguard the environment from unconsidered exposure or unintended release of dangerous agents and contagions. Despite the striking improvements, a notable amount of research is needed to understand the infectious agents and toxins related to biosafety and biohazards. In this respect, the researchers and the scientific community may profit from past teachings to predict potential problems. Implementation of the understanding of biosafety and biohazards and the use of suitable techniques and equipment will facilitate the research and scientific community to avert personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. Handling pathogenic microorganisms needs a comprehensive understanding of the containment methods and procedures. Applying this knowledge, techniques, and equipment will enable the scientific community to address biosafety- or biosecurity-related issues.

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Commercial Aspects and Market Pull of Biosensors in Diagnostic Industries

15

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Abstract

The extensive advancements in the micro-manufacturing process have led to the development of enormous miniaturized technologies for personalized care and health management. Several technologies have been reported for saving the environment and food from getting spoiled based on miniaturized devices across the globe. Moreover, the growth of the biosensor industry has tremendously affected the market pull of sensing devices around the world. In this chapter, we have discussed various such aspects of the biosensor development process in context to their mass manufacturing feasibilities and their commercial aspects. Herein, we have also discussed limitations and various challenges associated with the miniaturized biosensors in terms of commercial acceptance. Apart from this, we also provide the SWOT (strengths, weaknesses, opportunities, and threats) analysis of the various types of contemporary biosensors.

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P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_15

Keywords

Biosensors fabrication · Micro-manufacturing · Market-pull of sensor industries

15.1 Introduction

Early diagnoses of any disease always aid in the proper treatment of a patient, which eventually assists in improving health in minimum time (Mahato et al. 2018a; Kumar et al. 2019b). On-time detection of pathogens, lethal materials, chemicals, and metabolites plays a vital role in contending the disease progressions (Kumar et al. 2019d; Purohit et al. 2019a). Although being powerful tools, yet the conventional technologies used in the diagnosis lack various advantages to be applied for onsite applications (Mahato et al. 2016). The disadvantages, such as slow diagnosis, sophisticated machinery, skilled worker requirement, large sample requirement, and lack of miniaturization, hamper the onsite diagnosis and point-of-care applicability (Mahato et al. 2018d, c). Moreover, on-time diagnosis not only helps the health workers to act within the stipulated time but also aids in rapid testing during epidemic and pandemic crises (Mahapatra and Chandra 2020). Besides, the biosensors industry, a new domain of the diagnostic industry, provides an opportunity to overcome these limitations. In general, a biosensor is known as an analytical device with receptors and transducers quantifying the physiochemical reactions in a decipherable signal (Prasad et al. 2016b; Mahato et al. 2018d). Usually, a biosensor aims to generate digital/electronic signals that are in proportion to biochemical reactions occurring during diseased/physiological conditions (Kumar et al. 2018a). The leading advantages of biosensors, which make them dominant over conventional diagnostic approaches such as minimum sample requirement, rapid analysis, and robustness, make them more anticipated for marketable acclamation (Mahato et al. 2018d).

Currently, biosensors are accelerating their prospect by challenging numerous health complications by providing an accurate and selective analysis (Mahato et al. 2018b; Purohit et al. 2019b, 2020a, b, c; Kumar et al. 2020a). Yet, there are several challenges, such as narrow detection range, less sensitivity, and stability, which are needed to be fixed to reinforce the performance quality of conventional biosensors. To address these issues and challenges, several works have been done with the help of nanotechnology and material engineering that not only aid in the fabrication of robust biosensors but also enhance the generated signal (Kumar et al. 2019c; Mahato 2019; Mahato et al. 2019a). In recent decades, the expansion in interdisciplinary research paved the numerous approaches for robust biosensor fabrication, and due to the improvement of nanotechnology, significant milestones have been attained in the area of biosensor design (Kumar et al. 2018b; Mahato 2018c; Mahato et al. 2020a). Fabrication of the biosensors with nanomaterials provides a practical and capable solution for addressing the abovementioned limitations associated with conventional biosensors (Baranwal et al. 2016; Prasad et al. 2016a). The extraordinary mechanical and optoelectronic properties of nanomaterials can offer a firm and robust assembly

of bio-receptors on a sensing platform (Mahato et al. 2019a). Moreover, nanomaterials provide a higher surface area to interact during biochemical reactions while biosensing. Therefore, the development of nanomaterial-based biosensors has gained momentum in research and development as well as sensor industries (Kumar et al. 2015, 2019a; Kashish et al. 2017; Bhatnagar et al. 2018; Mahato et al. 2019b, 2020c, 2021; Purohit et al. 2020b).

