Buddhadev Purohit
Pranjal Chandra *Editors*

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Editors
Buddhadev Purohit
DTU Bioengineering
Technical University of Denmark
Kongens Lyngby, Denmark

Pranjal Chandra
School of Biochemical Engineering
Indian Institute of Technology
Varanasi, Uttar Pradesh, India

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Preface

Point-of-care (POC) analytical devices are rapidly becoming an essential part of clinical and industrial investigation due to their enhanced efficiency after integrating functional nanomaterials and surface engineering strategies. The development in the synthesis and characterization of nanomaterials in the last two decades has significantly enhanced these analytical devices' catalytic activity, efficiency, and performances. An interdisciplinary approach to design new surface chemistry and the design of biorecognition elements paved the way for developing highly selective detection devices for use in clinical, environmental, and food safety domains. This book focuses on the recent developments in advanced nanomaterials and surface engineering technology for designing more sensitive and selective biosensors for onsite analysis.

For a better understanding, Chapter 1 describes the most important surface engineering techniques for fabricating POC devices. This chapter has discussed in detail how the morphological or physical modification of the sensor surface regulates the sensor's interaction with the target biomolecule and affects the sensitivity of the POC sensor. Chapter 2 discusses the importance of nanomaterials and the specific functionalization strategy on the sensor surface development for an efficient orientation of the biorecognition molecules on the sensor surface. Surface design, biomolecular conjugations, and sensor-interface interactions have been discussed in this chapter, highlighting the recent breakthroughs in nanotechnology. The first two chapters glance at the recent advances in using nanomaterials and surface modification technologies in POC device development. The rest of the chapters in the book discuss the application of these two important domains in sensing various analytes. Chapter 3 deals with the detection of various biologically important gas molecules using three-dimensional nanostructured materials in reliable and cost-effective ways. The chapter has also highlighted the need for various designing frameworks to further these materials' development. Chapter 4 discusses the use of magnetic nanoparticles in developing POC for healthcare and environmental applications. The chapter also summarizes different synthesis procedures of the magnetic nanoparticles and the modification of these nanoparticles for different analyte detection using different modes of biosensing. Chapter 5 focuses specifically on optically active nanomaterials in POC sensor development, focusing on the optical detection of biomarkers for cancer, cardiovascular diseases, and neurodegenerative

diseases. Chapter 6 is also based on the optical detection of analytes, with major focus on using aptamers as the biorecognition element, and molecules related to food safety as a major target. The chapter has also discussed in detail the recent advances and limitations of currently used sophisticated analytical instruments. Chapter 7 discusses the recent POC sensor developments for emerging infectious disease diagnosis using nano-bio analytical methods. The chapters also discuss antibiotic susceptibility testing for antibiotic resistance determination of the causative viral and bacterial pathogens. Chapter 8 explores the design of POC for disease biomarker detection and discusses in detail the use of new sensing models for onsite detection. Chapter 9 discusses the use of custom-made peptides as a substitute for antibodies for the clinical detection of different biomarkers. The chapter discusses the development of different peptides, their immobilization, and their use in electrochemical biosensing. Chapter 10 focuses on the engineering of another biorecognition element, enzymes, and their use in POC device development to detect a number of clinically and environmentally important molecules. This chapter has compiled a very detailed account of the use of different nanomaterials to facilitate electron transfer for sensing applications. Chapter 11 focuses on aptamers for environmental pollutants detection using fluorescence. Chapter 12 discusses POC devices to detect some of the most commonly used pesticides. The chapter has compiled the recent developments in the domain focusing mostly on the interactions of the pesticide with the nanomaterial used on the sensor surface and the interaction of the pesticide with biological molecules. In the last decade, the smartphone has become an integral part of analytical studies, and the POC devices are getting integrated with the smartphone for data collection, analysis, and storage with other portable onsite features. Chapter 13 has compiled most of the recent breakthroughs in smartphone integration in POC for the onsite analysis and the use of smartphones in wearable sensors. The sensing mechanism, design principle of the smartphone-based portable and wearable sensing system, and implementation of biosensing strategies are discussed here. Likewise, lateral flow assays are undisputedly the most popular POC devices in the larger population for rapid testing in the post-COVID era owing to their simplicity in use. Chapter 14 discusses the nanomaterial and advanced sensing strategy to develop more sensitive lateral flow assays for clinical analysis. The book covers all the important aspects of POC design and development for the onsite analysis of clinical, environmental, and food safety samples. It will help the reader to understand the recent advances in POC devices and their use in analysis of different clinical, environmental, and industrial samples.

Kongens Lyngby, Denmark — Buddhadev Purohit
Varanasi, Uttar Pradesh, India — Pranjal Chandra

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Editors and Contributors

About the Editors

Buddhadev Purohit is currently working at the Department of Biotechnology and Biomedicine (DTU Bioengineering), Technical University of Denmark. He obtained his Master's degree in Life Sciences from Sambalpur University and pursued his Ph.D. at the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India. His research focuses on designing novel electrochemical and optical biosensing platforms. He is also involved in the development of microfluidics systems, smartphone-based biosensors, and portable, low-cost diagnostic devices.

Pranjal Chandra is an Associate 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 interdisciplinary, 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 / 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 is an expert member/ reviewer of various national and international funding agencies. He has also been appointed as Advisor for the Biomedical Sensors Domain and Sensor Networks

Systems at the Institution of Engineering and Technology (IET), Michael Faraday House, LONDON, United Kingdom. Prof. Chandra has authored over 140 high-impact publications, including research/review papers and invited book chapters and published 15 books on biosensors/medical diagnostics / material engineering. He has guided 5 Ph.D. and currently supervises 8 Ph.D. students. Prof. Chandra received many prestigious awards, coveted honors, and fellowships, such as Fellow of the Indian Chemical Society, Shakuntala Amirchand Award 2020 by the Indian Council of Medical Research, NASI-Prof. B.K. Bachhawat Memorial Young Scientist Lecture Award (2022); FSSAI Eat Right Award-2022 (Government of India); 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; Biotech Research Society India 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 Technological Innovation Award (GYTI) 2020. Dr. Chandra is also listed among the world's top 2% scientists in 2020 and 2021 by Stanford University, USA.

Contributors

Hussaini Adam Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Perlis, Malaysia

Tijjani Adam Faculty of Electronic Engineering Technology, Universiti Malaysia Perlis, Perlis, Malaysia

M. Asunción Alonso-Lomillo Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Burgos, Spain

Arpita Banerjee Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai, India

Pranjal Chandra School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India

Chen Cheng Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China

Yeng Chen Department of Oral & Craniofacial Sciences, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

Zetao Chen Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Olga Domínguez-Renedo Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Burgos, Spain

Ying Fang Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Arnab Ghosh Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai, India
Medical Specialist, Indian Navy, New Delhi, India

Surbhi Goel Indian Institute of Technology Delhi, New Delhi, India

Subash C. B. Gopinath Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Perlis, Malaysia
Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis, Perlis, Malaysia

Qiongzheng Hu Qilu University of Technology (Shandong Academy of Sciences), Shandong Analysis and Test Center, Jinan, China
School of Pharmaceutical Sciences, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China

Merin Joseph School of Materials Science and Engineering, National Institute of Technology Calicut, Calicut, Kerala, India

Neeti Kalyani Department of Biotechnology and Biomedicine, Technical University of Denmark, Copenhagen, Denmark

Mashooq Khan Qilu University of Technology (Shandong Academy of Sciences), Shandong Analysis and Test Center, Jinan, China
School of Pharmaceutical Sciences, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China

Ashutosh Kumar Department of Electrical Engineering, University of Notre Dame, Notre Dame, IN, USA

Guang Liu Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China

Qingjun Liu Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China

Yanli Lu Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China

Kuldeep Mahato Department of Nanoengineering, University of California, San Diego, La Jolla, CA, USA

Jinbiao Ma Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Sundas Munir Department of Chemistry, University of Mianwali, Mianwali, Punjab, Pakistan

Lakshmi V. Nair School of Materials Science and Engineering, National Institute of Technology Calicut, Calicut, Kerala, India

Seema Nara Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

A. Marta Navarro-Cuñado Physical Chemistry Department, Faculty of Sciences, University of Burgos, Burgos, Spain

Chumki Praharaj Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

Nivedita Priyadarshni Department of Biological Sciences, Alabama State University, Montgomery, AL, USA

Buddhadev Purohit DTU Bioengineering, Technical University of Denmark, Kongens Lyngby, Denmark

P. P. Muhammed Shafeeque Rahman School of Materials Science and Engineering, National Institute of Technology Calicut, Calicut, Kerala, India

Tushar Kanta Sahu Department of Materials and Environmental Chemistry, Stockholm University, Stockholm, Sweden

Preeti Singh Department of Bioengineering, The University of Texas at Dallas, Richardson, Texas, USA

Sanjay Singh Tulane University, New Orleans, LA, USA

Rohit Srivastava Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai, India

Winnie Edith Svendsen DTU Bioengineering, Technical University of Denmark, Kongens Lyngby, Denmark

Jiahao Xu Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Jie Xu Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China

Chengyun Yan Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Dehong Yang Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Fenni Zhang Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China

Advanced Surface Engineering Strategies for Point-of-Care Devices

1

Mashooq Khan, Sundas Munir, and Qiongzheng Hu

Abstract

Modification of substrate surfaces is significant for the immobilization and retaining the activity of biorecognition elements (BREs) such as antibodies, aptamers, enzymes, or others, which is an important part of the design of point-of-care (POC) devices. Morphological or physical modification of the surfaces increases the surface area and durability, allowing high loading of the BRE to ensure sufficient interaction with the biomolecule at the interface and sensitivity of the POC. In this contribution, the development of methods for controlling the spatiotemporal arrangement of the desired molecules to generate monolayers or multilayers of highly ordered nanostructures is of great significance. This chapter describes surface engineering techniques such as Langmuir-Blodgett, self-assembled monolayers, sputtering, molecular beam epitaxy, lithography, polymer brushes, and polymer hydrogels utilized to fabricate POC devices. Also, the immobilization techniques of BRE to the modified surfaces are described. The discussion on fundamentals and application of the surface engineering techniques for the application of POC is important to assist scientists working in the field as well as in other related disciplines.

M. Khan · Q. Hu (✉)
Qilu University of Technology (Shandong Academy of Sciences), Shandong Analysis and Test Center, Jinan, China

School of Pharmaceutical Sciences, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China
e-mail: huqz@qlu.edu.cn

S. Munir
Department of Chemistry, University of Mianwali, Mianwali, Punjab, Pakistan

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Keywords

Surface engineering · Monolayer · Self-assembled structure · Biosensors · Biomolecules

Abbreviations

5CB	4-Pentyl-4′-cyanobiphenyl
BRE	Biorecognition element
DTMS	n-Decyltrimethoxysilane
EDC-NHS	1-(3-(Dimethylamine)-propyl)-3-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide
ET-1	Endothelin-1
GOx	Glucose oxidase
ITO	Indium tin oxide
LB	Langmuir-Blodgett
LC	Liquid crystal
LCP	Liquid crystal polymer
LCP-b-PAA	Poly(4-cynobiphenyl-4′-oxy-undecyl acrylate-b-acrylic acid)
LCP-b-QP4VP	Poly(4-cynobiphenyl-4′-oxy-undecyl acrylate-b-quartanized-4-vinylpyridine)
MBE	Molecular beam epitaxy
MPTMS	(3-Mercaptopropyl)trimethoxysilane
MUA	Mercaptoundecanoic acid
OTAB	Octadecyl trimethyl ammonium bromide
OTMS	Octadecyltrimethoxysilane
OTS	Octadecyltrichlorosilane
PB	Prussian blue
PBA	4-Pentylbiphenyl-4′-carboxylic acid
POC	Point of care
RNA	Ribonucleic acid
R-SH	Alkanethiol
SAM	Self-assembled monolayer
SAMs	Self-assembled monolayers
SARS-CoV-2	Severe acute respiratory syndrome-coronavirus-2
ssDNA	Single-strand deoxyribonucleic acid

1.1 Point-of-Care Sensors

Point-of-care (POC) sensors enable the robust collection of information about a target analyte present in biological, environmental, food, and other samples (Adiga et al. 2018; Neogi et al. 2020; Vashist 2017). The development of the Clark electrode

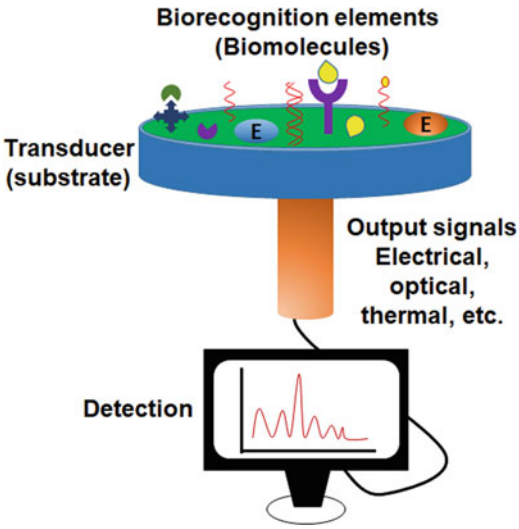

Fig. 1.1 Schematic of various components of a POC (biosensor)

system for the detection of glucose revolutionized the field of POC testing (Arya et al. 2008). POCs reduce healthcare cost and improve health access and quality of healthcare delivery. The POC has significant applications in the detection of glucose, cardiac marker, infectious diseases, and coagulation monitoring, among others. Rising incidences of infectious diseases drive the growth and demand for the advancement of point-of-care biosensors (Purohit et al. 2020b; Purohit et al. 2020a). Moreover, with the advent of the COVID-19 pandemic, the need for past and efficient POCs rises to a great extent for the monitoring of infectious diseases (Price and St John 2021; Valera et al. 2021).

Biosensor is the integration of a biorecognition element (BRE) with a transducer for the detection and quantification of biomolecules (Fig. 1.1). Biomolecules are chemical compounds produced by living organisms ranging from small molecules such as metabolites to large molecules, such as proteins, enzymes, and carbohydrates, which can be investigated for diagnostics (Bhalla et al. 2016; Lakshmipriya and Gopinath 2019; Naresh and Lee 2021; Parmin et al. 2019). The biomolecules are the building blocks of living organisms, and therefore, their presence at a normal level is significant for the normal function of the living cells. Any alteration in the concentration of a specific biomarker may result in malfunctioning the cells and organisms. The interaction of BRE with a target analyte at the transducer interface yields signals as a function of analyte concentration. Therefore, biosensors enable the detection of analytes in the chemical processes, environmental, body fluids, and food samples at a low cost. Over the past three decades, biosensors research has had a significant impact on both laboratory research and the commercial sector. Biosensors have modernized the care and management of diabetes through continuous glucose monitoring devices and impact several other areas of clinical diagnostics (Hadžović et al. n.d.; Lee et al. 2021; Purohit et al. 2022). The development of a biochip that measures the activity of both gene and

protein allows common users to make technically complex and reliable measurements with the minimum of intervention (Haga 2016). Similarly, biosensor can be applied for the detection of chemical or biological weapons (Pohanka 2019). The applications of biosensors have broadened with time making biosensors development a challenging interdisciplinary area attracting engineers and physical and biological scientists. Thus, the necessity for designing sophisticated tools for the monitoring of biological systems has become ever more demanding.

1.2 Transducer

Transducer translates the interaction between the BRE and biomolecules into physical detectable signals. Based on the detection procedure, the transducers are of different types such as optical, mechanical, electrical, electrochemical, electromagnetic acoustic, thermal, and others (Kawamura and Miyata 2016; Michelmore 2016). Optical transducers are the most common detection technique and integrated with spectroscopies. The optical transducer generates signals based on absorption, transmission, fluorescence, luminescence, and polarization of light and change in refractive index. The mechanical transducer uses a disk, cantilever beam, or double cantilever beam and measures the deformations of a surface, weight change, and the acoustic waves generated on a surface due to the interaction between the BRE and analyte. Electrical translation of the biochemical interaction events is based on the measurement of changes of electrical potential, conductance, or impedance. Electromagnetic responsive surfaces transduce the alteration in frequency, polarization, amplitude, or phase of the incident electromagnetic waves caused by the BRE and biomolecule interaction. Electrochemical responsive surfaces measure the changes in electrical properties generated from the movement of electrons or ions in a solution due to the chemical reaction between the redox-active molecules. Based on the detection mechanism, electrochemical transducers are of different types such as field-effect, ion change, impedimetric, conductometric, amperometric, and potentiometric transducers. Electromagnetic acoustic transducers are based on electromagnetic mechanisms, which do not need direct coupling of the material with the surface, but monitor the noncontact acoustic wave generation and reception in conducting materials. Thermal transducers analyze the absorption or evolution of heat due to the biological interaction at the surface. Enzyme thermistor is generally used, which measures the heat changes caused by the interaction between the immobilized enzymes and a target analyte.

Surface modification of a transducer is significant for the immobilization and maintained activity of the BRE (Sassolas et al. 2012). Morphological or physical modification of the surfaces increases the surface area of a transducer for high loading of the BRE to ensure sufficient interaction and enhance the limit of detection (Kumar et al. 2019a; Mahato et al. 2020). The stability of the BRE is dependent on the thermal and chemical environment of the substrate surfaces. Thus, keeping various environmental factors in mind, the different mode of immobilization of the BRE can be employed to enhance the activity of BRE and stability and durability of

the surface. In this chapter, recent trends in the engineering of biosensing surfaces are presented according to the surface modification technique and materials used.

1.3 Surface Engineering Techniques

The development of methods for controlling the spatiotemporal organization of the desired molecules on a substrate surface is highly significant for biomolecules diagnosis. The solid substrates or liquid-liquid interface is modified by various techniques such as Langmuir-Blodgett, self-assembled monolayers, grafting, lithography, polymer hydrogel-based binding matrix, layer-by-layer assembly, molecular beam epitaxy, electrodeposition, sputtering, thermal evaporation, and others to fabricate POC devices. A comprehensive description of the fundamentals of various surface modification and their applications for POC is given in the following sections.

1.3.1 Langmuir-Blodgett Technique

Thin molecular films (monolayer) of a thickness of a few nanometers are of great importance for the modification of substrates for application in POC devices (Basu and Sanyal 2002; Breton 1981; Roberts 1985). The Langmuir-Blodgett (LB) approach is a sophisticated monolayer deposition technology for the production of biologically, optically, or electrically active, densely packed, nanometer-scale transferable film onto the surfaces of the substrates (Ariga 2020). Figure 1.2a shows that the LB instrument is consist of a trough (to be filled with a subphase, generally water), two barriers that contract to produce a closely packed molecular monolayer, and a balance provided with a suspended Wilhelmy plate. The Wilhelmy plate is made of platinum metal and suspended in such a way as to touch the water in the trough. The LB technique can precisely control the thickness and homogeneous deposition of the monolayer over large areas and also enable the making of multilayer structures with varying layer compositions. Additionally, the LB technique has the advantage that monolayers can be deposited on almost any kind of solid substrates, and in some cases, the monolayers can be transferred on liquid interfaces (Collins et al. 2006).

Amphiphilic molecules such as surfactants consist of a hydrophilic head and a hydrophobic tail. The amphiphilic nature allows these molecules to form micelles, bilayers, vesicles, and other structures in solution and to accumulate at the air/water or oil/water interface. Most of the surfactant or other amphiphilic molecules can be spread on the surface of the water in the LB trough with the help of dissolving them in a volatile immiscible organic solvent such as chloroform, hexane, etc. (Alejo et al. 2013; Liang et al. 1994). The solvent evaporates from the surface of the water, leaving the hydrophilic group of the molecules immersed in the water and the hydrophobic part in the air (Fig. 1.2b). The formation of a monolayer required a long enough hydrophobic part (hydrocarbon chain) of the amphiphilic molecules.

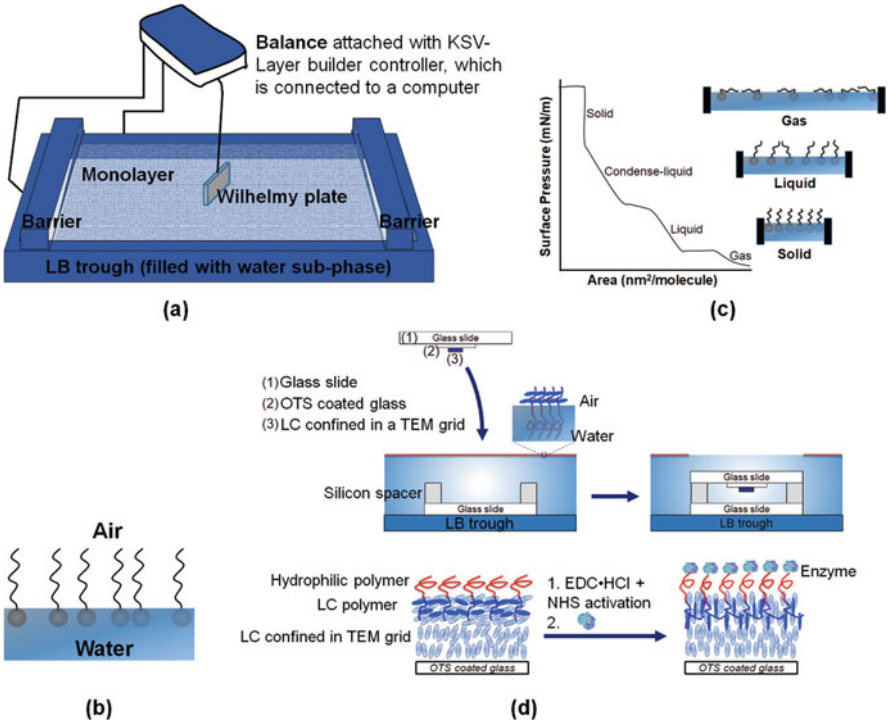

Fig. 1.2 Schematic of (**a**) LB instrument with a spread monolayer, (**b**) a monolayer of amphiphilic molecules formed on the surface of the water. (**c**) Phases of monolayer upon compression of the barriers. Adapted from reference (Basu and Sanyal 2002) with permission, Copyright 1990, Wiley Online Library. (**d**) LB monolayer of the amphiphilic block copolymer and its transfer onto liquid crystal (liquid substrate), followed by the immobilization of the enzyme

The length of the chain needs to be more than 12 carbons long. The shorter chain will form water-soluble micelles, which will prevent the formation of the monolayer. On the contrary, too long a hydrophobic chain does not form a monolayer and consequently crystallize on the surface of water. The generalization of optimal length of the hydrophobic chain length is difficult because the molecular monolayer formation is also depending on the length, size, and polarity of the hydrophilic part.