The biosensor industry, which was originated from the research carried out by Clark and Lyons in 1962 (Clark Jr. and Lyons 1962), found its way toward mediator-based sensing to improve the interferences from electroactive interfering molecules such as uric acids and neurotransmitters (da Silva et al. 2018). The well-designed techniques developed by early researchers formed the platform for the fruitful commercialization of Medisense's glucose testing pen (Spivey et al. 1988). Further, in the subsequent decades, researchers across the globe developed various biosensors for numerous applications for healthcare and food industries, such as cholesterol diagnosis, blood glucose testing, pregnancy kits, milk and meat quality analysis in food industries, and drug analysis (Buerk 2014). These applications have paved the way for the biosensor to play an influential systematic role in drug, farming, food safety, homeland security, bioprocessing, environmental and industrial monitoring, which are expected to impact the biosensor's market positively (Shukla and Suneetha 2017).

Moreover, the development of cutting-edge technologies in micro-manufacturing industries has further boosted the possibilities of biosensor industries (Nesaei et al. 2018). Also, the development in the micromachining fabrication techniques in the last few decades eventually caused the growth as well as mass production of electronic chips and helped the biosensor industry to grow in the commercial market (Cai et al. 2018). The new fabrication technique, such as laser-based micromachining, offers precise designing of chips and rapid production of electronic components quantitively over the traditional chip-making approaches (Fischer et al. 2020). With the advancement of such technology and manufacturing techniques, there has been an increase in unconventional biosensors such as nanomechanical/piezoelectric and wearables; however, the biosensor industry is mainly dominated by electrochemical, paper-based, and optical biosensors, as shown in Fig. 15.1.

In view of such advances and possibilities in the area of biosensor industries, this chapter has been documented to provide a glimpse of current business maneuvers in biosensor industries. Stress has been given not only on biosensor designing but also on the factors that can influence the market pull of the biosensor industries across the globe. Expectantly, the chapter will motivate further consideration in the way of biosensor designing and marketability. Importance has been given to validate the current status of biosensors in the development of doorstep diagnosis. In order to deliver a better understanding between researchers of interdisciplinary areas, the current trend and future perspective of biosensor industries have been explained.

Regardless of the exact research or area of study, developing a marketable technology to better healthcare, economy, and society is at the core of interdisciplinary research. Thus, there is a requirement for understanding the business viability associated with the biosensor industries, which will eventually enhance the research

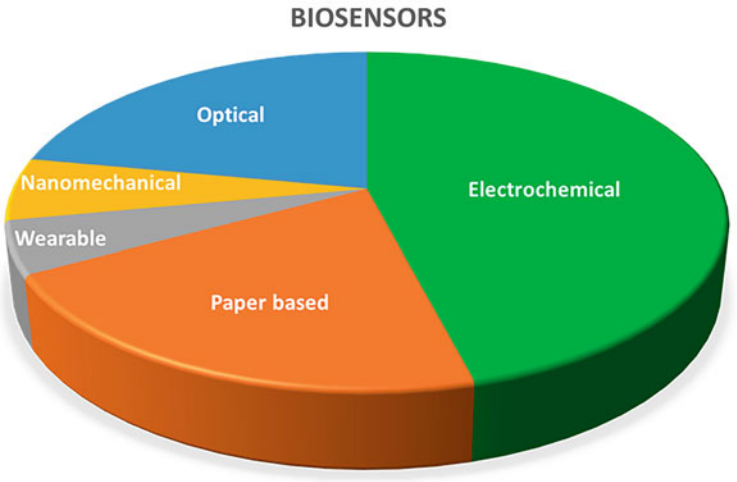

Fig. 15.1 Market share of different types of biosensors in 2021

quality and aid in the development of transferable technology for healthcare, farming, and environment monitoring.

15.2 Factors Influencing Market Pull of Biosensors

The successful biosensor must have enough versatility that can support substitutable receptors, miniaturization capability that permits computerization, and ease of operation at a modest cost. Additional anticipated features comprise automated, regular, and onsite sensing of multiple as well as complex analytes. These features are going to attract the market of biosensors (Luong et al. 2008). Further, the marketability and commercial viability of any product primarily depend upon two factors: (a) demand of the product and (b) supply of resource materials. The market pull of biosensors is also very much dependent upon these parameters, which are influenced by other factors and reasons. Figure 15.2 shows the major factors influencing the market pull of biosensors in diagnostic industries, which are explained as follows.

15.2.1 Increase in the Geriatric Population

The global human population is rising at a tremendous pace, which is around 83 million annually or by 1.1% every year (Roser et al. 2013). According to United Nations estimations, the total population of the world will become 8.6 billion by mid of 2030, 9.8 billion by mid of 2050, and 11.2 billion by 2100. This population growth is not only because of the increment of newborns but also due to the increased life span of older people (Simon 2019). Life expectancy has been steadily

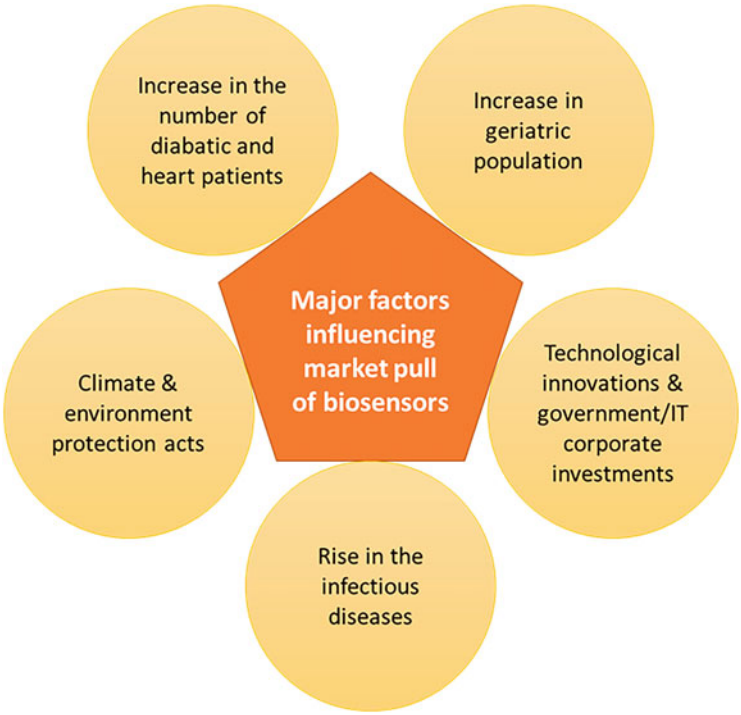

Fig. 15.2 Major factors that influence the market pull of biosensors in diagnostic industries

increased two-fold in the last two centuries. Initially, the increase in the population was driven by reductions in infant mortality because of advancements in medical facilities for infant care and childbirth (Coale and Hoover 2015). But, since mid-1950, the main driving force for population increment has been reductions in old-age mortality (Janssen et al. 2004). Old age is often coupled with diseases, which include osteoarthritis, chronic obstructive pulmonary disease, cataracts, loss of hearing, joint pain, hypothyroidism, depression, anxiety, dementia, and diabetes. Further, older people experience many symptoms together as they age. Also, older age is associated with the appearance of numerous multifaceted health conditions that are inclined to happen only in the later stage of life. These conditions do not fall into discrete diseases and are termed geriatric syndromes, which are caused due to several underlying factors such as fragility, urinary incontinence, falls, delirium, and ulcers (Monroe 2013). Geriatric syndromes seem to be better forecasters of death than the occurrence of specific diseases. Although, old-age mortality is highly reduced because of the availability of high-end sophisticated lifesaving medical instruments such as pacemakers, ventilators, cardiac defibrillators, etc. Yet, the availability of these high-end instruments is limited only in metro cities and big superspecialist hospitals. Also, the proper diagnosis of any disease within stipulated time highly affects the survivability of old age patients. Therefore, point-of-care

devices that can be employed onto the remote site and can be operated by the common man are highly desirable. This need is the root cause and source of motivation for recent inventions in electronics and wearable biosensors. Also, as per the 2018 report by the WHO, the worldwide population aged 60 years and more is likely to range to 2 billion by the end of 2050 (Rafalimanana 2020). In addition, the increasing older population in Asian countries such as Japan and China—with primary unexploited opportunities—is anticipated to drive the market for biosensors in upcoming years. As per the 2018 World Bank study, it was anticipated that more than 20% of the inhabitants in Japan were above 65 years, which is making it one of the top states with an elderly population (World Bank 2019). Since the old residents are extremely vulnerable to chronic illnesses, they require routine examinations and continuous monitoring. This, in turn, is anticipated to be a major reason to drive the biosensor market growth over the upcoming years. The biosensor industries associated with the fabrication of devices that can be applied for the diagnosis of old age diseases have better scope in biosensor market, which has been highly enhanced due to growth in geriatric population.