The monolayer formation is measured from the surface pressure against the area of the water available to each amphiphilic molecule through surface pressure-area isotherm (π-A) at a constant temperature (Behroozi 1996). The π-A isotherm is measured through the compression of the barriers to reduce the area at a constant rate. Several distinct regions called phases are immediately visible on observing the isotherm. Harkins has classified the monolayer of fatty acids in different phases (Fig. 1.2c). Generally, the monolayers exist in the gaseous state. Upon compression, a liquid-extended state is formed. Further compression leads to the formation of a condensed-liquid phase and finally a solid state. On further compression of the solid phase, the monolayer collapses into a three-dimensional structure. The collapse is

observed through a rapid fall in the surface pressure. In the case of a monolayer collapse in the liquid phase, a horizontal break in the isotherm can be seen. Several cycles of compression and expansion may be necessary to achieve reproducible results (Bibo and Peterson 1990).

Many studies have utilized the LB monolayer on solid substrates for sensor application. A multilayer well-ordered membrane from calcium chelator 1,2-bis-(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid and octadecylamine is synthesized through the LB method and for the detection of calcium in an aqueous solution (Kalinina et al. 2006). Sun et al. produced an active film of glucose oxidase (GOx) enzyme and transferred it to a platinum substrate for the detection of glucose (Sun et al. 1991). GOx immobilized on an inorganic-organic LB film of octadecyltrimethylammonium (ODTA) and nanosized Prussian blue (PB) is used for amperometric monitoring of glucose (Ohnuki et al. 2007). The enzyme immobilization onto LB monolayers for biosensor application has been summarized in detail elsewhere (Luciano 2018).

LB technique was also applied for the transfer of a monolayer onto the liquid-liquid interface. Park and coworkers synthesized amphiphilic liquid-crystal-*block*-polyelectrolyte block copolymers such as poly(4-cynobiphenyl-4′-oxy-undecyl acrylate-b-acrylic acid) (LCP-b-PAA) and poly(4-cynobiphenyl-4′-oxy-undecyl acrylate-b-quartanized-4-vinylpyridine) (LCP-b-QP4VP) and generated its monolayers using LB techniques (Khan and Park 2015; Khan and Park 2014). Then, the monolayer is transferred onto a nematic liquid crystal (LC) confined in a transmission electron microscopy grid. The LCP part of the polymer adsorbs into the LC, which anchored the hydrophilic part in the aqueous medium (Fig. 1.2d). The system was then utilized for the detection of proteins. Also, the enzyme was covalently or electrostatically immobilized on the hydrophilic part for biosensor applications.

1.3.2 Self-Assembled Monolayers

Self-assembled monolayers (SAMs) are the spontaneous assembly of highly organized layers of amphiphilic molecules on the surfaces. SAMs are prepared through the direct casting of organic amphiphiles molecules from the solution or gas phase onto a solid surface or liquid-liquid interface (Ulman 1996). The spontaneous deposition firmly anchored the amphiphilic molecules on the surfaces through a chemical reaction, physical adsorption, or the auto-organization through intermolecular forces, which results in an organized and compact thin film on a given surface. SAMs provide a simple, supple, and robust platform to modify the interfacial properties of transducer surfaces. Additionally, SAMs offer high packing, ordered structure, control at the molecular level, and easy synthesis and functionalization. Figure 1.3 summarizes that the SAMs of surfactant (Moreno-Razo et al. 2012), fatty acid (Karsi et al. 2006), alkanethiols (Guo and Li 2014), alkylsilane (Castillo et al. 2015), and catechol (Guardingo et al. 2014a) derivatives have been employed on different surfaces for POC application. These assemblies

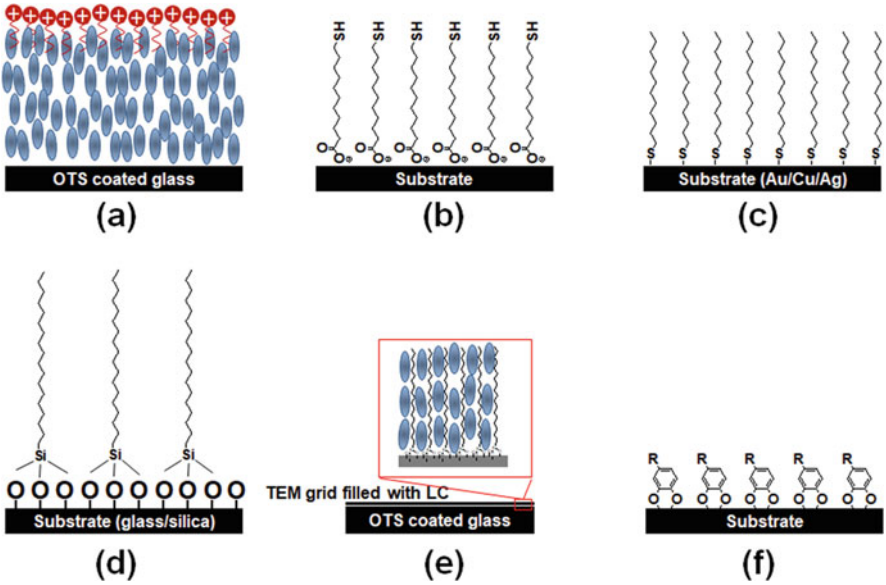

Fig. 1.3 Self-assembled monolayers of (**a**) surfactant on LC/aqueous interface, (**b**) carboxylic acid derivatives, (**c**) alkanethiols, (**d**) alkylsilane on substrate surfaces, (**e**) alignment of LC by alkylsilane-coated glass substrate, and (**f**) self-assembled monolayer of catechol derivatives

have been obtained on the surfaces like metals, metal oxides, silicon oxide, graphene, carbon nanotubes, carbon dots, fullerenes, polymers, liquid crystal, and others.

Zisman et al. obtained a surfactant monolayer on a metal surface in 1946 (Ewen et al. 2018). However, the potential of SAMs was not realized at that time. Sagiv prepared a monolayer of trichlorosilane on a silicon oxide surface in the late 1970s (Oyola-Reynoso et al. 2016), which opens a new direction, and a large variety of SAMs were obtained in the subsequent years until now. Since then, the assembly of organosulfur on gold and organosilanes on silicon and glass are the most popular combinations. Gillich et al. (Gillich et al. 2011) in late 2000 designed a series of catechol derivatives for the modification of metal oxides and successfully demonstrated the covalent linkage of nitrocatechols to metal and metal oxide.

The SAMs provide a suitable platform for the immobilization of BRE to retain its activity and thus provide reproducibility, stability, and high sensitivity to POC devices. The SAMs allow the fine-tuning of the interfacial phenomena at the molecular level and hence a surface of desired functionality and can be obtained for biosensor applications.

SAMs of Surfactant

Surfactants are versatile molecules due to their amphiphilic nature having a polar head group and a nonpolar organic tail. Therefore, surfactants adsorb at the interfaces and form a self-assembled structure such as micelles, vesicles, bilayer,

and others in bulk solution. Surfactant SAM on solid electrode substrates has been frequently applied for sensor application ((Gomez et al. 1991); (Anchev et al. 2019); (Atta et al. 2016)). Also, for the last two decades, surfactant SAM on liquid-liquid (aqueous/LC in particular) interface has been utilized for the design of biosensors. Surfactants form SAM on the LC/aqueous interface through the adsorption of the tail into the LC mesogens and the head group remain at the interface (Fig. 1.3a). The tail of the LC dictates the orientation of the LCs. At a critical value, the surfactants orient LC mesogen perpendicular to the surface. Thus, the manipulation of the SAM of surfactant at LC/aqueous interface has been the focus of many studies for the design of biosensors. A nematic LC 4-pentyl-4′-cyanobiphenyl (5CB) was doped with 4-pentylbiphenyl-4′-carboxylic acid (PBA) for the design of pH-sensitive biosensor for penicillinase detection. Hu et al. doped LC with dodecanal for the catalase detection (Hu and Jang 2012). Price et al. adsorbed single-strand deoxyribonucleic acid (ssDNA) onto a SAM of a cationic surfactant, octadecyl trimethyl ammonium bromide (OTAB) at the aqueous/LC interface (Price and Schwartz 2008). They observed the hybridization of the complementary ssDNA with the adsorbed ssDNA through the change in the orientation of the LC under cross-polarizers. This system is further utilized for the monitoring of p53 mutant gene segment, pathogens, and bleomycin hydrolase (Tan et al. 2014). Recently, the surfactant-SAM-laden interface with adsorbed ribonucleic acid (RNA) probe is used for the detection of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (Xu et al. 2020). Thus, surfactant due to its ability to form varieties of the nanostructure can form the basis for the design of POC devices at both solid substrates and liquid-liquid interface.

SAMs of Fatty Acids Fatty acids are saturated or unsaturated aliphatic carboxylic acids. The naturally occurring fatty acids consist of an unbranched chain of an even number (4 to 28) of carbon atoms (Moss et al. 1995). The SAM of fatty acids can be obtained by dipping a substrate in its solution (Karsi et al. 2006). The fatty acid molecules adsorb on the surface due to the presence of its reactive carboxylic acid (COOH) functional group (Fig. 1.3b). Because of the structure and well-packed density of the fatty acid monolayers, many studies have applied this approach for the manipulation of substrate surfaces for POC application. The SAM of 11-mercaptoundecanoic acid (11-MUA) on gold electrode was obtained through 1-(3-(dimethylamine)-propyl)-3-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide (EDC-NHS) chemistry. Subsequently, the SAM was functionalized with antibodies for the recognition of antigens (Ahmad and Moore 2012). Also, the 11-MUA on a gold disk substrate is functionalized with monoclonal anti-ET-1 antibody through EDC-NHS for the detection of endothelin-1 (ET-1), a colon cancer biomarker (Narayan et al. 2019). With the advent of the COVID-19 pandemic, the antibody against SARS-CoV-2 nucleocapsid protein is immobilized on 11-MUA for the monitoring of the viral protein (Eissa et al. 2021). Because of the simple substrate modification, sufficient BRE binding capacity and low-cost SAM of fatty acids is one of the most evaluated techniques for surface preparation.

SAMs of Alkanethiols

The natural affinity of sulfur-gold bonding due to the inert nature of gold makes the alkanethiol (R-SH) monolayer the most studied among others. The SAM of organosulfur molecules is formed by subjecting gold surface to a solution or vapor of an alkanethiol. Firstly, a gold film is obtained on a substrate of glass coverslips or silicon wafer followed by the deposition of alkanethiol monolayer. The sulfur and gold molecules interact at the metal surface, while the long alkyl chain makes an ordered structure in the air (Fig. 1.3c). The nature of the sulfur-gold bond is not yet clearly understood. However, as previously reported (Kane et al. 1999), we adopt the view that gold(I) thiolate molecules make the surface of metal gold after modification:

$$\mathrm{R-SH + Au(0) \rightarrow R-S-Au(I)Au(0) + \tfrac{1}{2}\,H_2}$$

Similarly, the alkanethiol SAMs have been generated on silver (Costelle et al. 2012) and copper (Colavita et al. 2005) metals. The R-SH of different functional head groups can be employed for the immobilization of various BREs as discussed in Sect. 1.4 of this chapter. For instance, oligo(ethylene glycol)-terminated carboxylate or amine thiolates were used to obtain SAMs with enhanced biocompatibility, prevent undesired adsorption, and achieve good surface wetting (Islam et al. 2014a). Attia et al. (Attia et al. 2021) obtained alkanethiol monolayer on gold. Subsequently, the alkanethiol was functionalized with oxytocin-dodecanoic acid for the selective detection of zinc(II). The long alkane chain of alkanethiol and the terminal amine group enhance the selectivity of the sensing platform. The gold substrate modified with the monolayer of an alkanethiol enhances its bio- and cell compatibility and durability of the surface. The alkanethiol SAM desorbs on heating at a temperature above 70 °C, with the UV irradiation in aerobic condition, and on exposure to atmospheric ozone. Therefore, the SAMs are required to be protected from high temperature or intense light before and during experiments.

SAMs of Alkylsilanes

Salinization is the spontaneous SAM formation of alkylsilane on a substrate through the formation of a siloxane bond. The siloxane bond is formed between the alkoxy hydroxide and oxide of the substrate by hydrolysis and condensation (Fig. 1.3d). Therefore, the alkylsilane monolayer on oxide surfaces provides high stability and durability of the surface through covalent bonding (Onclin et al. 2005). However, obtaining a homogenous alkylsilane monolayer on a surface is a fundamental challenge (Peng et al. 2015). For the formation of alkylsilane SAM, the surface is activated by strong acid, piranha solution (*Caution: Piranha is a mixture of three-part H_2SO_4 and one-part H_2O_2, formed by the slow addition (dropwise) of H_2O_2 into H_2SO_4 in a glass Petri dish. Piranha's solution should not be covered. It is highly corrosive and must be handled with care*), or oxygen plasma to clean the surface and generate hydroxyl, oxygen radicals, epoxide, and silanol groups on the surfaces (Zhuravlev 1987). This treatment makes the substrate's surfaces hydrophilic. Consequently, a thin water layer is formed on the surface, which guides the organosilane

into a well-packed monolayer. The deposition of the SAM layer depends on the type of solvent (Rozlosnik et al. 2003), temperature (Carraro et al. 1998), deposition time (Liu et al. 2001), solution age, and water content (Vallant et al. 1998). Also, the storage of alkylsilane solution aggregates due to the formation of polysiloxane (Glaser et al. 2004). The aggregates then cannot form covalent bonds with the surface but are adsorbing physically, leading to the formation of an inhomogeneous unstable coating.

Advanced surface manipulation techniques are used to obtain a homogenous and closely packed well-ordered silane monolayer on the silicon substrate. For instance, Si-H substrates are subjected to functional 1-alkene through thermally or visible light-assisted hydrosilylation (Eves et al. 2004). In this procedure, the alkene attacks the electrophilic positively charged Si sites on the surface, resulting in the formation of the Si-C bond. The spatiotemporal position of the monolayer on the substrate is controlled through UV irradiation (Scheres et al. 2007). Ring-opening click reactions are used for the grafting of heterocyclic silanes such as Si-N or Si-S on silanols substrate (Llorente et al. 2016). The heterocyclic silanes allow rapid functionalization of the porous silicon nanostructures. Amino- or thiol- functional silatranes are used for the immobilization of DNA and nanoparticles on the substrates.

Many studies reported the utilization of alkylsilane SAM on surfaces for sensing applications. For instance, the SAM film of octadecyltrichlorosilane (OTS) and octadecyltrimethoxysilane (OTMS) on a lithium niobate substrate was investigated for the detection of hydrogen through a surface acoustic wave sensor (Nihonyanagi et al. 2008). This study also demonstrated that OTS due to the abundance of local hydrochloric acid causes the etching of the metal surface, whereas OTMS does not show etching behavior on a metal substrate. Therefore, the compatibility of alkylsilane molecules is important for the design of biosensors that contain metal substrates susceptible to etching by acid released in the formation of a silane-based monolayer. The OTS monolayer was frequently used for the modification of glass substrates to align LC molecules perpendicular to the surface for biosensor applications (Fig. 1.3e) (Khan and Park 2014). Gabriunaite et al. (Gabriunaite et al. 2020) modified the surface of fluorine-doped tin oxide (FTO) with the SAM of OTS as a model system for biomedical and pharmaceutical analysis. Ashur and Jones (Ashur and Jones 2012) obtained a SAM of (3-mercaptopropyl) trimethoxysilane (MPTMS) and n-decyltrimethoxysilane (DTMS) on indium tin oxide (ITO) substrate and immobilized protein probe on the monolayer using protein film electrochemistry. A series of chemical reactions such as click chemistry, nucleophilic substitution, photochemical reaction, supramolecular modification, and other reactions can be applied for the formation of organosilanes SAM on hydroxyl-terminated substrates (Wang et al. 2021).

SAM of Catechol Derivatives

Catechol is also known as 1,2-dihydroxy benzene, pyrocatechol, or benzenediol containing a benzene core with two hydroxy groups ortho to each other. Catechols form a strong linkage with the substrate surfaces through oxygen atoms (Fig. 1.3f),

and this makes them significant anchors for surface modification. The adhesive properties of catechol moiety in mussel protein are well explored (Zürcher et al. 2006). Therefore, a SAM of upward-facing catechols thiol is achieved on epitaxial gold substrates for enhanced adhesion properties (Guardingo et al. 2014b). The catechol ligands on a surface are highly stable and therefore show resistance to oxidation at high pH. A zwitterionic catechol derivative is synthesized for the modification of iron oxide nanoparticles (Wei et al. 2013). The SAM of catechols on TiO_2 substrates is dependent on pH of the medium. With the hydroxyl group of oxidized TiO_2, the zwitterionic catechol adsorbs through hydrogen bonding at low pH. Subsequently, after subjecting the surface to high pH, the surface hydroxyl groups replace through deprotonation of the catecholic hydroxyl group to covert the hydrogen bonding to bidentate bonds. However, to date, few studies reported the potential of catechols monolayer for molecular detection, maybe due to limited availability. An electroactive SAM is synthesized on a gold surface through covalent attachment of protocatechuic acid for the detection of dopamine in an aqueous solution (Salmanipour and Taher 2011). Catechol derivatives are used to obtain centimeter-scale, well-defined, surface-chemical hydrophobic gradients from perfluoro-alkyl catechols and nitrodopamine on titanium dioxide (TiO_2) (Rodenstein et al. 2010).

1.3.3 Polymer Brushes

Polymer brushes are the assembly of polymeric chains of those one end is attached to the surface of a substrate or an interface (Milner 1991). For polymer brushes, the interchain distance needs to be much smaller than the chain length and the tethering density sufficiently higher to obtain crowded polymer chains which are forced to stretch away from the substrate surface or interface (Fig. 1.4a and b). Polymer brushes are a common name for many applied polymer systems such as grafted polymers on a substrate, block copolymer and graft copolymer at fluid-fluid interface, polymer micelles, and adsorbed diblock copolymer (Fig. 1.4c) (Zhao and Brittain 2000).

The polymer brushes are achieved by grafting polymer chains to a substrate through a chemical bond between the reactive end group of the polymer chain and reactive groups on the substrate surface or physical adsorption of block copolymers via sticky segments. This method is called the *grafting to* technique. The *grafting to* approach is simple but has several limitations. For example, it is challenging to obtain a high grafting density of the polymer brushes due to steric crowding of the reactive sites on a surface by already adhered polymers and reversibility of the non-covalent adsorption, and film thickness is limited by the molar mass of polymer in solution. Another approach to preparing polymer brushes is *grafting from* or surface-initiated polymerization. In this method, first, the substrate is modified with a SAM of the initiator. The SAM of the initiator can be obtained on almost any surface provided the right selection of the anchor functionality, for instance, silane on glass-, silica-, and plasma-treated polymers, thiols on gold, etc. as discussed in the previous

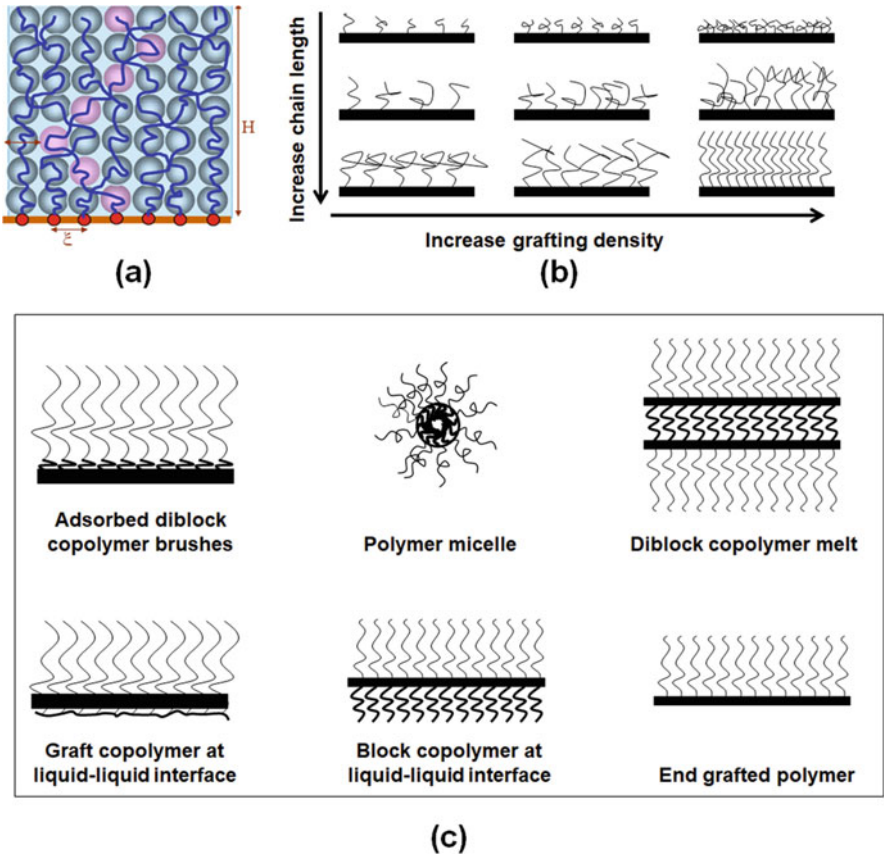

Fig. 1.4 (**a**) Schematic definition of the polymer brushes, (**b**) stretching away of polymer brushes from the substrate surface as a function of molecular mass and grafting density, and (**c**) different polymer brushes system for application in POC systems. Figure (**c**) is adapted from reference (Zürcher et al. 2006) with permission. Copyright 2000 Elsevier Publication

section. The initiator-functionalized surfaces are then subjected to the solution containing monomer and catalyst (Edmondson et al. 2004). In this approach, the polymerization is surface-confined and no polymerization occurs in solution. The polymer brushes can be achieved on various interfaces such as solid substrates, liquid-liquid, or liquid-air interfaces and between melts or solutions of homopolymers. On a solid substrate, the polymer brushes are formed with one block strongly interacting with the surface and another block extending away from the surface. For the liquid-liquid interface, similarly, one block has a high solubility in one liquid and the other block in another liquid; thus one block strongly anchored another block at the interface, which forms polymer brushes.