15.2.2 Increase in the Number of Diabetic and Heart Patients

The application of biosensors is rapidly growing in developed and developing countries, for instance, in the USA and Canada, there has been an increase in the number of lifelong illnesses such as diabetes and heart conditions (Shields et al. 2011). These health conditions are mainly caused by unhealthy routines and junk foods, which are responsible for obesity and overweight among youth and young children. Diabetes or hyperglycemia (an elevated level of glucose in the blood) is a greatly affecting disease found among various developing and developed countries. It has been categorized into two major groups, inadequate insulin creation either in the body (Type 1 diabetes) or by the inability of the body to utilize its produced insulin (Type 2 diabetes) (Chase and Maahs 2006; Frier and Fisher 2007; Danaei et al. 2011; Shaw and Cummings 2012). Further, according to the National Diabetes Statistics Report 2020, nearly 34.2 million persons in the USA alone were identified with diabetes in the year 2018, and 88 million individuals have shown prediabetic symptoms. Also, according to the International Diabetic Federation (IDF) and WHO, almost 350 million people globally have diabetes, and based on current predictions, diabetes will be the seventh leading reason for death as of 2030 (Oliver et al. 2009; Alwan 2011). Both precise observations as well as careful control of blood glucose are critical for the suitable diagnosis and treatment of diabetes. Accordingly, consistent testing of physiological glucose levels is important to avoid clinical emergencies such as hypoglycemic (deficient blood sugar levels) shock and prevent long-term complications from high-level blood glucose, including heart attack, stroke, high blood pressure, kidney failure, blindness, and limb amputation (Chase and Maahs 2006; Frier and Fisher 2007; Shaw and Cummings 2012). Accordingly, the collective burden of diabetes stresses more frequent blood testing. Although the current biosensor market is highly regulated by electrochemical

glucometers, in the future, the demand will upsurge as the number of diabetic individuals is going to increase in the upcoming days. The expanding incidence of diabetes as specified by the IDF is around 8.0%, yearly approximating almost 590 million individuals to acquire diabetes till 2035 (Edition 2017), thus encouraging considerable development of the biosensor market in upcoming decades. Moreover, most diabetes monitoring devices can be operated without the help of any trained person. Therefore, owing to their capability to evaluate health conditions and monitor diabetes onset and progression, the modern biosensing glucometers can have the potential to pull the biosensing market and, hence, are anticipated to enhance the market growth over the next decade (Yoo and Lee 2010).

15.2.3 Climate and Environmental Protection Acts

The rapidly increasing global warming and harsh effects of environmental pollutants in air, soil, and water are causing several chronic diseases among the vast population. The wastes and effluents from the industries often cause air, soil, and water pollution by adding undesirable and toxic chemicals into them. Also, there are several reports in which numerous individuals got sick because of heavy elements present such as Pb, As, Hg, etc. in soil and water resources (Vareda et al. 2019). Overexposure to these pollutants may cause pain, wakefulness, confusion, seizures, and life-threatening complications. Heavy metal poisoning is one of the main reasons behind neurological symptoms such as neural encephalopathy and peripheral neuropathy. Also, there have been several pulmonary diseases caused by these pollutants. To overcome these conditions, there is a need to closely monitor the soil, water, and air to ensure quality (Ali et al. 2019). Numerous governments, as well as non-government agencies, are working in Asia, America, and Europe to assess the quality of these resources. Moreover, several countries such as the USA, Canada, India, and the UK have formulated several laws to ensure the quality of these resources such as the Clean Air Act, Water Act, National Environmental Policy Act, Environment Protection Act, Air (Prevention and Control of Pollution) Act, etc. (Hernandez et al. 2010). Although, there are several methods to check the quality of these resources such as chemical titration, satellite imaging, and climate modeling. Yet, they lack spontaneity and often require time-taking protocols and trained personnel. Also, the simulated models based on image processing and coding can provide less accurate results in terms of quantification of pollutants in the air, soil, and water resources. Alternatively, biosensors can provide a better possibility for the accurate detection of pollutants. There have been several reports, where a biosensor was designed to assess the presence of heavy metals in water and soils, and the ability of biosensors to be applied onsite further supports the usage of biosensors for such type of environment monitoring (Rodriguez-Mozaz et al. 2005). Therefore, the laws constituted by the governments to continuously monitor the environmental resources also support the serious usage of biosensors in this domain (Patil et al. 2019). Further, the increase in global warming and emissions of greenhouse gases is going to impact the implementation of these environmental laws (Victor 1999). It is

anticipated that these laws are going to offer the market for biosensors with profitable growing chances in the upcoming decades.

15.2.4 The Rise in the Infectious Diseases

According to the report of WHO that was published in 2007, the world was warned of the emergence of infectious diseases at an alarming rate. There have been almost 40 infectious diseases that emerged since the 1970s, which include Zika, Ebola, Chikungunya, Swine flu, bird flu, severe acute respiratory syndrome, Middle East respiratory syndrome, and recently emerged Novel Coronavirus Disease (COVID-19) (Smith et al. 2014; Lafferty and Mordecai 2016). These diseases have the potential to spread quickly and their ability to become pandemic has caused a global catastrophic impact. Also, because of the population explosion in developing countries such as China and India, the chances of these diseases mutating into new forms are inevitable (van Oosterhout et al. 2021). Moreover, the rise in infectious diseases and their spread is threatening the worldwide economies and human lives, where several countries are still fighting for the repeated re-emergence of some of these epidemics. The freshly erupted COVID-19 has left the world bewildered, and even after more than 18 months, it has been affecting societies and economies across the globe, as several countries are still fighting the spread of the disease. Scientists are tirelessly probing for the key reasons accountable for this disease, standing against this condition (Mahapatra and Chandra 2020).

The catastrophic impact of these diseases on the economy and human lives should help as a reminder to be well organized to the arrival of identified and unidentified pathogens in the future. Therefore, the identification of these diseases before the pandemic is the core of the preparedness to control them. Quick detection and POC diagnosis of the infectious pathogens is not only a necessity to check the disease spread but also minimizes the challenges associated with the action plans of the healthcare sector. Detection and handling of infectious diseases are the main objectives of all community health agendas. Exclusive operative approaches of diagnoses are paramount in avoiding or mitigating the infectivity of a pathogen before the consequences make an impression on humanity (Holmes et al. 2017). Most of the diagnoses of these diseases are based on the real-time reverse transcription-polymerase chain reaction (RT-PCR) tests. Although, it is very accurate, it is not so fast in providing the results and is costly in operation. Even in the case of COVID-19, the rapid analysis of the disease is performed with the lateral flow-based antigen kits (Mahapatra and Chandra 2020). With the increasing demand for testing, several diagnostic devices are based on lateral flow techniques because of the market demand. The emergence of infectious diseases has positively impacted the market of biosensors. Henceforth, the international biosensor market for diagnoses of infectious diseases is anticipated to rise considerably in the upcoming years.

15.2.5 Technological Innovations and Investments from Government/IT Corporations

Technological innovations such as the Internet of Things (IoT), artificial intelligence (AI), machine learning, etc. are going to vastly influence the living standard of individuals. The industrial improvement in information technology to mechanize the progressions and data transfer wirelessly without human intervention has given rise to the formation of IoT. There have been serious applications of these innovations in the management of health care devices. Also, rapid technological progressions have led to the promotion of state-of-the-art product launches that are expected to fast-track the market progress in the upcoming years (Tushar et al. 2018; Jain et al. 2021; Javaid and Khan 2021). For instance, Medtronic, a company associated with the design and development of innovative health care devices, announced the launching of two onsite 780G-automated insulin delivery pumps, which can monitor diabetes and deliver insulin to the body (Collyns et al. 2021; Lockhart and Smith 2021). Similarly, the National Institute of Biomedical Imaging and Bioengineering, Maryland, has designed a miniaturized brain sensor that can record and transmit data wirelessly, through which activities of the brain can be monitored remotely (Dangi et al. 2018; Yu et al. 2020). These technological advancements can drive the biosensor market in the upcoming days.

Correspondingly, the IoT-based health care devices that are networked with the hospital server can provide onsite medical monitoring as well as preliminary treatments, simultaneously. The AI-based image processing diagnostic devices have paved the way for detecting life-threatening disorders within a stipulated time. Also, with the help of machine learning, new biosensing devices are being designed that can assess the severity of disease before severe conditions. The technological innovations in sensing technologies are going to bring the hospital to the doorstep of individuals. These new technologies are anticipated to drive the market for biosensors in the upcoming period (Jain et al. 2021).