Polymer brushes gain much attention for their application in biosensors, diagnostics, drug delivery, and medical implant over the past few decades

(Boujakhrout et al. 2015; Cui et al. 2011; Paul et al. 2013). Polymer brushes have the advantage of avoiding nonspecific interactions on the transducer surface and negating the false-negative or false-positive signal produced by the substrate (Welch et al. 2011). Tokareva et al. designed a nanosensor based on gold nanoparticles and responsive polymer brushes for enhanced transmission of the surface Plasmon resonance signals (Tokareva et al. 2004). Tam and coworker prepared grafted poly (4-vinylpyridine) brushes on indium-tin-oxide-coated electrode surface for reversible conversion of electrode surface between active and inactive state (Tam et al. 2010). This switchable electrode surface has the advantages of enhanced selectivity and biocompatibility for biosensor applications. The utilization of polymer brushes for electrochemical biosensors has been discussed elsewhere (Welch et al. 2011). Polymer brushes were also utilized for the design of LC-based biosensors. Khan et al. obtained the PAA-b-LCP block copolymer brushes at an aqueous/LC interface followed by the immobilization of glucose oxidase enzyme (Khan and Park 2014). The system was utilized for the detection of glucose in solution and human blood. Similar systems were utilized for the detection of cholesterol and urea (Khan et al. 2014; Munir et al. 2015). Also, mixed polymer brushes of PAA-b-LCP and QP4VP-b-LCP were obtained at an aqueous/LC interface (Khan and Park 2015). This system allowed the electrostatic immobilization of enzymes and was utilized for the detection of glucose. The polymer brushes enhanced the sensitivity and selectivity of the LC-based biosensor devices.

1.3.4 Lithography

The process of creating the desired pattern on a substrate surface through firstly exposure to light, laser, or ions followed by etching is called lithography (Dendukuri et al. 2007; Gale et al. 2018; Kane 2008; Virji and Stefaniak 2014). Based on the source to which the surfaces are exposed or pattern template, lithography is of different types such as photolithography, soft lithography, electron beam lithography, nanosphere lithography, and nanoimprint lithography. Among these photolithography is the most frequently utilized technique for the fabrication of microfluidic channels, micro-wells, micromotors, and other nanostructures (Qian et al. 2011). The photolithography technique is based on a photochemical reagent called photoresist. Figure 1.5 shows the patterning of a substrate through photolithography. In this approach, the photoresist is spin-coated on a substrate surface and then baked in an oven at a mild temperature to stabilize the photoresist material on the surface. A pattern mask is then placed on the photoresist-covered substrate and exposed to UV light. A mask is a black film with a transparent printed design or quartz with opaque patterns of chromium. There are two types of photoresist, a negative and a positive photoresist. After development, the positive photoresist will generate the same pattern as the printed or opaque pattern, while the negative photoresist will create the reverse pattern. The lithography technique got considerable attention for the development of POC devices (Mejía-Salazar et al. 2020). Recently, Chang et al. utilized the photolithography technique to obtain a fine grid pattern on a glass

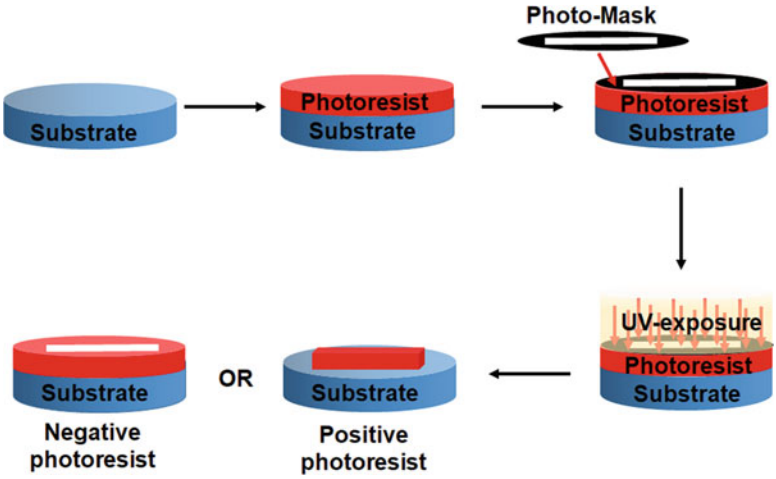

Fig. 1.5 Patterning on a substrate surface using lithography

substrate. The fine grid was then filled with LC to develop a LC-based sensor for the detection of mercuric ions in solution (Chang et al. 2021). The patterned grid LC-based sensor has excellent signal stability, a larger signal-to-noise ratio, and a lower detection limit.

1.3.5 Polymeric Hydrogels Binding Matrix

Hydrogels are a cross-linked polymeric network of hydrophilic homopolymers or copolymers. They exhibit highly open structure and large surface area and thus can accommodate a large number of molecules of specific functions. Also, these structures can be fine-tuned and integrated with other systems for the desired application. The polymeric hydrogels are responsive to external stimuli. Therefore, hydrogels are significant materials in numerous important fields such as drug delivery, pharmaceutics industry, biosensors, and tissue engineering (Stuart et al. 2010).

In biosensors, the hydrogels are employed at the substrate surface and were modified with the immobilized BRE for the recognition of a target analyte. In comparison to other types of surfaces, the hydrogel can accommodate a high amount of BREs and offer a more natural microenvironment to retain the activity of the recognition molecules (Wang et al. 2010). In addition, a class of hydrogels termed "smart gel" has gained considerable interest in the field of biosensors. Various polymers such as poly(acrylate) (Herrmann et al. 2021), poly(vinyl alcohol) (Vaddiraju et al. 2009), poly(4-vinylpyridine) (Arizaga et al. 2010), poly (N-isopropylacrylamide) (Islam et al. 2014b), poly(ethylene glycol) (Nam et al. 2018), dextran (Russell et al. 1999), and chitosan (Yang et al. 2021) were applied for hydrogel-based bio-interfaces. Deng et al. embedded LC droplets in chitosan

hydrogel for the detection of bile acid (Deng et al. 2016). Dan et al. stabilized LC droplets with a whey protein microgel for optical biosensor application (Dan et al. 2020). The LC-embedded hydrogel provided a label-free and portable sensing platform, which can be of great interest in the clinical diagnosis of disease.

Molecular Beam Epitaxy

Molecular beam epitaxy (MBE) is the process of deposition of a crystalline film on top of a crystalline substrate one layer at a time using an atomic or molecular beam in an ultrahigh vacuum (pressure 10^{-10} Torr, 10^{-8} Pa) ((Frigeri et al. 2011); (Mabe et al. 2018)). A continuous steady stream of atoms forms a beam due to a long mean free path and travels from source to the substrate. No physical or chemical interactions occur until the atomic beam reaches the substrate. This permits the uniform adsorption of atoms on the surface in a very controlled manner. The atoms interact with the surface and move around in a two-dimensional array until they combine with another atom. Islands of atoms are produced on the surface, layer by layer until they blend to form a complete film. New atomic islands generate on the first layer and the cycle repeats (Zhang et al. 2016). MBE has an extremely slow deposition rate but provides excellent control. Therefore, it is significant for the formation of highly crystalline film or a single crystal. It is typically used for the deposition of semiconductors (Seshan 2002). Because of the complexity of the instrument, slow deposition rates, high purity of the reactants, and high cost, this method is not often used in biosensors. However, MBE is an ideal approach for the development of high-value devices where performance is more preferable over cost.

Sputtering

Sputtering is used for the deposition of a variety of materials including refractory compounds having high melting points such as tungsten, niobium, tantalum, and tantalum nitride (Wingqvist 2010). A sputtering system is consisting of a chamber provided with a cathode made of material to be deposited. The substrate is loaded on the anode. As shown in Fig. 1.6, the chamber is degassed using vacuum and then filled with a working gas (inert gas or reactive gas for reactive sputtering). Inside the chamber, plasma and ions are generated. Then, the ions are accelerated through an electric field and impinge on the cathode (target). The accelerated ions dislodge atoms from the target; those fall on the substrate and grow into a deposited film (Abdul et al. 2018). Sputtering is useful for obtaining a uniform film on a substrate. Also, it is useful for the deposition of compounds. For example, compound targets are used to obtain a stoichiometric film. For some compound film, sputtering from an elemental target is achieved in the presence of a reactive gas instead of inert gas. The dislodged atoms from the target react with the reactive gas and form a compound film on the substrate. Sputtering allows the deposition of a dense film with strong adhesion. It is equally significant for the deposition and modification of metals, compounds, and alloys. Additionally, it offers high uniformity of the film (Abdul et al. 2018). Galdino et al. deposited gold nanoparticles on ionic-liquid functionalized graphene oxide along with cholesterol oxidase enzyme for the

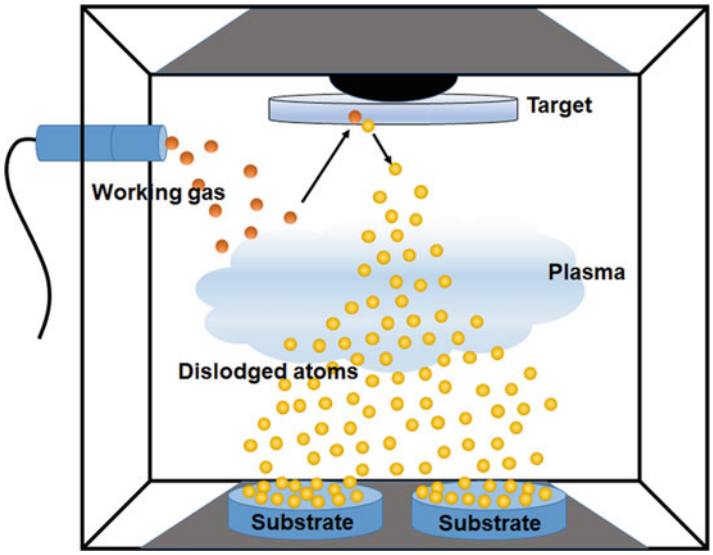

Fig. 1.6 Schematic of sputtering configuration with the target at the upper and substrate at the downside of the working chamber

detection of cholesterol (Galdino et al. 2017). The sputtered hybrid material allowed selective detection and has the potentials for clinical diagnosis.

1.4 Immobilization of the Biorecognition Element

The immobilization of BREs on the surfaces of the substrates is critical for the development of selective, sensitive, and stable POC devices (Kumar et al. 2019b). Many methods have been devised; however, challenges such as orientation, site specificity, binding density, and denaturation of the BREs remain to be solved. In this chapter, we described the common approaches utilized for the immobilization of the BREs, which can be categorized into five groups, physical, covalent, entrapment or encapsulation, cross-linking, and affinity immobilization (Fig. 1.7). The quest of which approach is superior to others is still open because it is difficult to apply one scheme for the immobilization of different types of BREs. Thus, the extrapolation of all immobilization methods is challenging because of the diverse characteristics of substrates and functional features of the BREs.

1.4.1 Physical Immobilization

Physical immobilization is the simplest and fastest way to attach BREs on the transducer surfaces. Physical attachment of the BRE on a surface can be achieved through hydrogen bonding, ionic bonds, intermolecular forces, polar, and

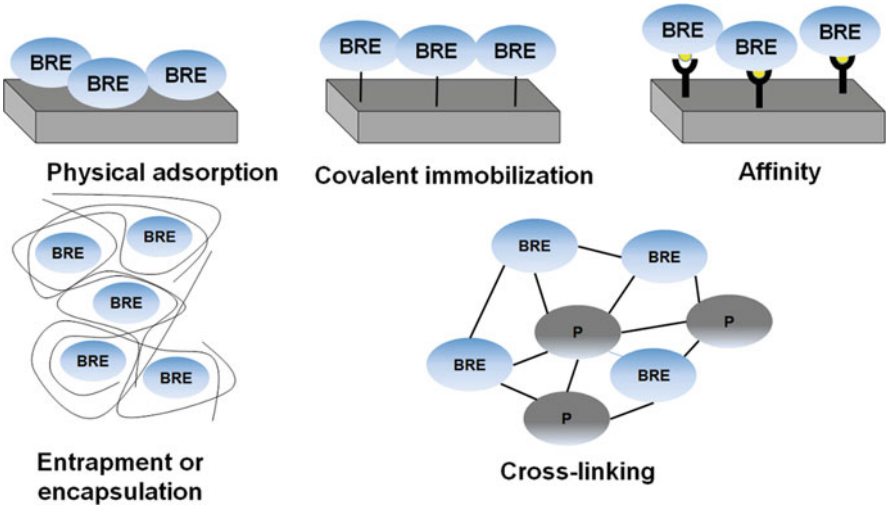

Fig. 1.7 Methods of BREs immobilization on substrate surfaces

hydrophobic interactions (Nguyen et al. 2019; Nimse et al. 2014). The physical immobilization has two major categories, physical and chemical adsorption. Physical adsorption is weak and occurs mainly via van der Waals forces between the BREs and the active sites of a surface. Chemical adsorption is stronger and occurs due to electrostatic interactions. The electrostatic immobilization method is a rare but more effective and fast method. In this method, the enzyme or protein is immobilized above or below their isoelectric point (PI) through the interaction with the oppositely charged surfaces. In this method the immobilized BREs exhibit high functionality and stability. However, the BREs may be desorbed with the change in the physiological pH of the medium.

Many substrates including charcoal, clay, cellulose, kaolin, glass, silica gel, collagen, and others adsorb BREs on their surfaces. The physical attachment depends on the nature of the surface and the type of BRE. Each BRE molecule can form many interactions with a surface to minimize surface energy (Nguyen et al. 2019). Therefore, the physical attachment of BREs is random and heterogeneous. Also, in this approach, the adsorption capacity and high-density immobilization are limited due to the geometric size of the BREs and sterically blocking of active sites on a surface, respectively. This approach has the disadvantages of random orientation and weak interaction. Furthermore, the background signals arising from the nonspecific interaction can cause interference and lead to false detection.

1.4.2 Covalent Bonding

In this method, a covalent bond is formed between the BRE and functional group of the engineered surfaces. The enzymes can be bonded covalently to the substrate

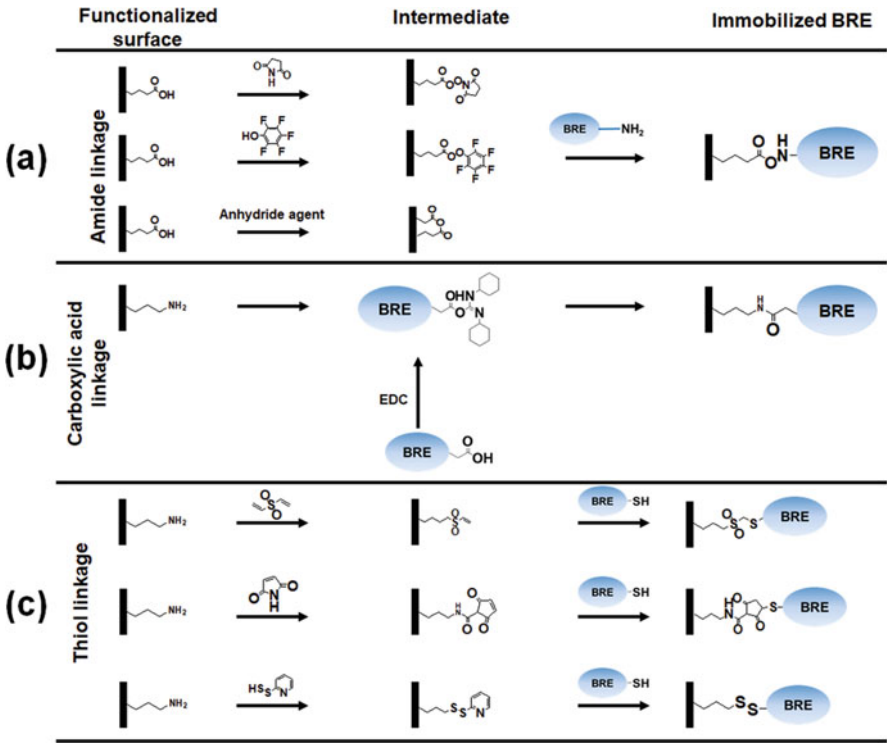

Fig. 1.8 Covalent immobilization of BRE through (**a**) amide, (**b**) carboxylic, and (**c**) thiol linkage of the BREs

surface through the enzymes sites which do not affect its normal activity. The covalent bonding can be achieved in mild conditions such as low temperature, low ionic strength, and pH in the physiological range for the attachment of BRE to the transducer (Huang 2019). As described below the covalent immobilization can be achieved through various types of chemical reactions.

Amide Linkage

The interaction between the carboxylic acid groups of a surface and the amine groups of a BRE is the most common type of covalent immobilization. The BREs are usually exhibiting numerous amine groups and it is easy to achieve this interaction. The amide linkage between BRE and surface carboxylic acid groups is achieved through different methods (Fig. 1.8a). The carboxylic acid groups on a surface are activated using NHS, which subsequently react with nucleophilic amine and form a stable carbo-amide bond (Rashid and Yusof 2017). The experimental conditions such as concentration, pH, reaction time, and ionic strength greatly influence immobilization. The reactivity of the NHS-activated carboxylic acid group can be enhanced by employing pentachlorophenol into the system. Besides, aldehyde-amine chemistry has been frequently utilized for the immobilization of

proteins on various substrates. The interaction between aldehyde and amine groups forms a labile Schiff's base. The bond is further reduced to form a stable secondary linkage (Melo et al. 2017). Furthermore, amide linkage can be formed through the interchain anhydride intermediate (Yan et al. 1997). In this approach, the surface carboxylic acid groups are dehydrated with trifluoroacetic anhydride, which yields interchain anhydride. Subsequent exposure of this activated surface to BRE forms amide linkage.

Linkage Through the Carboxyl Group

The immobilization of BRE can also be performed via activation of its carboxyl group. In this approach, a BRE-containing COOH group is activated with carbodiimide to form a reactive intermediate. The reactive intermediate is then attached to the amine groups on the surface of substrates through amide linkage (Fig. 1.8b) (Smith et al. 2020). A low concentration of the carbodiimide allows the easy immobilization of the BRE; however, a high concentration of carbodiimide significantly decreases the activity of BRE, particularly of enzymes (Tischer and Wedekind 1999).

Linkage Through Thiol Group

Proteins contain cysteine amino acids that exhibit sulfur (thiol) groups. The surfaces can be modified to form intermediates such as maleimide, disulfide, or vinyl sulfone (Fig. 1.8c). The maleimide intermediate then makes covalent thioether linkage with the thiol group of protein through an addition reaction (Rao et al. 1998). This reaction is feasible at pH 6.5–7.5, while at more basic pH, cross-reactivity occurs with the amine (Rao et al. 1998). Also, this reaction is fast but some problem arises in long-term operation due to hydrolysis. Disulfide linkage containing protein undergoes disulfide exchange with the surface in the presence of disulfide reagents and forms a new mixed disulfide bond. However, this linkage is unstable and reversible in the presence of a reducing agent. The surfaces containing vinyl sulfone react with thiol groups of protein through Michael's addition reaction. Michael reagent has the advantages of selectivity for thiol groups and stability in the aqueous medium (Pavlovic et al. 2003).

Linkage Through Epoxy Group

The epoxy group allows easy immobilization of the BREs onto the surfaces due to its high stability in an aqueous medium and neutral pH. Also, it forms a strong bond with different nucleophilic groups such as hydroxyl, thiol, amine, and others (Fig. 1.9a) (Chaparro Sosa et al. 2021). However, the reaction between the epoxy and the functional groups of a BRE is extremely slow. Mateo et al. developed a two-step immobilization technique. Firstly, the BRE is attached to the surface through hydrophobic interaction to obtain a high amount of recognition molecules on the substrate. Subsequently, the BRE reacts with the surface epoxy groups and forms a strong covalent linkage (Mateo et al. 2003).

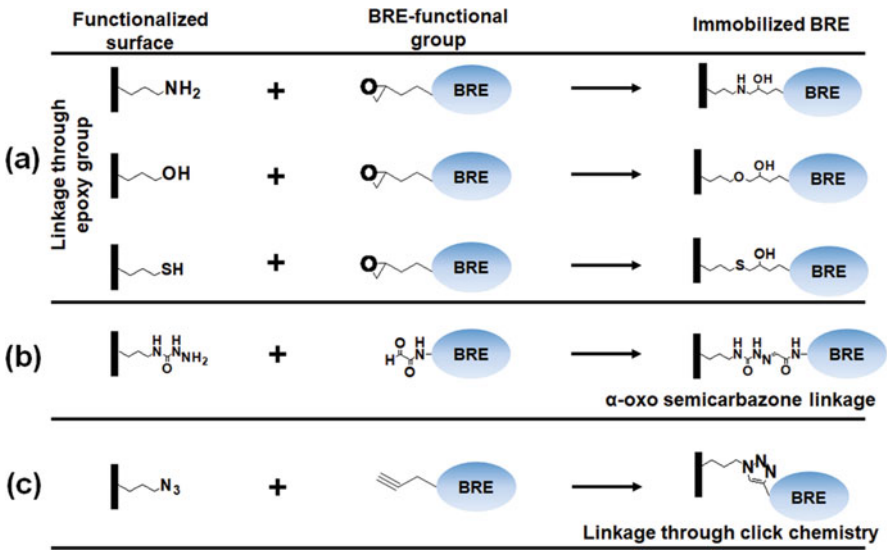

Fig. 1.9 Immobilization of BREs through (**a**) epoxy, (**b**) α-oxo semicarbazone, and (**c**) 1,2,3-triazole linkages

Linkage Through α-Oxo Semicarbazone Group

The BREs containing α-oxo aldehyde groups can form a covalent linkage with the surfaces of the substrate functionalized with semicarbazone molecules through condensation reactions (Fig. 1.9b). This linkage does not undergo hydrolysis and is therefore used in numerous bioconjugation including biotin (Rusmini et al. 2007), peptide ((Coffinier et al. 2007); (Olivier et al. 2003)), and antibody (Fertier et al. 2009).

Linkage Through Click Reaction

Click chemistry is the cycloaddition of an alkyne and azide to yield 1,2,3-triazole (Fig. 1.9c) (Rostovtsev et al. 2002). This method has simple reactivity and easy purification and has high stability because the formation of the product in this reaction is irreversible (Santra et al. 2020). In surface modification and immobilization of BRE, click chemistry is of great significance due to its ability to obtain a high density of BRE on a surface. Through this approach, many BREs such as recombinant proteins, biotin, lactose, polyethylene glycol, and DNA have been immobilized without producing any by-product (Jewett and Bertozzi 2010).

Affinity Immobilization

In this method, the surface and BRE have affinity bonds between the functional group (Andreescu et al. 2006). For example, avidin protein on the support has a high affinity for biotin attached to other proteins, enzymes, or DNA. This method allows controlled and orientated specific immobilization of biological components. This interaction is stable in harsh conditions of pH and temperature and allows well-

oriented immobilization of the recognition molecule (Loo et al. 2013). However, the drawback of this method is the need for the presence of specific groups on the enzyme. Furthermore, protein A or protein G is suitable for affinity-based immobilization of antibodies (IgG) (Saleemuddin 1999). The affinity immobilization of IgG has the advantage because protein A interacts with its Fc region and the Fab variable region remains accessible. However, the disadvantage of this method is protein A only interacts with certain classes of antibodies. In contrast, protein G shows diverse interaction with the IgG of different classes and species.

Entrapment or Encapsulation
In this method, the BRE is mixed with the monomer solutions followed by polymerization (Cosnier 1999). The BREs entrap inside the polymeric network. This method has the limitation of efficient diffusion of the analyte to the reaction site at the interface causing a delay in the reaction. Besides, the BRE can be leached out from the porous structure of the gel. Several polymer systems including starch gels, polyacrylamide, silastic gels, nylon, and conducting polymer can be applied for entrapment immobilization (Reetz et al. 1996).

Cross-Linking
In this approach, the BREs are labeled with the cross-linking agent followed by the linkage with solid or other support (Yang et al. 2010). Glutaraldehyde is the most used functional agent (Gade et al. 2006). The immobilized biomolecules are sufficiently stable; however, the use of excessive cross-linking agents can affect the activity of the BREs.

1.5 Summary and Conclusion

Biomolecules are chemical compounds such as glucose, urea, cholesterol, protein, enzyme, antibodies, toxins, DNA, and others making the building blocks of the living organism. These molecules control the normal functioning of the living cells. Therefore, monitoring the level of biomolecules and other pathogens such as bacteria, viruses, and fungi, which can influence the normal functions of a body, is of great significance. POC devices enable the robust collection of information about a target biomarker present in biological, environmental, food, and other samples. POC combines a biomolecule recognition element (BRE) with a transducer to achieve qualitative and quantitative analysis of the biomolecules. Modification of transducer surfaces is important to allow stable immobilization and retaining bioactivity of the BRE and to enhance the selectivity, sensitivity, and durability of the POC devices. Morphological or physical modification of the surfaces increases the surface area and durability, allowing high loading of the BRE. In this chapter, different modes of surface engineering such as the Langmuir-Blodgett technique, self-assembled monolayer, polymer brushes, lithography, polymer hydrogel, molecular beam epitaxy, and sputtering have been described along with their application for the design of POC devices. This list can be further extended with other

modification techniques such as chemical vapor depositions, thermal evaporation, solution casting, and others. However, these techniques were rarely adapted for the development of POC testing. Among surface engineering approaches, a self-assembled monolayer is simple, fast, and easy to achieve almost on any type of surface. Polymer brush-modified substrates exhibit high specificity by avoiding false-positive or false-negative signals generated from the direct contact of the target analyte with the surface. The techniques for the immobilization of the BREs on the modified substrates are also discussed. These approaches are categorized into five groups, physical, covalent, entrapment or encapsulation, cross-linking, and affinity immobilization. However, challenges such as orientation, site specificity, binding density, and denaturation of the BREs remain to be solved. The question of how one immobilization approach is superior to others is still open because it is difficult to apply one scheme for the immobilization of different types of BREs. Thus, the extrapolation of the immobilization methods is challenging due to the diverse varieties of the BREs and characteristics of the surfaces of the substrate.

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2 Functionalization Strategies for the Development of Nano-Bio-Conjugates in Contemporary Point-of-Care Analytical Devices

Ashutosh Kumar and Pranjal Chandra

Abstract

Nanobiotechnology has enabled the path for novel diagnostic strategies and nanomedicine to be developed and tested. Potential biomarker detection by existing nanosensors is significant in a wide range of industries, including healthcare and environmental monitoring as well as the food industry. Developing such systems frequently necessitates several functionalization stages for a large number of nano-bio-conjugates, which are then used in biomedical applications. Researchers must also look at the interactions that occur at the nano-bio-interface in order to take advantage of the usage of nanomaterials found in nano-bio-conjugates. Surface design, biomolecular conjugations, and sensor-interface interactions have been highlighted in this book chapter due to recent breakthroughs in nanotechnology. The limitations of operating at the nanoscale and the need for functionalization in biosensor fabrication are discussed in this chapter. The importance of deciphering the classic methodologies utilized for surface engineering and material functionalization has been highlighted. An additional table has been included to give readers of interdisciplinary fields a better understanding of the functionalization procedures and their applications.

Keywords

Nanomaterials · Biosensors · Surface functionalization · Nano-bio-conjugates

A. Kumar
Department of Electrical Engineering, University of Notre Dame, Notre Dame, IN, USA

P. Chandra (✉)
School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India
e-mail: pranjal.bce@iitbhu.ac.in

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2.1 Introduction

The subfield of nanotechnology known as "nanobiotechnology" has emerged to include all aspects of biological science and research involving the use of nanomaterials (NMs) (Kumar et al. 2018, 2019d, 2020a). The precise characteristics of what makes up an NM are not yet well-established, and there is a lack of standardization or definition of nanomaterials. NMs were defined as any material with at least one dimension in the range of 100 manometers, regardless of whether they were organic, inorganic, or both. In addition, the materials go through a process called nanofabrication in order to acquire the desired physical, chemical, mechanical, and optoelectronic properties (Cao and Wang 2004). In their categorizations of materials, international institutions such as International Association of Advanced Materials, International Centre for Diffraction Data, etc., have established an upper size boundary of 100 nanometers (nm) in at least one dimension. Despite this, there is not enough information that has been systematically gathered to support this upper size limit for any of the NM kinds (Cao and Wang 2004). We defined NMs as any substance in the submicron dimension that can be interfaced with biomolecules in order to generate a new "value-added" unit using any biotic or abiotic approach. In addition, the material needs to be purposefully sculpted in the nano-dimension to have distinct functional or structural entities arrayed on its exterior or interior, as well as unique features or configurations that cannot be obtained from a similar bulk material. Therefore, the surface modification of nanomaterials is of great importance to realize the true potential of nanotechnology in biological and analytical applications.

The main goal of nanobiotechnology is to build a novel assembly of biomolecules that are capable of acting in a way that has never been seen before by utilizing the intrinsic physical, mechanical, and catalytic qualities of NMs (Labhasetwar and Leslie-Pelecky 2007; Mahato et al. 2016; Savaliya et al. 2015), for instance, the use of NMs to assist drug distribution to the intended place, in vivo imaging, and the production of biosensors with the intention of removing hurdles in contemporary healthcare applications (Chandra 2015; Mahato et al. 2018a, 2018c). The subsequent goal focuses on using the unique features of biomolecules that come from their biomolecular interactions to create organized nano-devices with unique or novel properties (Alkilany et al. 2013; Hajipour et al. 2014; Tang et al. 2015). In the quest to develop single molecular electrical devices at the sub-lithographic scale, nucleic acid assemblies are being used to regulate the location and arrangement of nanoparticles (NPs). These NPs can be as small as one atom in size (Simmel and Dittmer 2005; Veerapandian and Yun 2011). Triglycerides, fatty acids, carbohydrates, proteins, peptides, and other biomolecules, whether monomeric or polymeric, are discussed as important materials to design nano-devices. Nucleic acids (DNA/RNA/PNA/LNA as genes, oligomers, aptamers, and ribozymes/DNAzymes) are also deliberated as promising materials to fabricate nano-devices (Simmel and Dittmer 2005). In a nano-bio-conjugate (NBC), the biomolecules can either serve as a functionally active entity that provides a place for immobilization, catalysis, or therapeutic action (e.g., antibodies or enzymes) or they can serve as a

passive cover or support material (Baranwal et al. 2016, 2018c). In order to accomplish the applications described above, it was necessary to construct a number of NBCs, any one of which might be put to use in the planning, creation, or improvement of a specific healthcare technology. Additionally, the development of the biosensor trade has greatly impacted the market pull of diagnostic devices across the globe (Kumar et al. 2022; Mahato et al. 2022; Purohit et al. 2022a, 2022b). The significance of using NMs, especially NPs, in the synthesis of NBCs stems from the exceptional size-dependent physical, chemical, and optoelectronic properties that they can contribute to the final NBCs (Baranwal et al. 2018a, 2018b; Kumar et al. 2018, 2019a, 2019b, 2019c, 2019d, 2020a, 2020b; Mahato et al. 2020; Mahato et al. 2020a, 2020b; Mahato et al. 2018b, 2021; Purohit et al. 2019a, 2019b, 2020a, 2020b, 2020c; Wadhwa et al. 2019; Chandra et al. 2010). Examples of size-dependent features include quantum-confined properties like photoluminescence of nanocrystals, surface plasmon resonances (SPR) of gold (Au) NPs, electrical conductivity of carbon-based NMs, and magnetic properties, as well as the catalytic properties of some metal nano-alloys and metal oxide NPs. In addition to this, the NPs have active interaction areas and high surface-to-volume ratios (S/V). This incredible S/V ratio not only provides greater interaction areas, but it also prepares the door for multi-functionalities on the NMs. Because of their small size, NMs make it possible to include a large number of catalyst surface or chemical functionalities onto a single particle. For instance, dendrimer NPs, which can have many reaction and chemical sites for interfacial interactions, are one type of particle that can do this (Kumar et al. 2019a).

The purpose of this book chapter is to discuss about the many different strategies that go into the synthesis of NBCs so that biological applications can be made better. Because it also explains the bio-physicochemical interactions of biomolecules that are used in the manufacture of NBCs, this book is appropriate for readers from a variety of interdisciplinary fields. In the hope that it will do so, this book chapter will probably generate more interest in the form of perceptions associated with the usage of NMs, which will allow for a better understanding of the difficulties in the design of NBCs and their applications in biological research. Focus has been placed on the introduction of nanomaterials, bio-conjugation, and nano-bio-interface in order to provide a fresh perspective on the multidisciplinary activities that have been done in the field of applied biology and material engineering. It has emphasized the importance of nano-bio-conjugate not only for the nanomedicine development, but it has also emphasized its crucial possibilities in current healthcare devices, such as the development of nano-biosensors. Both of these developments are related to the advancement of nanomedicine and healthcare technologies. In this article, we focus on the linkage chemistry that is employed for biomolecules to interact with NMs. This chemistry is ultimately what determines whether or not nearly all NBCs are successful in the applications for which they have been claimed. The procedures (targeted functional group, reactive group, product, and related mechanism) that are routinely utilized in the manufacture of NBCs have been compiled into a table for the purpose of assisting readers of interdisciplinary research in their comprehension of these methods. Regardless of the exact value or aim of the research being conducted,

the multidisciplinary research effort is focused on finding ways to interface biomolecules with NMs in order to develop novel functional units. As a consequence of this, it is essential to have an understanding of the challenges that are inherent in working with NMs, which need to be accounted for throughout the production of NBCs.

2.2 Challenges in Working with NMs for Biological Applications

There are many challenges related with the preparation of NBCs, all of which are significant enough to be taken into account in order to make the most effective use of them. Not only in terms of their sizes and shapes but also in terms of the constituent parts that make them up, NPs exhibit a great deal of morphological variety (Mandal et al. 2018). Some nanoparticles (NPs), including those made of gold (Au NPs), silver (Ag NPs), and platinum (Pt NPs), as well as other monometallic nanoparticles (NPs) like those made of iron (Fe NPs) and palladium (Pd NPs), and so on, are uniform because they are composed of just one kind of element. In certain situations, NPs are composed of single or multi-metallic oxides, which not only influence the physical and chemical activities of NMs but also play an important role in determining the form and size of the NPs (Kumar et al. 2019c; Mandal et al. 2018; Saxena et al. 2018). The NMs are designed in such a way that they are able to give particular qualities for the purposes for which they are intended. Core-shell nanostructures are produced and constructed specifically for the applications in which they are to be used (Kumar et al. 2019b; Mandal et al. 2018). The core-shell nanostructures were designed with the specific applications in mind for which they were intended, with the external shells being designed to defend and safeguard the core structures, which are sometimes necessary for biological reasons. Because a number of NMs, including those made of metals, semiconductors, and carbon, have hydrophobic surfaces, it is necessary to modify those surfaces in order to make the NMs hydrophilic and compatible with biological contact (Ghosh Chaudhuri and Paria 2012; Levin et al. 2009; Oliver-Tolentino et al. 2018). A common example is the process of affixing or substituting hydrophobic structures with hydrophilic ligands or other layers in order to make it capable of facilitating aqueous distribution. This can be done either directly or indirectly. It is a difficult and challenging process to generate hydrophilic ligands onto hydrophobic surfaces because it requires modifying NMs at the level of small, charged molecules in order to produce dendrimers, which can completely encapsulate the NPs (Sapsford et al. 2013). This process is difficult and challenging because it involves modifying NMs. The covering ligands that are used in the construction of NBCs additionally deliver novel chemical entities that have the potential to operate as active sites for later bio-conjugation, in addition to acting as a stabilizer for NMs (Sapsford et al. 2011). The purpose of NBC synthesis is to attach desirable biological molecules to the surface of NPs and to conjugate these molecules directly with the assistance of surface-attached coating ligands. This can be accomplished either directly or through the utilization of minute cross-linkers or

other mediators. In general, bio-conjugation helps in the precise targeting of nanoparticles (NPs) in a complex biological environment by utilizing bioreceptor elements (such as antibodies or aptamers), or it allows NBCs to carry out biologically derived interactions, such as catalysis (using enzymes or DNAzymes), more effectively. The assembly of nano-bio-conjugates has the potential to become extremely complicated due to the possibility of numerous configurations and iterations of the structure of the coating, ligand, or biomolecule (Kuldeep Mahato et al. 2020a). In this rudimentary architecture, the biomolecules have a direct interaction with the core of the NM. Either the NMs encase the biomolecules in their diverse design or the biomolecules encase the NMs in their own structure.

The chemistry of ligand conjugation, in addition to post-conjugation ligand-NMs interactions, has significant repercussions for the stability of colloidal systems, especially in cellular contexts. In addition, the interactions between ligands and NMs are frequently very different from one another, and they cannot be uniform across the entire surface area. As a consequence of this, it is self-evident that NBCs of this kind should be created, as they not only have the potential to improve bio-conjugation but also to contribute to the overall system's stability (Medintz 2009).

The following characteristics can be utilized to affix biological molecules to NMs in the context of a broad and nondescriptive bio-conjugation:

(a) *Biomolecules ratio monitoring for NMs binding*. Different ratios are required for different uses of NBCs during making; one ratio cannot be used for several bio-coupling techniques. Conjugation interactions and avidity are heavily influenced by the number of biomolecules present. Over-bio-conjugation can also harm coupling connections, even though the NM surface and their curvature can help mitigate this to a degree (Medintz 2006a).
(b) *Alignment controlling onto the NMs*. The organization and affixing capability of aptamers, enzymes, proteins, and antibodies are dependent on the coverage of their binding sites to the environment, which can enhance interactions. Nonspecific binding or electrostatic relations in the production of NBCs might lead to heterogeneous adherence, which can influence the final application's activity (Medintz 2006a).
(c) *Monitoring the separation space from the NP*. Targeted medication administration and sensing using Förster resonance energy transfer are some applications that require controlled detachment at a precise distance (Medintz 2009).
(d) *Attachment affinity controlling during NBCs formation.* Lasting or labile coupling between NMs and bioreceptors is required depending on the application of NBCs. For example, where drug distribution is needed, labile coupling can deliver better execution, while in the development of immunoassays, lasting connection is required for bioreceptor adherence (Sapsford et al. 2013).
(e) *Best utility and activity conservation of NMs and bio-conjugates*. The job and action of each comprising component should not be modified or changed during the fabrication of NBCs. The lack of structural stability of NMs or the catalytic/

catching activity of bioreceptors may have an impact on NBCs' intended use (Medintz 2006b).

(f) *Reproducibility of NBCs*. The full potential of an NBC development technique can only be realized if the same strategy can manufacture multiple types of NBCs with different types of biomolecules (Sapsford et al. 2011).

As previously said, the criteria are unquestionable; nonetheless, the issue lies in comprehending and devising a novel fabrication approach for NBCs.

The term "nano-bio-interface" is frequently used in the field of nano-bio-conjugations to mean "nanosized connections to biological entity" (Hajipour et al. 2014; Nel et al. 2009). The poorly studied component of NM behavior in biotic environments is that when they are subjected to biological settings like blood, serum, or saliva, NMs engage in a range of bio-physicochemical activities with biological molecules (Gagner et al. 2012). The cornerstone of this research was laid by the interconnections between NMs and biotic molecules, which produced the characteristics that NMs and biotic compounds have on NBCs.

Columbic forces, hydrogen bonding relations, London dispersion, the environment's pH, and the lone-pair electrons available on the ligands plus NMs for the formation of NBCs all play a role in how biomaterials adhere to NMs (Alkilany et al. 2013; Tang et al. 2015).

When it comes to creating and developing nanomedicine, this particular study area has even more consequences. Furthermore, each type of NM's bio-conjugation chemistries must be improved because the nano-dimensional construction of NMs significantly affects reaction rate on NM surfaces (Purohit et al. 2020c; Wadhwa et al. 2019). Additionally, current findings have shown the effects of protein surface chemistry and NP dimension, which was corroborated by later NBC reception in macrophages (Behzadi et al. 2014). The problem can be made more difficult because each NM and NBC are anticipated to have highly different interactions based on their unique physical and chemical features (Purohit et al. 2019b). Biological counterparts may also shed their function during the synthesis of NBCs as a result of sequestration, unfolding, denaturation, or blocking of active regions (Hajipour et al. 2014). As a result, it is critical to devise and decode new methodologies that can be used to build and construct a new NBC that can endure these constraints.

2.3 Bio-conjugation Approaches for NBCs

While functionalization with biological molecules, NPs with material properties such as external color, SPR, conductivity, and affinity for attachment are greatly improved (Cai et al. 2018). It is an important phase that not only determines the operational activity of NBCs but also allows for the development of innovative nano-devices having enhanced sensing capabilities (Akhtar et al. 2018). The fabrication of any nano-conjugate or the design of an NM-based sensing system can be oriented toward an analyte that requires the binding of bio-recognition elements to its functional surface (Xu et al. 2012). The type of bio-coupling frequently demands the

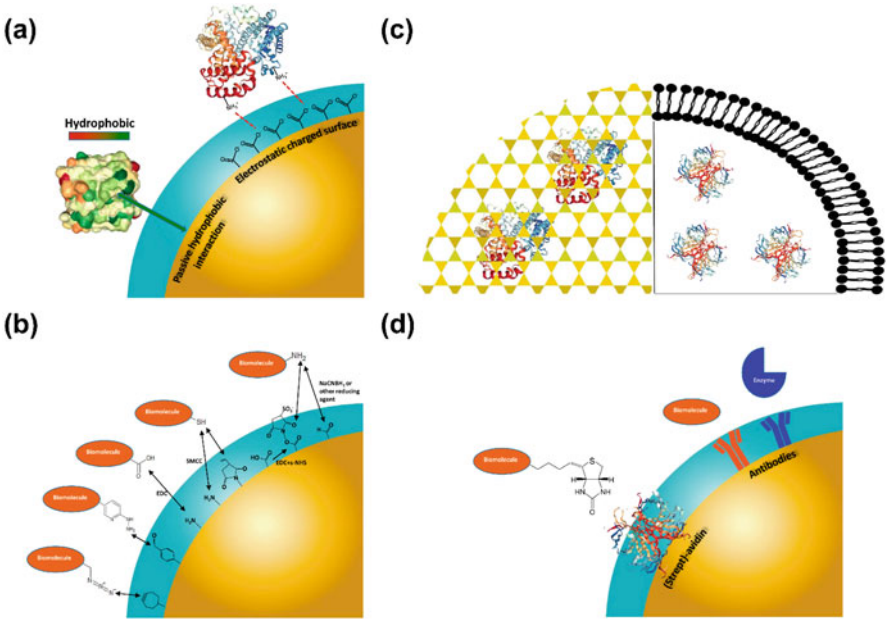


Fig. 2.1 (**a**) Adsorption, (**b**) covalent attachment, (**c**) physical entrapment (in left, sol-gel approach, and in right lipid vesicle entrapment), and (**d**) bio-affinity attachments such as EDC/NHS and SMCC-NHS. Reprinted with permission from Creative Commons Attribution from Magro et al. (2020)

use of specific linkers in addition to the NM interface (Aubin-Tam and Hamad-Schifferli 2008). The conjugation approach is essentially determined by a sequence of aspects involving shape, size, morphologies, interface interaction, and the inherent components of the NMs themselves (Hassan and Singh 2014). Furthermore, the type of biomolecules selected for NBC synthesis is influenced by the nature of surface ligands and their available, functional groups on NMs (Sapsford et al. 2011). Biomolecules are chosen based on their size, chemical bindings, and the final purpose expected in the end application. Covalent bonding to the surface of NM or noncovalent bio-conjugation is possible such as electrostatic interactions, as well as other kinds of adsorption and encapsulation. In order to correctly acclimatize and modify the outer surface of NMs, researchers have developed a variety of approaches to incorporate the projected functional outcomes in the NBCs (Nel et al. 2009). The most frequent techniques utilized to functionalize NMs are shown in Fig. 2.1. The NMs functionalization approach uses electrostatic activities at the interface to add a peptide; electrostatic charges that are opposing one another are used to assist electrostatic NP-peptide assembling in this process. The direct interaction approach is widely used for AuNP functionalization. Because of their strong affinity for NM surfaces, some protein peptides, such as free thiols, can connect to the surface of AuNPs by this technique. This approach involves defined

ligand-receptor interactions, such as biotin-streptavidin coupling, and depicts a covalent attachment strategy that employs traditional nano-functionalization chemistry such as EDC-based carboxyl coupling or NHS- and maleimide-mediated amine and thiol coupling. NMs have been widely functionalized with a variety of materials, including artificial polymers, biopolymers, dendrimers, and small molecules, using the methods described above. Fixing enzymes and antibodies to the functional surface of NMs requires a certain functional group, such as amine, carboxyl, thiol, or hydroxyl, which is controlled by the functionalizing process and chemicals employed for NBC synthesis.