Further, governmental initiatives intended to support the growth of genomics and proteomics centers are expected to fuel the market pull of biosensors in upcoming years. For instance, the Australian government promoted the research associated with data mining, networks, and embedded system through National Information and Communication Technology Australia. Likewise, the Chinese government is also funding seriously its computing networks started by the Chinese Academy of Sciences. In the same way, the China National Grid is getting support from the Chinese Ministry of Science and Technology. Similarly, the Department of Biotechnology, India, heavily funded the project associated with the development of diagnostic devices for COVID-19 (Morales-Narváez and Dincer 2020). Furthermore, most information technological (IT) corporate giants such as TCS, IBM, and Infosys are also heavily capitalizing on the biosensors market after COVID-19 (Paul 2021). Therefore, such promising initiatives are anticipated to have an optimistic effect on the biosensor market.

15.3 Biosensors' Market Pull Based on Sensing Strategy

Commercial feasibility will depend on the versatility and inexpensiveness of a biosensor for a variety of applications. Different application often requires different strategy during sensing. At present, the commercial biosensors are of the following types based on the sensing strategy adopted.

15.3.1 Paper-Based Biosensors

The paper-based biosensors provide an affordable diagnostic platform by using cheaper raw materials for the fabrication of devices (Mahato et al. 2017). The large-scale fabrication of the paper is attained by the processing and reprocessing of raw materials obtained from bamboo woods, jute, sisal, cotton, etc. The processing of raw materials is very much required to remove the impurities associated with lignin, which can cause difficulties in device fabrication due to its intrinsic fluorophores properties (Mahato et al. 2020b). Most of the paper-based biosensors have been designed using chromatographic and filter-grade papers because of the lignin-free cellulose mass. Also, quality paper processing is required to attain improved mechanical and chemical properties, such as tensile strength, stability, and the ability to functionalize receptors through flanking groups. The paper is a cheap raw material, which can provide a platform for the design and development of cost-effective biosensors.

Interestingly, the paper-based sensors have obtained a great progression in the last few years, spreading from the development of the unsophisticated dipstick kind testing strips to the origami-based multifaceted biosensors. The incorporation of higher intricacy has empowered paper-based sensors for handling multi-step sample purification and multi-analyte detections (Mahato et al. 2021). Also, the integration of the peripherals has converted these paper-based sensors to be more precise and capable of providing qualitative as well as quantitative detections. Nowadays, paper-based biosensors have been greatly applied for the diagnoses of numerous diseases using origami-based modules. The majority of paper-based biosensors are in the format of dipsticks, lateral flow devices (LFD), and μPads (Mahato et al. 2020b, 2021). The market pulls of paper-based biosensors can be estimated by the sale of pregnancy test kits. Almost all of the pregnancy test kits manufactured across the globe are based on LFD-based technologies. The increasing number of the global population and sexual awareness among youths can positively impact the sale of these biosensors in the upcoming decades (Hillscher et al. 2021). Figure 15.3 shows the increase in the growing research of paper-based biosensors across the globe.

15.3.2 Electrochemical Biosensors

In the last decade, numerous advances were achieved in terms of microfabrication methods, material engineering, and electronic chip fabrication. This progresses

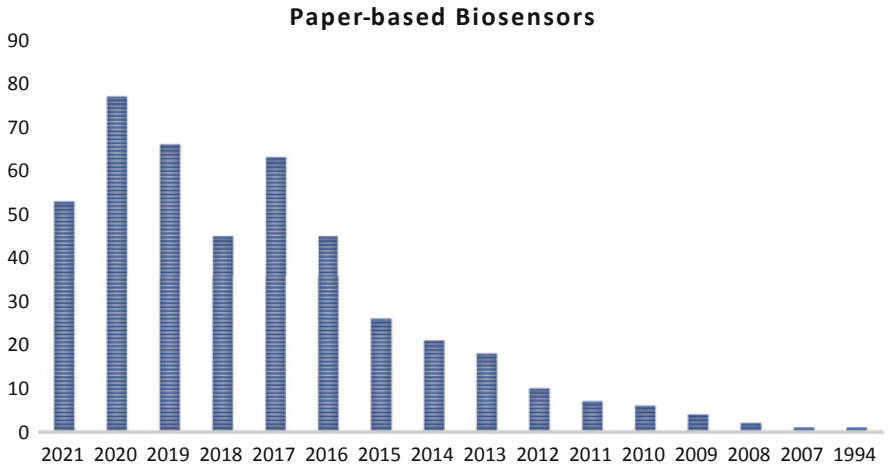

Fig. 15.3 Increase in the growing research of paper-based biosensors across the globe (Source: Scopus)