Chemicals with free amine groups, such as 3-aminotriethoxy propyl silane (APTES), chitosan, and serine, are used to provide amine groups to the NBCs (Punyani and Sathawane 2013). Similarly, reagents with free carboxyl groups are employed to supply carboxyl groups to the final NBCs. It is essential to monitor and control the functionalizing agent throughout this adherence because its structure can influence electron transfer through the surface that can change the biosensor's response generation. Immobilizing bioreceptors, such as immunoglobulin and enzymes, is a crucial step in the development of biosensors (Veerapandian and Yun 2011). By keeping an eye on the general movement of bio-recognition components and maintaining it in a relatively distinct region using bio-functionalization, the adhesion of a bioreceptor to a sensor structure can be controlled in the context of sensor development, which can increase sensor probe consistency and extensibility.

To put it another way, the external surface of NMs must be adjusted to supply firmness, compatibility, and functional activity in order to harness their features in medicinal arenas. Some essential bio-functionalization options for the development of NBCs are explained in the next section. There are several ways to functionalize the nanomaterials to form NBCs; some of them are physical binding approaches such as encapsulation, surface adsorption, etc., which are also known as non-covalent binding approaches. The benefit of non-covalent functionalization is that they are straightforward and do not compromise the integrity of the molecules used or how they interact with the intended biological materials. However, a variety of variables, including pH and ionic strength, can potentially impact non-covalent alterations. The most common chemical methods for immobilizing biomolecules onto nanoparticles are summarized graphically in Fig. 2.1 (Magro et al. 2020).

The interactions used to couple bioreceptors to the NM interface for analytical purposes are typically based on a small number of key bio-conjugation reactions (Obermeyer and Olsen 2015). In the following sections, we will go over some extremely important covalent coupling techniques that are commonly utilized to develop NBCs for biosensors and nanomedicines (Jung et al. 2017).

2.3.1 EDC/NHS Covalent Binding

Carboxylates and primary amines are used in the bulk of conventional NMs synthesis methods because they contribute to both homogeneous NM size distribution and

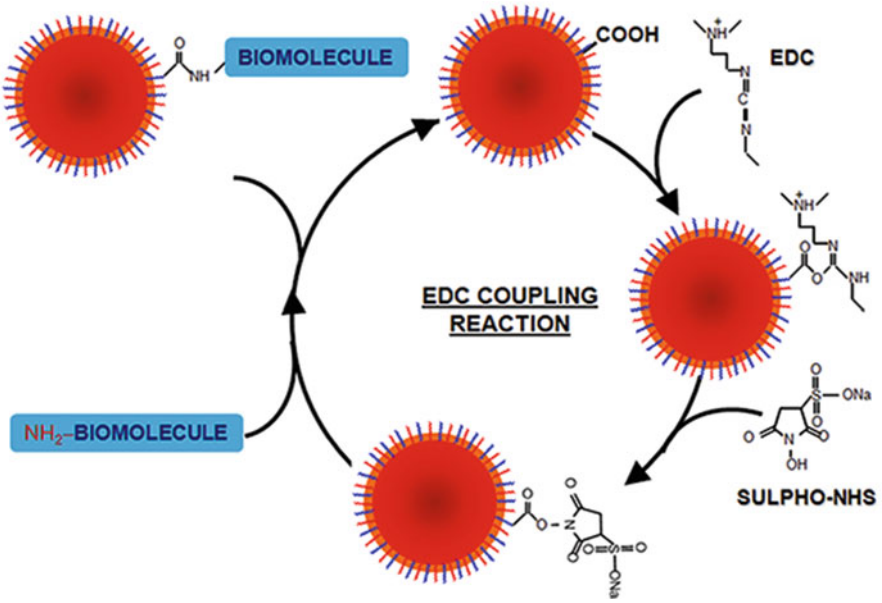


Fig. 2.2 EDC/NHS coupling method: EDC can be used to convert carboxyl groups of NMs/biomolecules to amine-reactive sulfo-NHS esters in the presence of sulfo-NHS. Reprinted with permission from Conde et al. (2014) with permission from ACS

surface stability. Because there are so many carboxylates and primary amines on NM surfaces, similar ligands on bioreceptors can interact with one another. One of the most common covalent chemistry techniques for producing functionalized NMs for diagnostics and sensor design is the generation of amide bonds. 1-Ethyl-3-(3-(dimethyl amino) propyl) carbodiimide (EDC), a zero-length bridging reagent that creates an active ester which can connect with primary amines in a succession of interactions, is the typical chemistry for establishing amide bonds on NM surfaces (Conde et al. 2014). The only esters that softly interact with primary amines and commonly trigger hydrolysis by-products, which block amide binding, are EDC-activated esters. As a result, NHS (N-hydroxysuccinimide) esters are frequently mixed with EDC to strengthen the hardness of the started ester intermediary (shown in Fig. 2.2).

Primary amine nucleophiles react violently with the NHS-activated ester intermediate to generate a long-lasting amide bond. To put it another way, the addition of NHS alleviates the amine-reactive intermediary by transferring it to an amine-sensitive sulfo-NHS ester, so improving the EDC-mediated binding processes' competence. To eliminate additional chemicals and interference caused by spinoffs, water is gently utilized. Cross-linking can be accomplished in standard physical circumstances with no use of supplementary chemical diluents once EDC becomes soluble in water. The EDC/NHS binding method is effective for making both antibody- and nucleic acid-based NBCs for biosensing probes (Hackett et al.

2017). Because primary amines are present in antibodies in sufficient amounts, they can readily attach to carboxylated NMs with no pretreatment. Furthermore, the EDC-imidazole bonding allows for the fixing of ethylene diamine to nucleic acids via 5′ phosphate groups, resulting in 5′-amine-functionalized oligonucleotides that can be linked to carboxylated interfaces of NMs (Subbiah et al. 2010).

In the fabrication of POC sensing devices, semiconducting NPs, magnetic NMs, and polymer NMs all have been utilized. These materials are frequently synthesized with carboxylate or amine groups on the surface to enable bio-conjugations via EDC/NHS coupling. Dihydrolipoic acid derivatives or other copolymers are frequently employed to stabilize quantum dots that attach carboxylate groups to a surface. In order to facilitate more cross-linking with pharmaceuticals and biomolecules that include a primary amine in their composition, carboxylated polymers are coated onto magnetic nanoparticles (NPs) that are also employed in biomedical diagnostics (Thanh and Green 2010). On NPs or sensing materials, silica shell compounds with carboxylate/amine ligands can also be employed as an option. In order to produce aminopropyl-silanol surfaces, silica oxide monomers like tetraethyl orthosilicate (TEOS) or APTES are coupled (Hsiao et al. 2007). A customized sensor with surface covered in aminopropyl-silanol can be connected to bioreceptors using EDC/NHS bonding. Silanol wrapping of NPs is an ideal method for developing diagnostic sensing devices since it not just supplies functional groups for additional bio-conjugation but also enhances biocompatibility and safeguards nuclear components.

2.3.2 Thiol Covalent Binding

The attachment of thiols existing on bioreceptors or on the NMS interface is another important coupling reaction for functionalizing NMs and sensor surfaces. DNA, RNA, and aptamers can be thiolated utilizing the similar method as described in the amine-functionalization procedure for nucleic acids (Bhand and Singh 2019). To add a disulfide bond, cystamine is utilized instead of ethylene diamine for thiol coupling, which can then be reduced using dithiothreitol (DTT) to yield a 5′-thiolated oligonucleotide probe. To produce thiols for bio-conjugation of NMs or onto the sensor surfaces, antibodies must be thiolated (using Traut's chemical) or cut so at interchain disulfides link (using DTT or papain). Biomolecules are typically thiol-coupled to noble metallic NMs (such as AuNPs or AgNPs) using coordinate dative bonds, where the sulfur lone pair electrons form robust bonds to the NM surface (Fig. 2.3a). Alternatively, a hetero-bifunctional linking chemical made from maleimide, such as succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), can be employed to intercalate compounds with thiol groups or NMs (Markwalter et al. 2019). A strong thiol-ether bond is created in this process when the thiol group joins with the maleimide functional group. The sulfo-SMCC NHS-ester component additionally provides interaction sites for reactions with primary amines seen in bioreceptors (Fig. 2.3b).

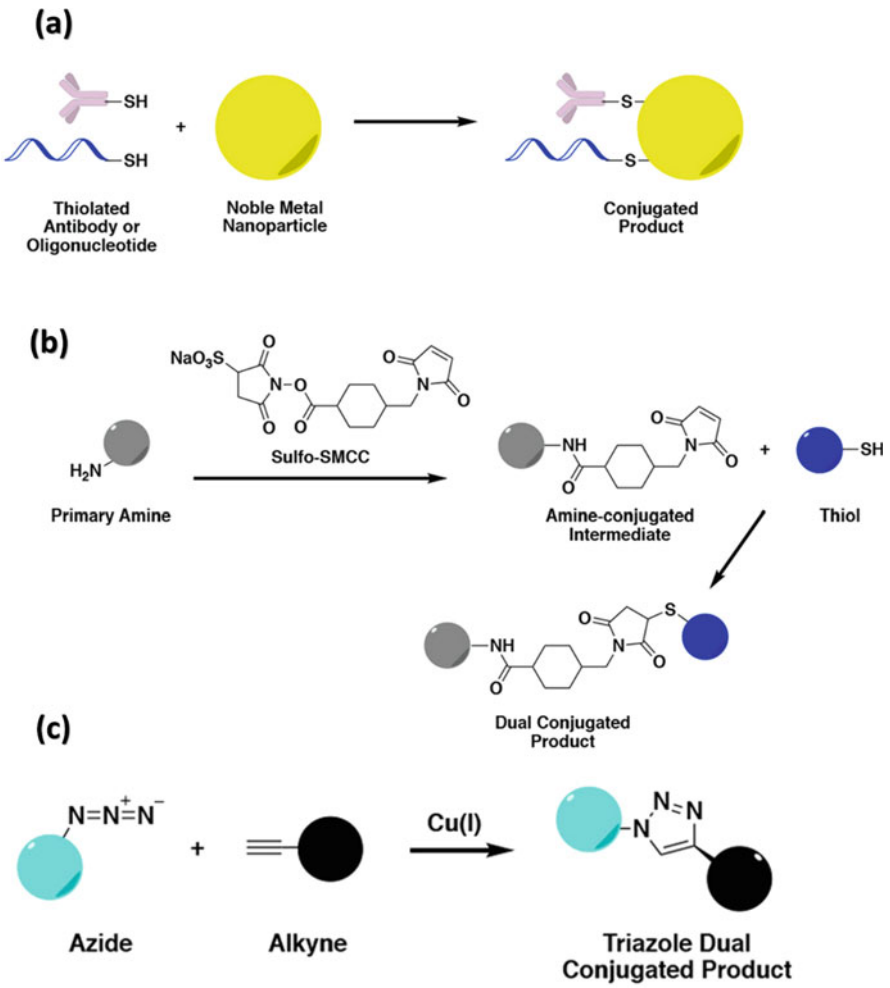


Fig. 2.3 (**a**) Antibody/oligonucleotide-mediated thiol covalent coupling to an NP; (**b**) sulfo-SMCC-mediated thiol conjugation of primary amine via hetero-bifunctional cross-linker; and (**c**) "click" chemistry attachment between an azide and an alkyne in the presence of a copper catalyst. Reprinted with permission from Markwalter et al. (2019) with permission from ACS

2.3.3 Click Chemistry and Photochemical Cross-Linking

Utilizing photochemical cross-linking processes along with "click chemistry," several NMs-based diagnostic sensors have been developed. When UV light is introduced to chemical compounds like phenyl diazirines and phenyl azides, they generate sensitive carbenes and nitrenes, separately, which aid in the production of new bonds between carbon, nitrogen, and hydrogen. These photochemicals can be included into hetero-bifunctional cross-linkers, such as sulfo-NHS-LC-diazirine, for

the purpose of bio-conjugation of NMs and immobilization of bio-recognition components. “Click” chemistry refers to bio-orthogonal chemical reactions that have high yields, little by-products, and low temperatures at which they operate. In the presence of a Cu(I), which is utilized for catalysis, the most common “click” chemistry-based chemical reaction is the addition of an alkyne to an azide (Fig. 2.3c) (Jung et al. 2017; Xu et al. 2019). There are numerous more chemical reactions that connect NMs and bioreceptors that are connected to “click” chemistry.

2.3.4 Biotin-Streptavidin Coupling

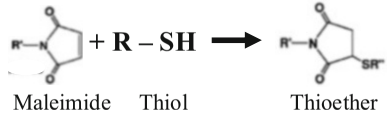
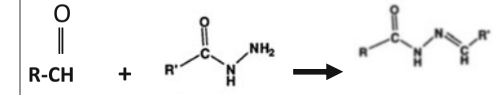
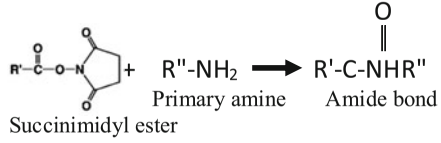
Biotin-streptavidin linkage is the most popular affinity-based coupling method for NMs functionalization and is ultimately applied in the fabrication of modern diagnostic tools. The tetrameric bacterial peptide streptavidin has a molecular mass of roughly 60 KD, while the small protein known as biotin has a molecular mass range of 1.3 to 10–15 KD. An extremely stable bond is produced by the stoichiometry of 4: 1 between biotin and streptavidin. Creating streptavidin-biotin combinations is a common technique for affixing biomolecules like DNA and antibodies. The first and most widely used technique involves joining a streptavidin-functionalized NM with a biotinylated biological molecule. It’s important to remember that streptavidin’s early adhesion to the NM may obscure one or more biotin connecting sites, and when combined in conjunction with the whole heterogeneity resulting from the technique, the final streptavidin-functionalized NM will surely reveal a plethora of different alignments. Streptavidin is often applied to the interacting faces of NMs via inert adsorption or a hetero-bifunctional coupler such as sulfo-SMCC. Attaching sulfo-NHS biotin or NHS-(PEG) n-biotin to a primary amine is the most popular method for biotinylating a bioreceptor. Although the streptavidin-biotin binding is a relatively anxiety-free procedure, a (PEG) n insertion can be used to improve biotin solubility (Gao et al. 2011; Haun et al. 2010; Wilchek et al. 2006).

There have been a variety of methodologies for NBC development, as shown in Table 2.1, where an apparent inventory of a variety of common NBCs and their expected functionality effectively mimics the scope of anticipated purposes and the strength underlying at the heart of this research (Huang et al. 2021).

2.4 Conclusions and Future Perspectives

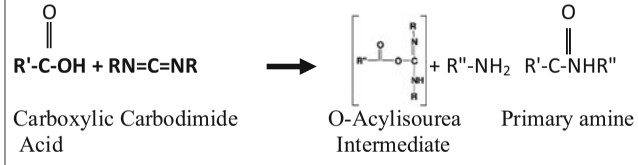
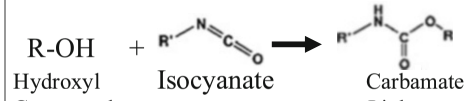
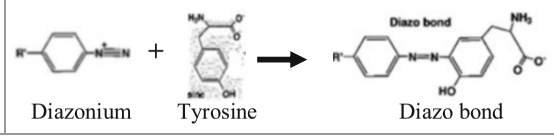
The different forms and shapes of the NMs and their composites influence the physical, chemical, and optical aspects of the synthesized NBCs. To design an intended device for diverse uses and while operating on the interface at nanoscales, it is essential to manage the variables that regulate the formation of NBCs, like the ratio of biological molecules, rate of cross-linking, alignment of biological macromolecules and NMs, connection predilection, and repeatability of the fabrication process. The well-thought-out functionalization strategy not only produces the required NBCs but also reaps the benefits of simple fabrication techniques and low

Table 2.1 List of common bio-functionalization techniques and reaction mechanisms used in the fabrication of NBCs

Aim for	Active group	Outcome	Reaction process
Free thiol	Maleimide Haloacetyl/alkyl halide Arylating agents Aziridine Acrylol derivatives Disulfide exchange-pyridyl disulfides, 5-thio-2-nitrobenzoic acid (TNB)	Thioether Thioether Thioether Thioether Thioether Mixed disulfides	
Aldehyde/ketone	Hydrazine Amines	Hydrazone Schiff's base (imine)	
Free amine	N-Hydroxysuccinimide ester (NHS) Acyl azides Isocyanates, isothiocyanates Sulfonyl chlorides Aldehydes, glioxals Epoxides, oxiranes Carbonates Arylating agents Imidoesters Carbodiimides, anhydrides	Amide Amide Urea, thiourea Sulfonamide Imine, secondary amine Secondary amine Carbamate Arylamine Amidine Amine	

(continued)

Table 2.1 (continued)

Aim for	Active group	Outcome	Reaction process
Carboxylate	Carbodiimides, carbonyldiimidazole Diazoalkanes, diazoacetyl	Amides Ester	
Hydroxyl	Epoxides, alkyl halogens Periodate Isocyanates, carbonyldiimidazole N,N′-Disuccinimidyl carbonate, N, N′-hydroxysuccinimidyl chloroformate	Ether Aldehyde Carbamate or urethane	
Reactive carbon (on a phenol, e.g., tyrosine)	Diazonium	Diazo bond	

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manufacturing costs. We sought to provide a quick picture of present methodologies in the production of various NBCs in this chapter. We come to the conclusion that NM of different shapes and sizes, along with their bio-conjugates chemically modified with linker components, can offer a unique perspective in research and the formulation of contemporary scientific strategies, with significant analytical and medicinal implications. It might encourage the development of revolutionary analytical tools and alter the course of bioengineering. The combined efforts of nanotechnologist and biotechnologist are greatly anticipated to generate new breakthroughs in the field of biomedical research. The desired characteristics in developed NBCs are necessary to boost the various interactions at the interfaces, even though it is challenging to create novel methodologies and manage the characteristics of NMs at this magnitude. Due to the dedication made to the investigations, we have solid reasons to believe in NM and associated bio-conjugates for the development of contemporary medical tools. The biocompatibility of a variety of NBCs is critical in a variety of in vitro and ex vivo research involving cells, serum, blood, tissue slices, and other materials that will determine the use of NBCs in in vivo situations.

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3 Advances in Three-Dimensional Metal Oxide-Based Nanostructures for Biological Gas-Sensing Applications

Tushar Kanta Sahu

Abstract

Nanostructured materials have been widely studied and established for various sensing applications in the last few decades. Nevertheless, there are still outstanding problems that need to be dissected to further boost their performance, reliability, and cost-effectiveness. Herein, three-dimensional (3D) metal oxide-based nanostructured materials and their implementation in various sensing devices are discussed. Furthermore, the significance of apprehension and modulating the 3D design frameworks is explored to achieve more effective sensing devices for different healthcare issues.

Keywords

Breath biomarkers · Volatile organic compounds · Disease diagnosis · Metal oxide-based semiconductors · Environmental sensing · Food quality and safety

3.1 Introduction

Metal oxide-based semiconductors (MOSs) are known for their tunable morphological, optical, and electrical properties and, hence, most popular for gas-sensing applications. Among various MOSs, n-type metal oxides such as WO_3, In_2O_3, ZnO, Fe_2O_3, and SnO_2 are known for their promising performances in various gas-sensing applications (Yuan et al. 2019; Ren et al. 2020; Li et al. 2021; Ma et al. 2020; Zhao et al. 2020; Zhou and Zhang 2021). The gas-sensing applications of p-type metal oxides are relatively limited due to their low sensitivity as compared to

T. K. Sahu (✉)
Department of Materials and Environmental Chemistry, Stockholm University, Stockholm, Sweden
e-mail: tusharkanta.sahu@mmk.su.se

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n-type metal oxides. Design and construction of nanomaterials with special morphology and nanostructure has a crucial role in enhancing the selectivity and limit of detection (LoD) (Zhou and Zhang 2021). Generally, three-dimensional (3D) nanostructures with variable geometry and crystal facets provide higher surface area and a greater number of active sites which results in efficient gas-sensing applications (Zhou and Zhang 2021). In this chapter, we will briefly discuss about the recent advances in 3D nanostructure-based materials for biological gas-sensing applications.

3.1.1 Principles of Gas Sensing

The general functioning principles of a MOSs-constructed device for gas-sensing detection comprise the variations of electrical properties. So, when a specific gas comes in contact with the sensor, it will change the resistance of materials which is sensitive to gases (Neri 2015; Zhang et al. 2020). If the chemical adsorption of the gas molecules on the semiconductor surface is strong enough, then they exchange electrons between them. This directed to an alteration in the concentrations of charge carriers and as a result conductivity of the materials also changes (Fig. 3.1a) (Neri 2015). The amount of gas adsorbed directly depends on the concentration of the target gas, which in terms set the affiliation between the resistance value and concentration of target gas.

The mechanistic path involving n-type MOSs is shown in Fig. 3.1b (Kim and Lee 2014; Gurlo et al. 2005). In this type of sensing device, the oxygen molecules from air are adsorbed on the surface and interact with the electrons of sensing materials. These adsorbed oxygen molecules ionize to O_2^-, O^-, and O^{2-} depending on the working temperature. These working temperatures are generally high for MOSs-based sensing devices (Zhou and Zhang 2021). The interlinkage within the sensing surface and target gas results in the formation of a depletion layer, which further consequences in the increase of resistance due to an increase in potential barriers. Based on these mechanisms, reducing gases such as CO, H_2, acetone, ethanol, etc.

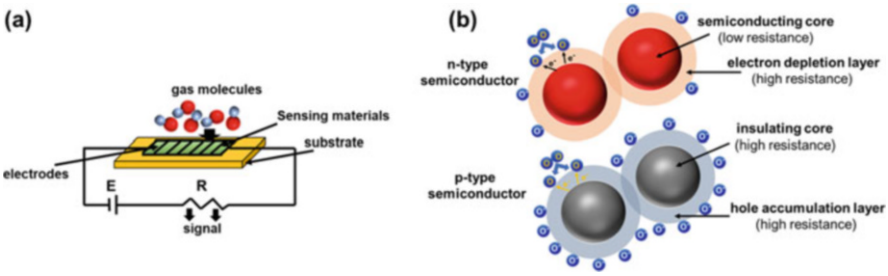

Fig. 3.1 (**a**) Detection circuit of a MOSs-based sensor [Reproduced with permission from Ref. (Zhang et al. 2020), copyright Elsevier] and (**b**) working mechanism for MOSs-based sensors. [Reproduced with permission from Ref. (Kim and Lee 2014), copyright Elsevier]

reduces the resistance, whereas oxidizing gases such as SO_2, NO_X, O_3, etc. increase the resistance (Zhang et al. 2020).

Besides MOSs, for other gas-sensing materials based on carbon and MXene, the oxygen molecules adsorption is not necessary for sensing. Herein, the mechanism involves physisorption of target gases on the surface of sensing devices, and they interact through van der Walls or the donor-acceptor interaction. Presence of various functional groups such as -OH, −COOH, and = O also increases the gas molecules adsorption. Also, the number of adsorption sites on the sensing material surfaces and higher binding energy between target gases and sensing surfaces gives rise to efficient detection of gas molecules (Zhou and Zhang 2021; Degler et al. 2019).

3.2 Analytical Performance of Gas Sensors

The performance of sensor devices can be identified by numerous constraints which include the limit of detection, selectivity, sensitivity, cross-sensitivity, response and recovery times, stability, and lifetime. Based on these constraints, the functioning qualities of gas-sensing devices are quantified.

The lowermost concentration of the gas analyte that can be measured by the sensing devices is termed as the limit of detection (LoD). The LoD is a central constraint to know the aptness of any type of gas sensor. For example, when gas sensors are premeditated for air quality nursing, based on their LoD values, it can be checked if they are suitable for the sensing or not. Table 3.1 shows the threshold boundaries of the key air pollutants set by the National Ambient Air Quality Standards (NAAQS) in the USA and also by the European Union (EU) in Europe (Isaac et al. 2022). Although Table 3.1 only shows the LoD values, it is necessary to consider other parameters such as selectivity, cost-effectiveness, and robustness before employing the sensors for air quality nursing.

Table 3.1 European Union and US agencies approved threshold limits for some common pollutants with LoD of best-performing sensors reported in the literature

Pollutant	Threshold limits as per EU	Threshold limits as per the USA	Best-performing sensor (in terms of LoD)	References
CO	10 ppm	9 ppm	1 ppm	Lin et al. (2018)
CO_2	N/A	N/A	150 ppb	Willa et al. (2015)
SO_2	130 ppb	75 ppb	38 ppb	Prajapati and Bhat (2018)
O_3	120 ppb	70 ppb	20 ppb	da Silva et al. (2017)
NO_2	50 ppb	53 ppb	5 ppb	Rossinyol et al. (2007)

The sensitivity of a sensing device can be demarcated as the alteration in electrical signal with respect to the concentration of the target gas. It is represented as the ratio between the response signal when the sensing device is exposed to the target gas analyte and the response signal in the presence of air:

$$S = \frac{R_g}{R_a} \tag{3.1}$$

where R_g represents the resistance of air containing a fixed concentration of target gas and R_a represents the resistance in pure air. The sensitivity of a sensing device is dependent on external factors such as humidity and operating temperature and also on the intrinsic properties of the sensing materials (Wang et al. 2010).

The dynamic behavior of a gas sensor can be expressed by its response and recovery times. The definition of response time is the time required to attain a stable signal by the sensing devices when exposed to a specific amount of target gas, whereas the recovery time is the time required for a sensing device to reappearance to its original value in the absence of target gas. Typically, the response time is calculated when the signal takes 90% of the saturation signal and the response time is 10% of the saturation signal. This is for the reason that vast majority of the sensing devices take quite a few hours to reach the concluding saturation value due to slower kinetics after certain time (Isaac et al. 2022).

The aptitude of a gas sensor to identify a target gas among other various gases is titled its selectivity. It is a crucial parameter because numerous gaseous species present with the target gas can change the resistance with a similar way as of the target gas (Seinfeld and Pandis 1998). The reproducibility or stability of a gas sensor is also important for real-world applications as it reflects the ability of the sensing devices to reproduce the same signal when exposed to the target gas several times (Bochenkov and Sergeev 2010).

3.3 3D Nanostructures for Gas Sensing

Although many low-dimensional sensing devices including nanostructures of 0D, 1D, and 2D have been utilized in gas sensing, the main issues which hinder the functioning of these gas-sensing devices are structural stability, dispersibility, and sensibility. However, 3D nanostructures developed by incorporation of low-dimensional particles can overcome the abovementioned drawbacks with excellent gas-sensing applications.

3.3.1 3D Gas Sensors for Environmental Applications

Pollution of air by pollutant gas such as CO, CO_2, SO_2, NO_x, CH_4, etc. is a serious global concern as it is harmful to the environment as well as humans due to hasty industrialization, usage of agrochemicals, and the incineration of fuels. Besides this,

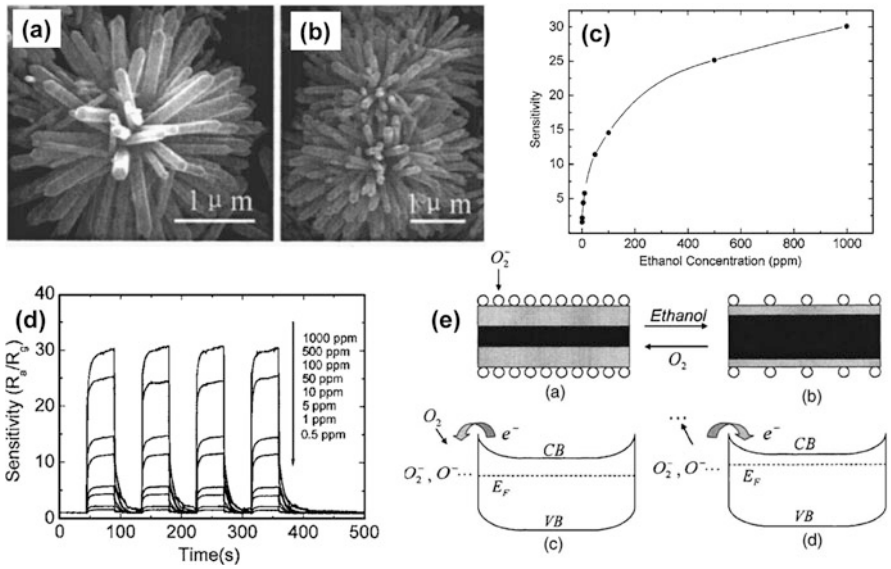

Fig. 3.2 (**a**, **b**) SEM images of flowerlike ZnO nanostructures composed of nanorods, (**c**) the sensitivity of sensors vs ethanol concentration, (**d**) the sensitivity response of sensors vs ethanol concentration, and (**e**) the mechanism involving the sensing of ethanol. [Reprinted with permission from Ref. (Feng et al. 2005), copyright AIP Publishing]

other gaseous pollutants such as NH_3, benzene series, CH_2O, etc. originating from industries also severely affect the human health when the exposed limit is exceeded (Mirzaei et al. 2016). Therefore, it is essential to oversee the concentration of these harmful gases for better environment.

3D nanostructures create a better ohmic contact with the substrate it is grown as compared to 1D nanostructures. For example, it was found that 3D hierarchical superstructure of ZnO grown over an FTO substrate has a lower ohmic resistance as compared to 1D ZnO nanowires as reported by Ansari et al. (Ansari et al. 2018). Besides, 3D hierarchical superstructure of ZnO with a large surface area and higher electron mobility results in better NH_3 sensing as compared to 1D nanowires of ZnO. Besides morphology, the defects and porosity existing in the 3D ZnO mesoporous thin films are also beneficial for sensing of NO_2 gas (Patil et al. 2018; Mane et al. 2021). Also, the 3D morphology of ZnO gives rise to porous structure and high surface area as compared to lower-dimensional ZnO nanostructures which facilitate better transport of mass and diffusion of gas in the sensing material (Mane et al. 2021). Krishnakumar et al. premeditated the effect of various 3D nanostructures of ZnO (flowerlike, starlike, spherical) for sensing of CO gas. All these nanostructures were prepared by a microwave-assisted method. It was found that the nanostructure with flowerlike morphology is more efficient for CO gas sensing than the other two nanostructures due to its trivial crystallite size, larger surface area, and modified barrier in potential (Krishnakumar et al. 2009; Feng et al. 2005). Figure 3.2 shows

the sensitivity of ethanol sensing with 3D flowerlike nanostructures of ZnO. SnO_2 nanostructures also have potential applications as gas sensors due to its upright selectivity as well as sensitivity to various gases. SnO2 with different morphologies have been extensively explored for gas sensing (Firooz et al. 2009; Periyasamy and Kar 2020; Wang et al. 2016; Yuliarto et al. 2015). Also, 3D nanostructures of WO_3 are efficient in sensing NO_2 gas with superior selectivity (Cao et al. 2020).

Although, many gas sensors reported in the literature are effective in terms of lower value of LoD and good selectivity, however, operating temperatures for these sensors are very high. For actual environmental applications, gas sensors with lower operating temperature are needed to construct a long-term stable monitoring device.

3.3.2 3D Gas Sensors for Breath Analysis

Exhaled breath samples of human are very complex as it consists of carbon dioxide, nitrogen, oxygen, nitric oxide, and thousands of volatile organic compounds (VOCs). Among them, many VOCs have clinical significance as a biomarker for identification of some specific diseases (Alam et al. 2019). For example, a high level of acetone, nitric oxide, ammonia, hydrogen sulfide, and toluene are typically associated with diabetes, asthma, renal disease, halitosis, and lung cancer, respectively (Awano et al. 2008; Shin et al. 2012; Narasimhan et al. 2001; Gouma and Kalyanasundaram 2008; Peng et al. 2009; Yoon and Lee 2017). Table 3.2 shows some exhaled breath biomarkers related to diseases with their permissible limit (Das and Pal 2020). So, analyzing human respired breath samples through gas sensing characterizes a simple, economical, and faster method for disease diagnosis and prevention. However, the concentrations of disease-associated biomarker are very little (in ppb level). Therefore, gas sensor used for this type of operation should be highly selective toward a specific VOC with very low LoD (Konvalina and Haick 2014).

Table 3.2 Exhaled breath biomarkers related to diseases and their permissible limit

Biomarker	Related diseases	Maximum permissible limit
Acetone	Diabetes	0.9 ppm
NO	Asthma, lung cancer	25 ppb
H_2S	Ischemia, asthma, Down syndrome, Alzheimer's disease, halitosis, etc.	8–16 ppb
NH_3	Cirrhosis of the liver, halitosis, peptic ulcer, liver dysfunction, renal failure, etc.	250 ppb
Methane	Liver diseases, breast cancer, colon- and intestine-related diseases, asthma, etc.	–
Aldehydes	Alzheimer's disease; cancers, viz., lung cancer, breast cancer; Parkinson's disease; Wernicke's encephalopathy; etc.	–
Isoprene	Diabetes, hypercholesterolemia	105 ppb

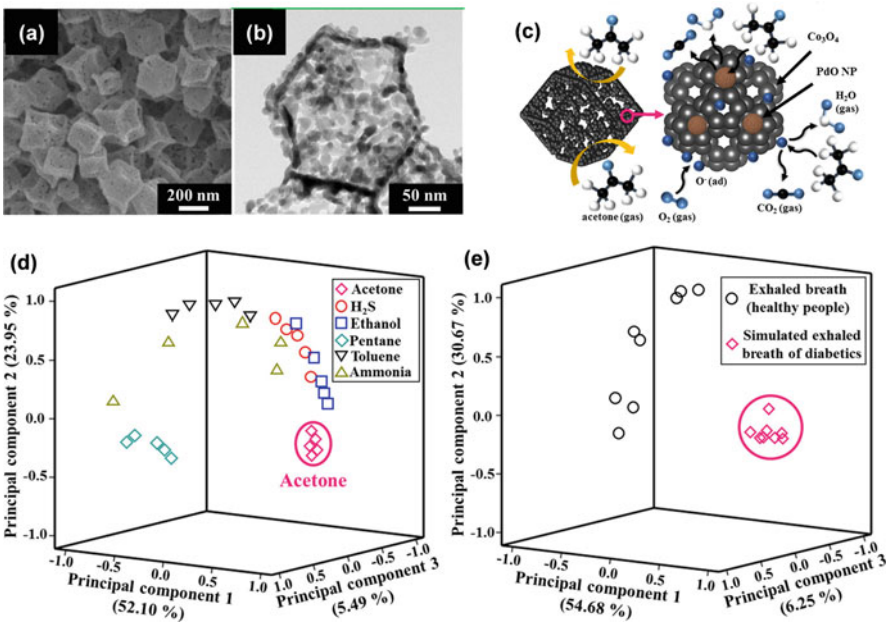

Fig. 3.3 (**a**) SEM image of PdO nanoparticle-functionalized Co_3O_4 hollow nanocages, (**b**) TEM image of PdO nanoparticle-functionalized Co_3O_4 hollow nanocage, (**c**) schematic illustration of acetone-sensing mechanism for PdO nanoparticle-functionalized Co_3O_4 hollow nanocage, (**d**) acetone sensing in presence of various interfering gases, and (**e**) acetone sensing of exhaled breath. [Reprinted with permission from Ref. (Konvalina and Haick 2014), copyright American Chemical Society]

Using metal-organic framework (MOF) templates, Koo et al. synthesized PdO nanoparticle-functionalized Co3O4 hollow nanocages (Koo et al. 2017). The morphological features are shown in Fig. 3.3a and b. The mechanism involving acetone sensing is shown in Fig. 3.3c. In this study, acetone sensing was demonstrated to be highly selective against other interfering gases by using hollow Co_3O_4 nanocages with high surface area and higher catalytic activity (Fig. 3.3d). Moreover, as shown in Fig. 3.3e, the sensor was also capable of distinguishing diabetic exhaled breath from that of normal people. Similarly, PdO-loaded $NiO/NiCo_2O_4$ truncated nanocages, SnO_2 multichannel nanofibers loaded with PtO_2 catalysts, and 3D inverse opal In_2O_3 microspheres show excellent acetone-sensing capabilities due to larger specific surface area, size-tunable pores, periodic porous structure, and highly 3D interconnection (Chen et al. 2018; Jeong et al. 2018; Wang et al. 2018).

The abnormal concentration of hydrogen sulfide (H_2S) in exhaled breath is a biomarker for diseases such as ischemia, asthma, Down syndrome, Alzheimer's disease, and halitosis (Jha et al. 2008; Huber et al. 2017; Kamoun et al. 2003; McGeer and McGeer 2010; Choi et al. 2014). For selective H_2S sensing, Yang et al. designed and fabricated inverse opal 3D WO_3 structures modified with ZnO@Au nanoparticles derived from MOF (Yang et al. 2020). The detection limit

was 50 ppb at 170 °C and attributed to increased adsorption energies, a higher negative charge of O, and improved conductivity. Similarly, ZnO encapsulated in MOFs containing Pt nanoparticles showed good H_2S-sensing response (Zhou et al. 2022). Smaller Pt nanoparticles present in the MOF cavities activated the H2S sensing. The molecular-sieving effect of MOF (ZIF-8) resulted in high selectivity. It was also found that MOF-derived metal oxides are effective in gas sensing because of their high surface area (Wang et al. 2021; Shi et al. 2022; Wu et al. 2022).

3.3.3 3D Gas Sensors for Food Quality and Safety

Thousands of people are affected by food poisoning every year by consuming food contaminated by parasites, bacteria, and viruses. Early recognition of spoiled food can prevent many serious health issues. The spoiled food which creates poison and the vapors released from this poison can be distinguished using gas sensors (McCabe-Sellers and Beattie 2004).

Many kinds of seafood including fish, due to biodegradation during spoilage, release gases like dimethylamine (DMA) and trimethylamine (TMA) (Messenger et al. 2013). These gases can be used a marker for determining the freshness of this kind of foods. As reported by Sui et al., 3D α-MoO_3 flowers with hierarchical structure can effectively detect TMA among various other VOCs with LoD as low as 0.5 ppm (Sui et al. 2015). Literature report suggests that 3D MoO_3-based semiconductor is very effective for various amine-based gas sensing (Malik et al. 2021). Due to excellent permeability involved to yolk-shell structure, the gas sensor based on yolk-shell nanoboxes of SnO_2/Au/Fe_2O_3 can detect a LoD of 50 ppb with a rapid response and recovery properties (Liu et al. 2019). ZIF-8-derived ZnO-$CsPbBr_3$ (Z-CPB) polyhedrons were found to be effectual in sensing TEA with response time of 2 seconds (Liu et al. 2022). The morphological features of Z-CPB are shown in Fig. 3.4a, b. The selectivity was very prominent for TEA for all the sensors as related to other analytes such as formaldehyde, ethanol, acetone, benzene, and acetonitrile as shown in Fig. 3.4c. In the mechanism, when $CsPbBr_3$ and ZnO come in contact, the free electrons from $CsPbBr_3$ transfer to ZnO until they reach equilibrium. Thus, at the interface, an electron depletion layer formed for $CsPbBr_3$ and an electron accumulation layer formed for ZnO. This interface between materials leads to the formation of an n-n heterojunction where more oxygen molecules can be adsorbed onto ZnO. The abundant electron resources on ZnO make it easier for this extra oxygen to be absorbed, improving gas-sensing performance (Fig. 3.4d). Spoiled meat product also releases gas like H_2S. Mesoporous hierarchical architectures of SnO_2 were fabricated using waste scallion root bio-template (Song et al. 2020). The working temperature of the gas detection system was set at 92 °C, which allowed for the successful detection of H_2S concentration with a limit of 0.5 ppb. This SnO_2-based sensor was effective in tracking H_2S concentrations during its decay process in fresh pork and human exhaled breath and within an environment containing high humidity levels.

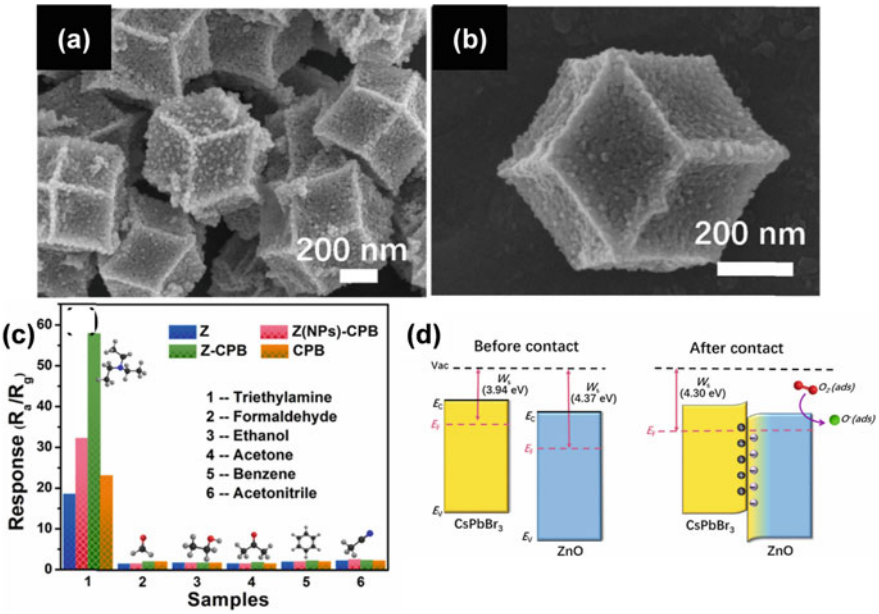

Fig. 3.4 (**a**, **b**) SEM images of Z-PCB, (**c**) selectivity of as-prepared sensors to different gases at 180 °C, and (**d**) energy-level diagram of Z-CPB. [Reprinted with permission from Ref. (Liu et al. 2022), copyright Elsevier]

Similarly, Cr_2O_3-based sensors were synthesized following the same bio-template method and successfully monitored the chicken freshness (Song et al. 2022).

From the reported literature, metal oxide-based gas sensors are far ahead of other sensing materials for various applications. However, 3D morphology-based gas sensors are not explored much as compared to 0D, 1D, and 2D materials. Further exploration on 3D nanostructure-based materials may result in better selectivity, sensitivity, response, and low working temperature.

3.4 Summary and Outlook

This chapter has summarized some topical 3D nanostructure-based materials for applications in gas sensing. Metal oxide-based semiconductors are found to be dominant in many sensing applications due to the ease in synthesis and fabrication, morphological tuning, good sensitivity, and faster response time. As most of the gas-sensing devices have higher operating temperature, the stability of MOSs-based materials in high temperature makes them suitable candidate for gas sensing. Besides pristine nanomaterials, heterojunctions, surface functionalization, doping, and composite materials also tailored the gas-sensing ability of many materials. It was found that the morphological and structural strategy such as exposed facet, porosity, hollow structure, and hierarchical structure can lead the way to a greater number

of active sites and higher activity. In disease diagnosis, noninvasive exhaled breath gas sensors make them an ideal alternate to current standard methods. Still there are many critical issues which need to be improved further to get better selectivity, sensitivity, and response. Simultaneous detection of multiple gas analytes in a single device will be more beneficial. Long-term stability of these devices in ambient atmosphere is very poor which limits the applicability outside of lab environment. Materials with low operating temperatures will decrease the power consumption with a better stability, and this could be achieved by hybridization of metal oxides with various carbonaceous materials, chalcogenides, MXenes, metal nitrides, etc. Looking toward the future and with the recent global pandemic, innovations in this field will certainly lead to rapid, portable, and inexpensive diagnostics and accessibility all over the world.