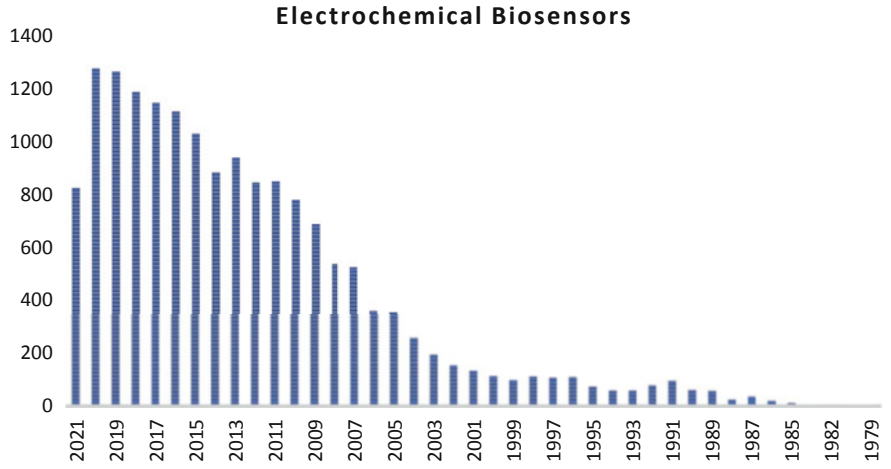

Fig. 15.4 Increase in the research in electrochemical biosensors (Source: Scopus)

simplified the manufacture of tiny, portable, and inexpensive electrochemical biosensors (da Silva et al. 2018). There has been an increase in the research on electrochemical biosensors across the world, as shown in Fig. 15.4, which indicates the increase in the publications on electrochemical biosensors. The incorporation and miniaturization of electrochemical reaction units were essential for the designing of portable diagnostic devices, which helped to overcome the challenges associated with large-scale manufacturing plants. In other types of biosensors such as optical biosensors, the signal generation in the sensing system is highly governed by the electron transfer associated with the color change or fluorescence originated because

of charge transfer during physiological reactions (Romanholo et al. 2021). Unlike this, in the electrochemical biosensors, the signal is often generated by the capture of electrons released during redox reactions, which is amplified using an electrochemical device.

Furthermore, it is easy to functionalize sensing electrodes precisely planned to address the detection of specific analytes (Kumar et al. 2020b; Mahato et al. 2020a).

Subsequently, the production of planar integrated circuit design technology has developed with widespread growth because of the development in micromanufacturing. Also, the electrochemical biosensors can achieve the lowest detection limits compared to other biosensors. This feature has made this sensing method a most striking technology in terms of costs and miniaturization. So, contemporary electrochemical-based biosensing technology showed its fortitude to satisfy the stresses of the biosensors market (Hammond et al. 2016).

The role of electrochemical sensors in the biosensor market can be estimated by the sale of glucometers across the globe, which are mainly electrochemical. Also, the market pull for electrochemical biosensors is anticipated to enhance at a compound annual growth rate (CAGR) of 9.7% between 2016 and 2022. Also, there is a possibility that the market size can achieve a $23707.2 million market value by the same time (Luong et al. 2008; Shukla and Suneetha 2017). At present, the market of electrochemical biosensors can be primarily categorized in diagnostic and monitoring applications. It is anticipated that the market size of electrochemical sensors can reach nearly $33 billion by the end of 2027. Further, the test strip, utilized in electrochemical sensors, is projected to capture 35% of the market (Jain et al. 2021). In addition, the attention in wearable electrochemical biosensors for the nonstop monitoring of biomarkers, united with the computerized wireless data communication, signified a substantial step forward in the sensing area toward innovative market potentials.