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Magnetic Nanoparticle-Based Sensing Strategies for Clinical Analysis and Environmental Safety Assessment

4

Nivedita Priyadarshni, Preeti Singh, and Kuldeep Mahato

Abstract

In recent days, magnetic nanoparticles (MNPs) have found great attention to the chemical sensing and biosensing modules, which not only help in signal amplification but also offer sample concentration and accumulation, especially in complex matrices. This chapter covers various aspects of developing analytical techniques using the MNPs. This chapter also summarizes the synthetic procedures using MNPs, where coprecipitation, hydrothermal, microemulsion, thermal decomposition, and biosynthetic-based methods have been briefly described. A brief discussion on the sensing strategies has also been included to give insight into the various types of sensors/biosensors for the readers. In addition, the analytical strategies and the sensing applications have been elaboratively described in this chapter by citing the recent and important developments of MNP-based sensors using adequate examples, illustrations, and tables, which will help the readers to get a comprehensive idea of the MNP-based analytical techniques/sensors developments. The future direction in this field relies on the exploration of various techniques for biomarker

Nivedita Priyadarshni and Preeti Singh contributed equally with all other contributors.

N. Priyadarshni
Department of Biological Sciences, Alabama State University, Montgomery, AL, USA
e-mail: npriyadarshni@alasu.edu

P. Singh
Department of Bioengineering, The University of Texas at Dallas, Richardson, Texas, USA
e-mail: Preeti.Singh@UTDallas.edu

K. Mahato (✉)
Department of Nanoengineering, University of California, San Diego, La Jolla, CA, USA
e-mail: kmahato@ucsd.edu

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estimations by addressing the limitations in contemporary diagnostics employing the MNPs.

Keywords

Electrochemical sensor · Fluorescent biosensor · Biomarkers diagnosis · Nanomaterial synthesis · Surface functionalization

4.1 Introduction

Nanoparticles tend to react to an external stimulus and are of great interest for developing analytical assays. The advancements in the nano-based techniques have leveraged the synthesis of various types of nanostructures such as carbon dots, metallic, polymeric, and magnetic nanoparticles (Akhtar et al. 2018; Chandra et al. 2010; Chandra and Prakash 2020; Chatterjee et al. 2017; Mahato et al. 2019; Prasad et al. 2016). Among all, magnetic nanoparticles (MNPs) have found great attention due to their ability to respond in presence of both electrical and magnetic stimuli which eventually helps in different analytical applications (Behrens 2011; Behrens and Appel 2016). Magnetic nanoparticles, a nanoscale-dimensional structure, have successfully grabbed the researchers' attention for more than two decades which could be evidenced by a large number of publications (Almomani et al. 2020). Various morphologies have been reported for MNPs including nanoparticles with dispersed matrix, colloidal crystals, core-shell, and macroscales spheres (Hoan et al. 2016). Magnetic nanoparticles mainly consist of magnetic metal nanoparticles, often iron, nickel, cobalt, chromium, manganese, gadolinium, and functionalization using a chemical component (Kudr et al. 2017). The super-paramagnetic property of magnetic nanoparticles is due to the nanoscale size that has great potential to offer different applications, either in bare or coated form with functional groups for definite uses like detection or drug delivery (Vatta et al. 2006). For example, the commonly explored ferrite MNPs can be changed into magnetic beads by clustering single nanoparticles.

The MNPs can be synthesized using a chemical component as functional molecules that permit transportation to the targeted location with the help of an external magnetic field, i.e., either electromagnets or permanent magnets. To minimize the interaction of the nanoparticles with the system environment, the surface coating has been introduced which in turn has improved the stability, biocompatibility, and even the solubility of the MNPs (Zhu et al. 2018). To increase MNPs stability in solution, surface modification is often employed by using surfactants, silica, phosphoric acid derivatives, and silicones. The most common applications of coated magnetic nanoparticles are analyte detection, separation and purification (Soelberg et al. 2009), drug delivery (Salmani et al. 2020), cell isolation (Unni

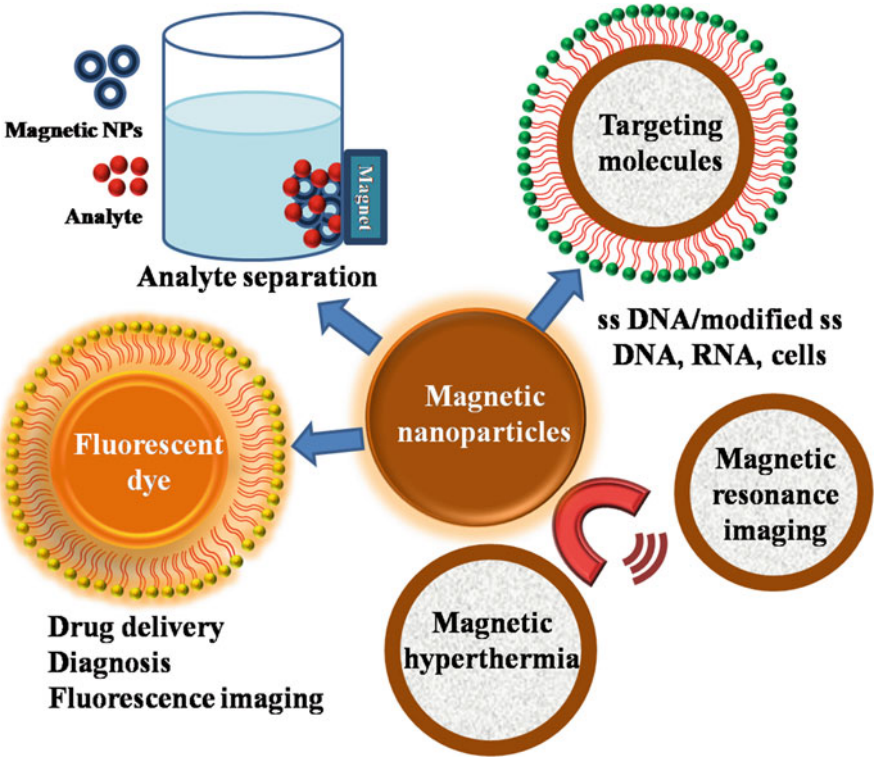

Fig. 4.1 Different applications of magnetic nanoparticles

et al. 2020), diagnostic testing (Fathi Karkan et al. 2017), imaging (MRI) (Avasthi et al. 2020), and immunoassay (Wang et al. 2011; Ali et al. 2021) (Fig. 4.1).

The MNPs comprising a solid magnetic core coated with (bio)organic shell is the easiest way of particle modification via physical adsorption. However, the covalent binding of (bio)organic molecules to the solid magnetic core parts is most suitable as it provides stable covalent immobilization. The MNPs, viz., Fe_3O_4 and γ-Fe_2O_3, are the most common core parts that contain flanking hydroxyl groups (OH) at their surfaces which allows silanization of particles and facilitates covalent binding of (bio)organic molecules. The presence of magnetic properties in MNP-conjugated (bio)organic molecules induces both catalytic and recognition features that work more efficiently together for the detection of clinical analytes and heavy metal ions (Akbarzadeh et al. 2012). The synthesis of MNPs with controlled size, specific morphology, and magnetization has been achieved by using various synthetic procedures (Nadar et al. 2021) based on the properties and applications which include catalysis and biomedical (Fernandez et al. 2017; Haun et al. 2010; Katz 2019; Tian et al. 2019). For example, chitosan-coated MNPs have been designed and developed as highly efficient nano-adsorbent for heavy metal ion detection and

removal (Nasirimoghaddam et al. 2015). The MNPs embedded in a polymer matrix have also been employed in biomedical applications for precise drug delivery and release (Liao and Huang 2020). Another example is for heavy metal ion detection, where a polymer-modified MNP is developed and used for sensing and removal of heavy metals (Ni, Cu, and AL) from secondary effluent wastewater (Lofrano et al. 2016), which may impact monitoring public health and hazards.

MNPs properties can be adjusted by varying ratios of chemical composition (with metal ions) and thickness of the coating materials. The modified ferromagnetic FeCo nanoparticles possess excellent properties as a promising sensor platform which is better than iron oxide-based MNPs for magnetically assisted separation methods, analysis, and electrochemical applications (Hu et al. 2020; Katz 2019). Sensor platforms with metal nanoparticles like gold, copper, iron, and silver enhance the surface area, chemical stability, high electrical conductivity, and biocompatibility of the final nanomaterial (Roy et al. 2021; Singh et al. 2021a, 2021b). Recently, researchers have explored the functionalization of MNPs with metallic nanoparticles (Li et al. 2017; Sanchez and Alvarez 2019) and found its potential applications in sensing biomolecules and heavy metal ions and using colorimetric (Kang et al. 2014; Su et al. 2012), fluorescence (de la Rosa-Romo et al. 2016), and electrochemical methods (Arvand and Hemmati 2017; Kudr et al. 2017).

Due to the extensive potential of MNPs in the detection and analyses, these have also been explored in the detection of the markers of clinical biomedical importance. The incorporation not only facilitated rapid detection but also provided accurate sensing, which can help in the monitoring of disease, removal of toxic moieties, and design of personalized medicine. The magnetic susceptibility of the DNA, RNA, cells, and biomolecules (glucose, glutathione, creatinine, etc.) has also been reported, which could be employed for the development of various novel sensors for clinical and biomedical applications (Mahato et al. 2016). This chapter incorporates and discusses various aspects of the MNPs including the properties, synthesis, and sensing strategies with the help of illustrations and recent state-of-the-art examples.

4.2 Synthesis of Magnetic Nanoparticles

To attain the desirable properties of MNP, different synthesis methods are employed (Ali et al. 2021), where the synthesis of these magnetic nanoparticles is broadly classified under top-down and bottom-up approaches. The top-down approach involves the shattering of bulk materials to nano-range particles using different physical techniques such as high-energy ball milling. Top-down offers large-scale production but controlling the size and shape of the MNPs is difficult. However, these top-down strategies are limited due to their time-consuming and expensive protocols (Ali et al. 2021; De Castro and Mitchell n.d.), thus not always preferred. On the other hand, in the bottom-up approach, atoms or molecules are assembled to build complex magnetic nano-constructs, which are easy and scalable processes. Thus, the bottom-up approaches have found great attention for the development of

MNPs. The bottom-up synthesis includes various approaches including chemical (coprecipitation, microemulsion, hydrothermal, thermal decomposition, and sol-gel) (Akbarzadeh et al. 2012) and biological methods and is more popular to produce well-dispersed magnetic nanoparticles (Ali et al. 2021; Wu et al. 2008). In this section, we discuss various synthetic procedures for magnetic nanoparticles which are most commonly used.

4.2.1 Chemical Synthesis

4.2.1.1 Coprecipitation Method

For the synthesis of MNPs, coprecipitation is the most convenient and widely used method to prepare the monodispersed MNPs (Lu et al. 2007; Sandeep Kumar 2013). This method of MNPs synthesis involves the addition of a base solution in an aqueous precursor (usually salt solution) under an inert and temperature-controlled environment (as shown in Fig. 4.2a) (Faraji et al. 2010; Majidi et al. 2016), which involves the following reaction (Eq. 4.1) (Indira and Lakshmi 2010):

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \tag{4.1}$$

The alkaline medium (pH 8–14) and nonoxidizing oxygen environment favor a complete precipitation process with a 2:1 stoichiometric ratio of Fe(III):Fe(II) (Faraji et al. 2010; Iida et al. 2007; Laurent et al. 2008). The synthesized Fe_3O_4 (magnetite) nanoparticle is not much stable, and in the oxygenic environment, it turns to γFe_2O_3 (maghemite) following the reaction as shown in Eq. (4.2):

$$Fe_3O_4 + 2H^+ \rightarrow \gamma Fe_2O_3 + Fe^{2+} + H_2O \tag{4.2}$$

The size, shape, and composition of these synthesized MNPs can easily be controlled by varying the type and concentration of precursor salt, pH, and temperature. By tuning these parameters, various kinds of MNPs can be synthesized for several biomedical applications (Gul et al. 2019).

4.2.1.2 Hydrothermal Method

Another commonly used process is hydrothermal-based synthesis, which is also known as the solvothermal process. This is a solution-based synthetic approach that produces ultrafine magnetic nanoparticles (Butter et al. 2005; Majidi et al. 2016; Mao et al. 2006; Zhu et al. 2007). In this process, the MNPs are prepared by hydrolysis and oxidation of precursor salt under high pressure and temperature (Reddy et al. 2012; Zhang et al. 2016). An illustration of hydrothermal synthesis is demonstrated in Fig. 4.2b. The MNPs with uniform size distribution and desired surface coating have been obtained by this method. In a report, Zheng and co-workers have synthesized MNPs of Fe_3O_4 with 27 nm coated with the sodium bis(2-ethylhexyl) sulfosuccinate (Zheng et al. 2006). Similarly, Li et al. have reported

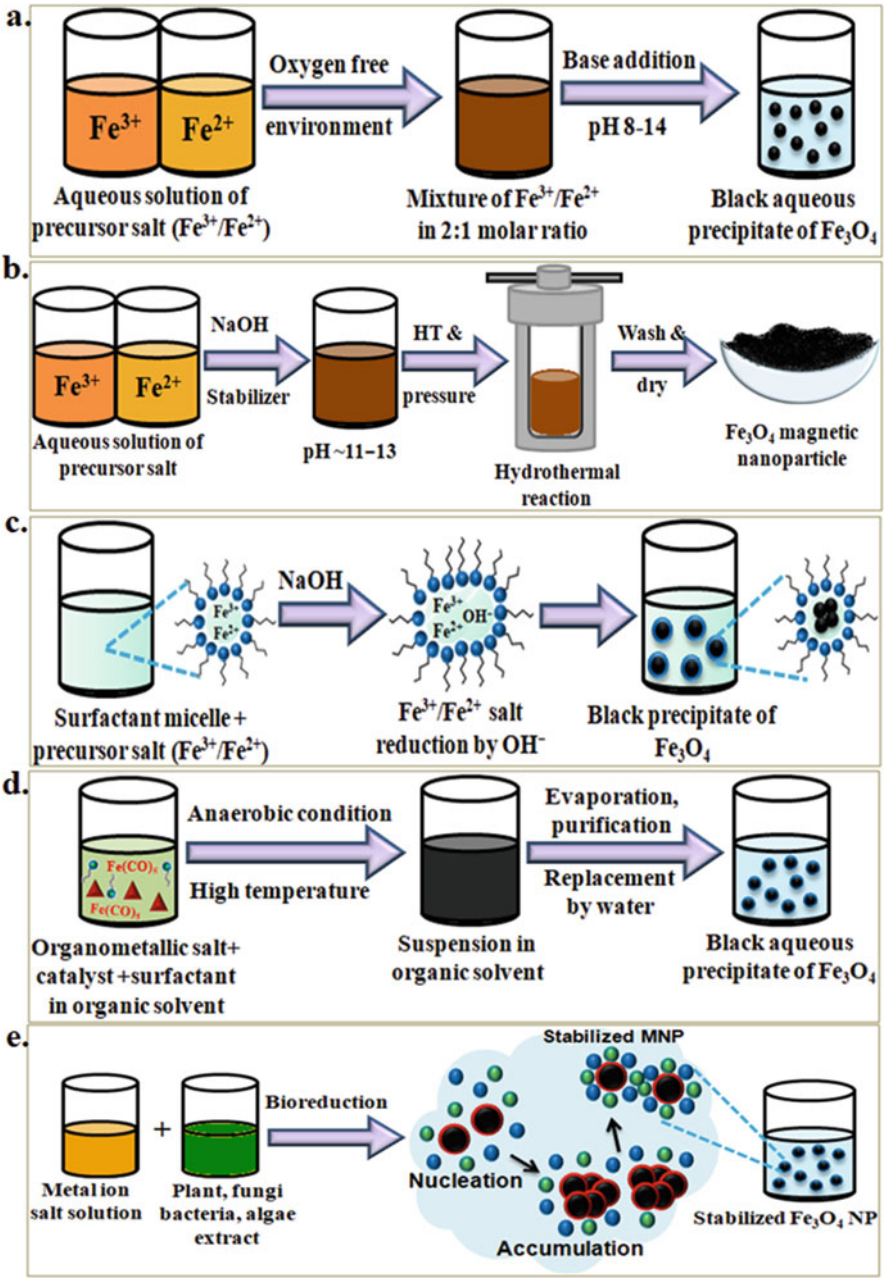

Fig. 4.2 Schematic representation of different synthesis routes of magnetic nanoparticle. (**a**) Coprecipitation method, (**b**) hydrothermal, (**c**) microemulsion, (**d**) thermal decomposition, and (**e**) biological synthesis

spherical Fe_3O_4 MNP of size 15 nm (Li et al. 2013). For obtaining the linker molecules on the surface of the MNPs, different passivating materials have been used in the recent past. In an example, chitosan was employed to Fe_3O_4 MNPs and was demonstrated in the immobilization of enzymes (Li et al. 2008). Since this synthetic procedure is carried out under high pressure and temperature, safety is required during experiments. It requires special equipment called a hydrothermal autoclave reactor for synthesis. Despite these hassles, the hydrothermal method is extensively used as it produces desirable morphology, surface coating, and composition of MNP by adjusting the pressure, temperature, and time of reaction (Ali et al. 2021; Zahid et al. 2019).

4.2.1.3 Microemulsion Method

Another common method used is the microemulsion which is the isotropic dispersal of two immiscible solvents consisting of lipophilic and hydrophilic phases in presence of a surfactant. In this method, oil and water are mixed with surfactant and stirred at room temperature (de Toledo et al. 2018; Gul et al. 2019). The surfactants are amphiphilic compounds consisting of a hydrophilic head and hydrophobic tail that assemble themselves as a monolayer at the oil-water interface. In monolayer formation, the hydrophilic head is directed toward an aqueous medium and the hydrophobic tail toward the oil phase (as shown in Fig. 4.2c) (Solans et al. 2005; Williams et al. 2016; Wu et al. 2008). Here, surfactant acts as an oil-water interfacial tension-lowering agent to result in a transparent solution (Faraji et al. 2010; Majidi et al. 2016). Based on the amount of water and oil, three types of microemulsions can be synthesized: (1) oil in water (O/W), which consists of a few droplets of oil in an aqueous medium; (2) water in oil (W/O) also called as reverse micelle formation, which consists of oil with small amount of water; and (3) contains an equal amount of water and oil (Ali et al. 2021; Lopez Perez et al. 1997; Mosayebi et al. 2017). The microemulsion method has been used in the synthesis of important magnetic nanocrystals like MFe_2O_4 (M: Ni, Mn, Cu, Mg, Co, Cd, or Zn) which are extensively used in electronic applications (Lu et al. 2007). For example, Liu et al. reported the synthesis of $MnFe_2O_4$ MNPs of 4–15 nm size using water-in-toluene reverse micelle formation in presence of sodium dodecylbenzene sulfonate (NaDBS) as a surfactant (Liu et al. 2000). The use of binary arrangement (oil/surfactant or water/surfactant) allows the formation of a variety of structures including spherical, cylindrical, lamellar, and bi-continuous microemulsions (Majidi et al. 2016; Solans et al. 2005). The type of surfactant used in reaction controls the size and shape of magnetic nanoparticles (Lu et al. 2013). The MNPs synthesized by microemulsion are uniformly dispersed but are of low quality (Ali et al. 2021).

4.2.1.4 Thermal Decomposition Method

This approach utilizes organometallic compounds to produce monodispersed magnetic nanocrystals. The organometallic salt precursors are dissolved in organic solvents and decomposed in presence of surfactants (as a stabilizing agent) in high temperature and anaerobic conditions (as shown in Fig. 4.2d) (Effenberger et al. 2017; Laurent et al. 2008). In the absence of oxygen, the first metal hydroxide is

formed which is eventually oxidized by protons present in water to produce metal oxides. For example, during the synthesis of iron oxide, the ferrous hydroxide is obtained which is eventually oxidized to obtain different types of iron oxides (Gul et al. 2019). For controlling the size growth of the MNPs, co-stabilizing agents are used, which slows down the nucleation of MNPs (Ali et al. 2021; Effenberger et al. 2017). For this, several stabilizing agents like fatty acids, oleic acid, and hexadecylamine are commonly used during the thermal decomposition process. Apart from these, synthetic polymers have been used for obtaining monodispersed MNPs. For instance, polymer-catalyzed thermal decomposition of $Fe(CO)_5$ produced monodispersed iron oxide MNPs with sizes ranging from 6 to 20 nm (Huber 2005; Smith and Wychick 1980). The zerovalent precursor-like $Fe(CO)_5$ firstly forms metallic iron nanoparticles which are followed by oxidation resulting in iron oxide MNPs with high-quality yield (Effenberger et al. 2017). This method has proved best for large-scale production of uniform-size and homogenous-shaped magnetic nanoparticles (Ali et al. 2021; Kudr et al. 2017). However, organic solvents at extreme temperatures, pressure, and volatile vapors utilized during synthesis are prone to risky and safety issues (Dong et al. 2015; Gul et al. 2019).