15.3.3 Wearable Sensors

Although at present, non-wearable sensors are dominated in the foremost of the market share, with current progressions in wearable technologies, it is anticipated that wearable sensors will develop as fast-rising devices (da Silva et al. 2018). The development of non-invasive or minimally invasive wearable sensors for onsite and nonstop monitoring has been getting huge consideration from scholars of diverse divisions (Ling et al. 2020; Teymourian et al. 2021). With the fortitude of wearable sensors, invasive blood analysis or tissue sampling is going to be replaced by other biological fluids for analyte detection such as saliva, tear, sweat, urine, etc. (Mahato and Wang 2021). Moreover, wearable sensors can be designed with suitable materials by utilizing numerous signal transduction procedures such as resistance, capacitance, and piezoelectric/triboelectric estimation (Ling et al. 2020). Ultimately, it is going to provide a pain-less analysis of valuable physiological parameters. There have been several pieces of research reporting the application of wearable sensors on daily-basis items such as contact lenses, oral guards, bandages, skin tattoos, and textile clothing (Poongodi et al. 2020; Mahato and Wang 2021). In order to develop

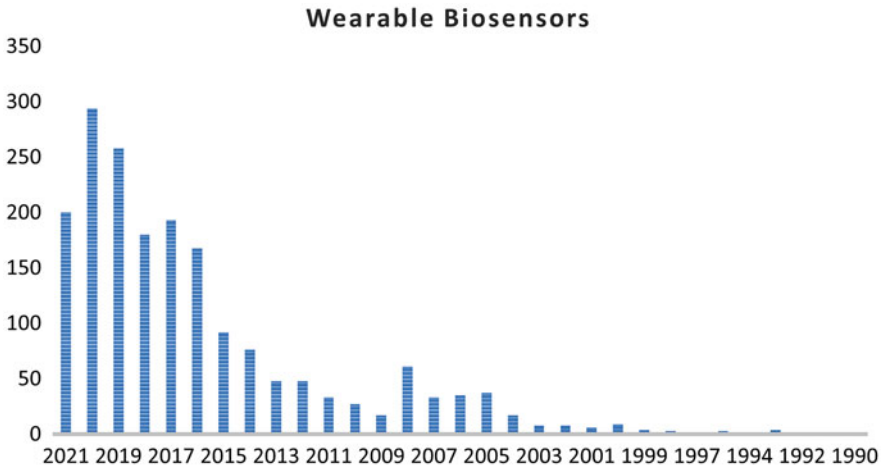

Fig. 15.5 Increase in the research in wearable biosensors (Source: Scopus)

a wearable sensing device, there is a need for sensing module integration directly or indirectly to the biological fluids or intimate contact of tissue that releases the relevant analyte. Although wearable devices are appropriate for application in different fields such as animal health analysis and bio-threat control, it appears that the most positive applications are in personalized monitoring and personal health analysis (da Silva et al. 2018) (Fig. 15.5).

For a diabetic patient, the continuous measurement of blood glucose levels has been always a matter of interest among academic/industrial researchers. The freshly introduced electrochemical wearable sensors for diabetic continuous monitoring have revolutionized the market of glucometers. These minimally invasive microneedle-based wearable sensors measure glucose nonstop in interstitial fluid, which ultimately aids in the effective control of diabetes (Teymourian et al. 2021). Particularly, the sensor market is enhanced by increasing awareness and consciousness about the benefits of healthcare support, transitioning from existing clinic-based disease detection to patient-centered diagnostics. The number of wearable sensors in the worldwide market has touched 125 million in 2016, and the end-users are projected to reach 900 million by 2021 with a current CAGR of 23%. As per the report of IDTech Ex, the market share of the wearable sensor is anticipated to rise to greater than $75 billion by the end of 2025 (Jain et al. 2021).

15.4 Conclusions and Future Trends

In conclusion, the biosensor market is going to expand due to the rise of infectious diseases, ease of operation at the user end, population rise, arrival of innovative technologies, and government and industrial investments. New technologies such as IoT, AI, machine learning, etc. are going to overcome the current limitations in

biosensor technology. The fortitude of upcoming sensor technologies is going to govern and expand the market share of biosensor industries. Ultimately, new jobs and better healthcare services will be available in developing and developed countries. Overall, an improved combination of biosensing platforms and fabrication using micro-manufacturing is going to positively impact the growth sensing industries. In the upcoming years, extensive development of sensing devices is expected, which will eventually transform the world of medical diagnostics and ease the disease burden in rural areas, even in low-income countries. Currently, the research efforts are focused on the development of new and smart sensing technology with novel nanomaterials, bio-active sensing platforms, and computational models to attain easy operation and performance enhancement. It is required to combine the efforts of material scientists, biologists, and computer engineers to develop new sensing technology. With the current trends and projections, the sensing industry is in the exponential phase of growth in terms of market pull and financial scope.

Acknowledgments Dr. Pranjal Chandra thanks Prof. Pramod Kumar Jain, Director IIT(BHU), for encouragement and for providing the necessary facility for the completion of this work. AK acknowledges IITG for a Ph.D. fellowship.

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