4.2.2 Biological Synthesis

Biological syntheses are also known as green synthesis and are an eco-friendly method of MNPs preparation that utilizes living organisms such as plants and microbes (fungi, algae, bacteria, and actinomycetes) instead of toxic, hazardous chemicals ((Ali et al. 2021; Gul et al. 2019; Nassar et al. 2016; Tadic et al. 2014; Verma et al. 2021). A biological synthesis is a bottom-up approach that is formed by the assembly of precursor atoms in a cluster and eventually to magnetic nanoparticles (Fig. 4.2e). These biological materials contain a variety of bioactive compounds and molecules which act as both stabilizing and reducing agents for MNPs (Yew et al. 2020). Different parts of plant extracts (Shameli et al. 2012), exudates (Lukman et al. 2011), tissue (Padil and Černík 2013), and other substrates are extensively exploited for MNPs syntheses. For example, Mahdavi et al. synthesized cube-shaped Fe_3O_4 MNPs with 18 ± 4 nm diameter using brown seaweed extract and $FeCl_3$ precursor. Seaweed contains various functional groups containing sulfate, aldehyde, and hydroxyl moieties which may be used as reducing and stabilizing agents during synthesis (Mahdavi et al. 2013). Similarly, Buazar et al. have also reported the synthesis of Fe_3O_4 MNPs of 40 ± 2 nm size using potato extract as a reducing agent (Buazar et al. 2016). The properties of MNPs can be tuned easily by altering the concentration of precursor and bio-extract, temperature, time, and pH of the reactant mixture during the synthesis process (Yew et al. 2020). The biologically synthesized MNPs are nontoxic and environmentally friendly. Thus it shows increased biocompatibility and can be employed for various biomedical applications (Mahdavi et al. 2013; Salam et al. 2012).

4.3 Nanosensors and Sensing Strategies Based on MNPs

The sensors are miniaturized devices for detecting the target analytes sensitively. These are constantly replacing the lab-based protocols, where cumbersome equipment and dedicated infrastructures are required (Kumar et al. 2019a, 2019c, 2020a; Mahato et al. 2020a; Purohit et al. 2020a). The detection process uses the transducer which is mainly nanomaterials (Purohit et al. 2019b; Mahato et al. 2020b; Kashish et al. 2017), nanocomposite (Kumar et al. 2019b, 2020b; Purohit et al. 2020b), engineered surfaces (Mahato and Chandra 2019), etc. Employing these materials offers miniaturization and improves optoelectronic properties for obtaining enhanced sensitivity to the sensors (Baranwal et al. 2016; Mahato et al. 2018a, 2018b; Purohit et al. 2019a; Purohit et al. 2020c). Sensors are devices that convert an analyte's chemical or physical properties into a signal corresponding to the analyte concentration (Jayabal et al. 2015). Commonly the sensors are devised of the recognition element, transducer, and readout. MNPs play a role in the transduction in most of the sensor formats (Mahato et al. 2018b; Willner and Vikesland 2018). Based on the signal generation mode, the MNP-based sensors are classified under the colorimetric, fluorescent, and electrochemical sensors.

4.3.1 MNP-Based Colorimetric Sensing

A colorimetric sensor measures the color change linked with the chemical interaction that happens between the analyte and the MNP sensor probe. In the past few decades, the colorimetric sensor has gained huge attention because of its easy usability, affordability, and rapid detection time. In addition, these sensors work in the visible range of electromagnetic radiation, and therefore, any change can be directly detected by the naked eye (Jayabal et al. 2015; Kim et al. 2012; Xu et al. 2020). The magnetic nanoparticles do not have intrinsic color change properties like some of the noble metal (e.g., Au, Ag, Pt) nanoparticles. However, it exhibits enzyme-like catalytic activity that catalyzes some reactions and produces a prominent color change in presence of the target analyte. These MNPs mimic the working mechanism of some well-known in vivo enzymatic reactions and therefore also known as nanozymes (i.e., nanoparticles that act as an enzyme) (Xu et al. 2020). Christus et al. reported a colorimetric sensor for mercury (Hg^{II}) using Fe_3O_4@ZIF-67 (magnetic-MOF nanocomposite) as a peroxidase mimic. Fe_3O_4@ZIF-67 catalyzes the oxidation of tetramethylbenzidine in presence of hydrogen peroxides to form blue color. After the addition of glutathione (GSH), depending on the concentration of GSH, the produced blue color disappeared. When Hg^{II} is added to the Fe_3O_4@ZIF-67 − GSH complex, Fe_3O_4@ZIF-67 is replaced by Hg^{II} due to Hg^{II}-thiol complex formation, thereby releasing TMB from GSH, and subsequent oxidation results in reappearance of blue color (Fig. 4.3a) (Christus et al. 2018). In another work, As(V) detection using Fe_3O_4 MNP was demonstrated to mimic a Fenton-like catalytic reaction. The oxidation of methylene blue indicator was catalyzed by Fe_3O_4MNP in presence of H_2O_2 and turned colorless due to power

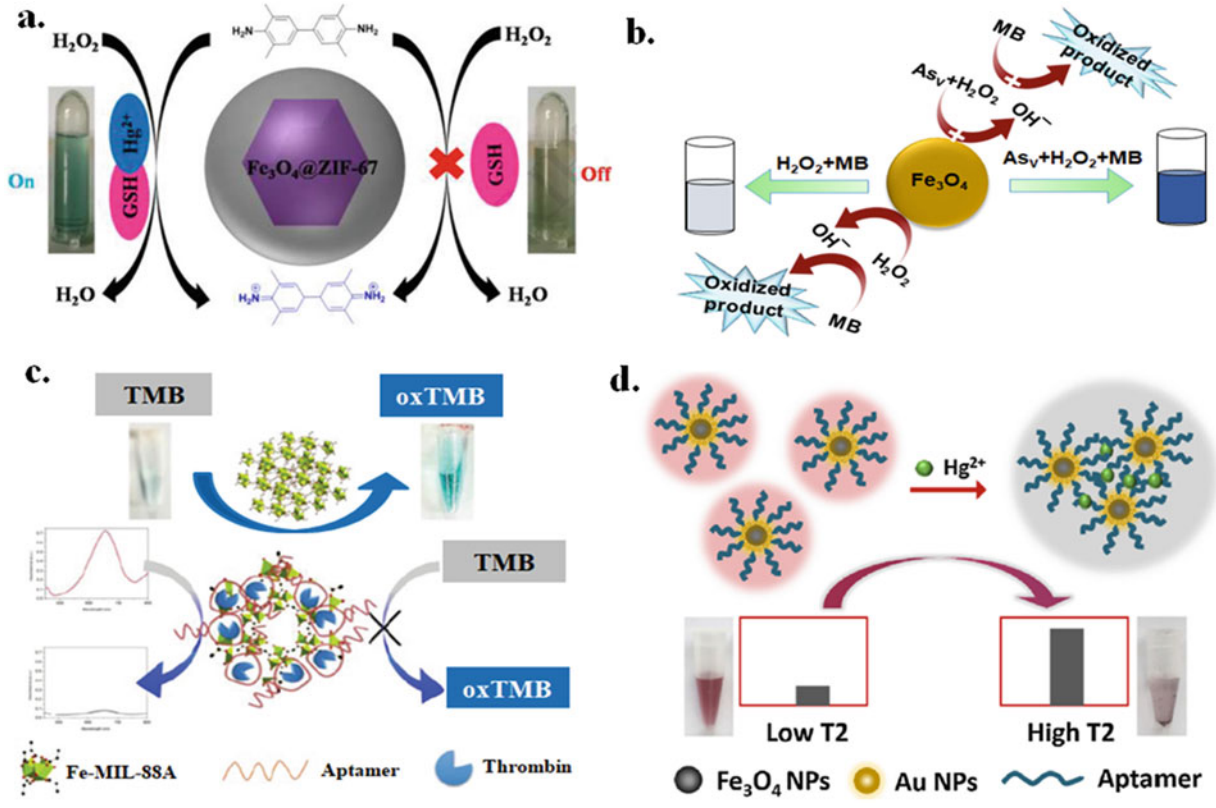

Fig. 4.3 Magnetic nanoparticle and conjugates used in colorimetric sensing of analytes. (**a**) Fe_3O_4@ZIF-67 magnetic-MOF nano-conjugate as peroxidase mimic for detection of Hg^{II}, (**b**) Fe_3O_4 MNP mimicking Fenton-like catalytic reaction for detection of As^{V}, (**c**) Fe-MIL-88A nano-conjugate as peroxidase

mimic for detection of thrombin, and (**d**) thiolated-aptamer-modified Au@Fe_3O_4 magnetic nanocomposite that selectively detects Hg^{II} by forming T-Hg^{II}-T base pairs and concomitant color change from red to colorless. (Reprinted with permission from references a. (Christus et al. 2018), c. (Wang et al. 2016b), d. (Liu et al. 2020), b. adapted from Babu Christus et al. (2018))

oxidant radical ($^{\bullet}OH$) generation. When As(V) is added, it adsorbs on Fe_3O_4 MNP reducing the catalytic activity of MNP, and the solution turns back to blue color (Fig. 4.3b) (Babu Christus et al. 2018). Wang et al. reported Fe-MIL-88A (MNP-based MOF material), a label-free colorimetric sensor as peroxidase mimetic nanozyme for detection of thrombin (Fig. 4.3c). The oxidation of TMB in presence of H_2O_2 is catalyzed by Fe-MIL-88A nanocomposite developing blue color solution. Introduction of thrombin to the reaction mixture, complexes with Fe-MIL-88A, inhibits its catalytic activity and turns the solution colorless. The extent of decoloration is dependent on the concentration of thrombin; the higher the concentration, the more the color will fade. The detection limit of thrombin using Fe-MIL-88A was 10 nM with the naked eye (Wang et al. 2016b). Another method of colorimetric detection using magnetic nanoparticles is by nanohybrid of MNPs with noble metal nanoparticles. These nanohybrids explore the optical property of noble metal nanoparticles and show a prominent color change in presence of a specific analyte. Liu et al. synthesized thiolated-aptamer-modified Au@Fe_3O_4 magnetic nanocomposite to selectively detect Hg^{II} by forming T-Hg^{II}-T base pairs (Fig. 4.3d). The aptamer-Au@Fe_3O_4 appeared red-colored due to the presence of Au-NP in the MNP nanocomposite. After interaction with Hg^{II}, the red color of a solution becomes colorless. Hg^{II} forms T-Hg^{II}-T base pairs with aptamer present on the surface of aptamer-Au@Fe_3O_4 nanocomposite and forms aggregates that leads to precipitation of aptamer-Au@Fe_3O_4 − Hg^{II} (Liu et al. 2020).

4.3.2 MNP-Based Fluorescence Sensing

The fluorescence sensor consists of a fluorophore as a signal-transducing element, which exhibits photoluminescence. When the fluorophore is irradiated with electromagnetic radiation, it absorbs the photon energy and excites the orbital electrons to a higher energy level. Fluorescence occurs when the excited electron relaxes to a lower energy state by emitting a photon. MNPs lack fluorescent property, therefore functionalized with a fluorophore to obtain a fluorescent sensor (Xiong et al. 2019; Willner and Vikesland 2018). Liu et al. reported oligonucleotide-functionalized $CuInS_2$ quantum dot@magnetic Fe_3O_4 (ssDNA-CuInS2 QDs) nanocomposite as a "turn off-on" fluorescent nanosensor for detection of As(V) (Fig. 4.4a). Oligonucleotide-modified $CuInS_2$ QDs exhibit fluorescence property which is quenched ("turn off" mode) after binding with Fe_3O_4 MNP. As(V) can bind with Fe_3O_4 MNP by displacing ssDNA-$CuInS_2$ QDs and results in fluorescence "turn on" (Liu et al. 2015). Liu et al. reported fluorescein amidite (FAM)-labeled DNA oligonucleotides adsorbed iron oxide (FAM-DNA-Fe_3O_4) through phosphate backbone and used for arsenic detection. The binding of iron oxide MNP to FAM-DNA quenches its fluorescence. When FAM-DNA-Fe_3O_4 is treated with As(V), it binds with Fe_3O_4 MNP by displacing adsorbed FAM-DNA enhancing fluorescence (Fig. 4.4b) (Liu and Liu 2014). Kim et al. synthesized rhodamine derivative-functionalized Fe_3O_4 (Rh-Fe_3O_4) nanohybrid and used it for simultaneous detection and removal of Hg^{II}. When (Rh-Fe_3O_4) nanohybrid was treated with Hg^{II}, it showed

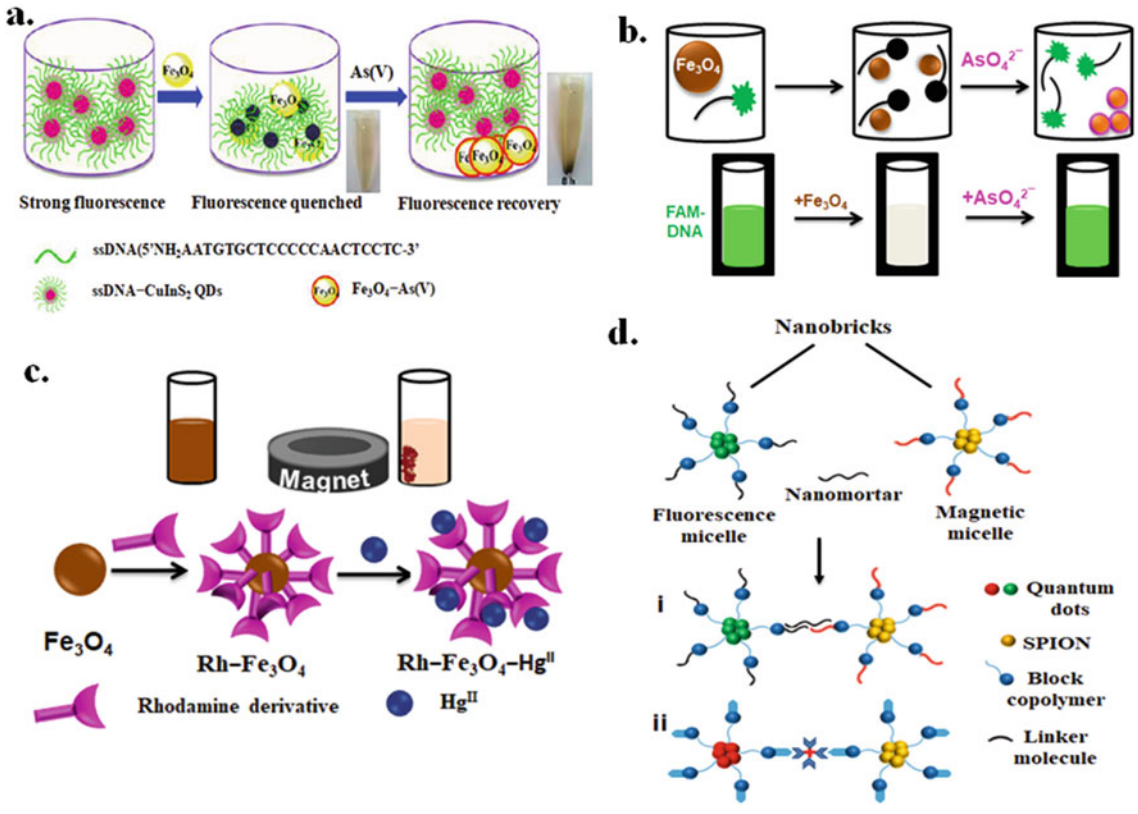

Fig. 4.4 Magnetic nanoparticle and conjugates used in florescent sensing of analytes: (**a**) oligonucleotide-functionalized $CuInS_2$ quantum dot@magnetic Fe_3O_4 (ssDNA-CuInS2 QDs) nanocomposite as "turn off-on" fluorescent nanosensor for detection of As^V, (**b**) fluorescently labeled DNA oligonucleotides

Fig. 4.4 (continued) adsorbed iron oxide (FAM-DNA-Fe_3O_4) showing enhanced fluorescence in presence of As^V, (**c**) rhodamine derivative-functionalized Fe_3O_4(Rh-Fe_3O_4) nanohybrid showing enhanced fluorescence in presence of Hg^{II}, (**d**) super-paramagnetic iron oxide nanoparticles (SPIONs) and quantum dots (QDs) showing micelle-based aggregation assay for detection of p53 DNA (i) and streptavidin protein (ii) forming a hierarchical structure. (Reprinted with permission from reference a. (Liu et al. 2015), adapted from b. (Liu and Liu 2014), c. (Kim et al. 2016), d. (Mahajan et al. 2020))

enhanced fluorescence due to the formation of highly conjugated xanthene moiety. After conjugation, Hg^{II} can be removed simultaneously by placing Rh-Fe_3O_4 – Hg^{II}-containing solution near magnetic field (Fig. 4.4c) (Kim et al. 2016). Mahajan et al. prepared a magneto-fluorescent biosensor/separation platform containing super-paramagnetic iron oxide nanoparticles (SPIONs) and quantum dots (QDs) for streptavidin protein and p53 DNA analyte (Fig. 4.4d). Two different micelles of SPIONs and QDs (red and green QD) using amphiphilic block co-polymer were prepared. For sensing of streptavidin protein, SPIONs and QDs (red) were functionalized with biotin, while to capture p53 DNA, SPIONs and QDs (green) were functionalized with ssDNA. The detection was achieved by enhanced fluorescence via aggregation sandwich assay by forming fluorescent/magnetic complexes: SPION-streptavidin-QD (red) and SPION-p53DNA-QD (green) (Mahajan et al. 2020).

4.3.3 MNP-Based Electrochemical Sensing

Electrochemical detection is widely used for the trace quantification of compounds because it provides a rather easy procedure for direct and selective detection with fast response, affordable, high sensitivity, and user-friendly characteristics (Rajeev et al. 2019). In the electrochemical detection technique, the anodic/cathodic peak for the target analyte is easily distinguishable from other compounds that coexist using a modified electrode with magnetic nanoparticles specific to the target analyte (Rajeev et al. 2019). The electrochemical sensors are designed by immobilizing DNA, antibodies, tissue, enzyme, and ligands for the clinical analyte and heavy metal ion detection which are attached to the working electrode surface (Rajeev et al. 2019). For improving the signal amplification and sensitivity of electrochemical devices, MNPs are used. MNPs can be used either as direct contact with the working electrode surface or the formation of a thin film and transport of chemical species to the electrode surface. The electrochemical techniques which are commonly used for analyte quantitative estimation in the MNP-based electrochemical sensors are cyclic voltammetry, electrochemiluminescence (ECL), potentiometry, amperometry, and electrochemical impedance spectroscopy (EIS) (Arvand and Hassannezhad 2014; Rocha-Santos 2014; Yang et al. 2014). For example, Santos et al. (2021) demonstrate electroanalytical strategies for sensing and quantifying an important bioethanol (antioxidant), i.e., glutathione (GSH), which is found in humans and takes part in many biological processes including the defense mechanism (Singh et al. 2016). The selective detection of GSH was based on developing a magnetic molecular imprinted polymer (mag-MIP) due to its coupling property with high selectivity for rapid analyte detection of interest from complex solution, by using an external magnetic field. The electrochemical sensing of GSH was achieved by adding mag-MIP and mag-NIP (non-imprinted polymers) mixture in 0.1 mmol/L GSH solution. The separation of analyte was achieved by the external magnetic field using a magnetic graphite-epoxy composite electrode (Fig. 4.5a) in 1 min turning the solution transparent. The mag-MIP selectively binds with the GSH in the solution,

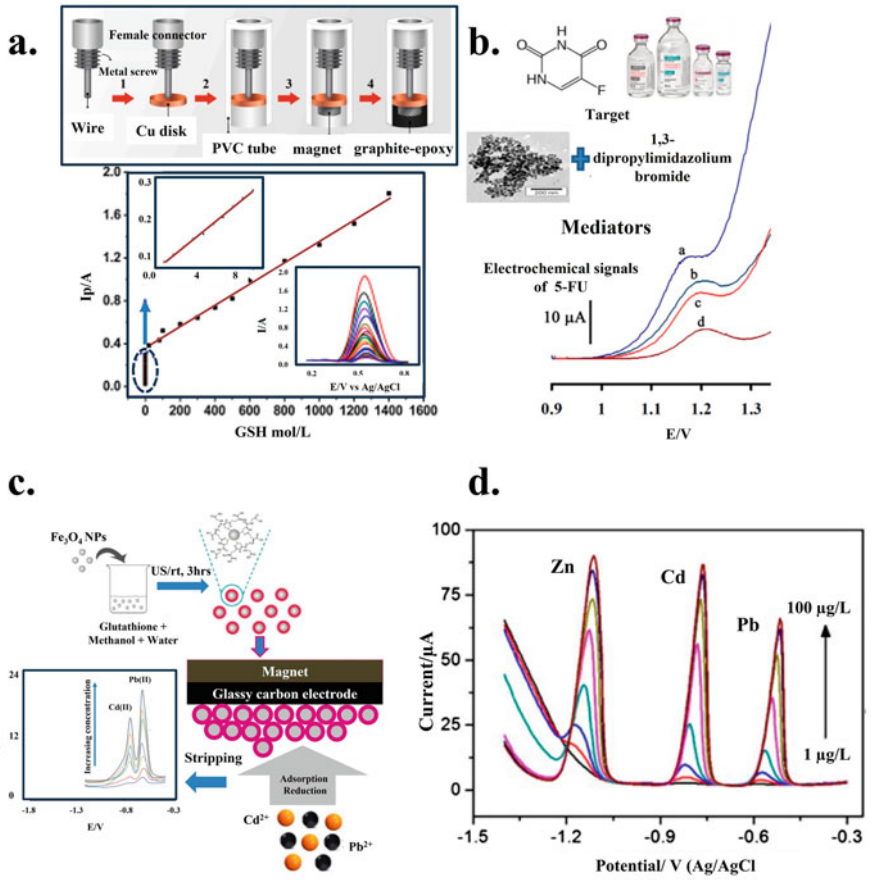

Fig. 4.5 (**a**) Schematic representation of fabrication of the magneto-sensor, i.e., mag-MIP; calibration curves for GSH concentration (1–1400 µmol/L) (inset, DPV); (**b**) electrochemical behavior of 5-FU study using $ZnFe_2O_4$/MNPs/IL/CPE; (**c**) electrode fabrication, i.e., GSH@Fe_3O_4/MGCE and simultaneous detection of Cd^{2+} and Pb^{2+}; (**d**) simultaneous detection of Zn, Cd, and Pb using Fe_2O_3/G/Bi as sensor platform. (Reprinted with permission from references Santos et al. (2021), Fallah Shojaei et al. (2016), Baghayeri et al. (2018), and Lee et al. (2016))

and the quantification of GSH was determined using CV and DPV techniques (Fig. 4.5a). The linear response for the GSH was recorded from 1 to 1400 µmol/L with a detection limit of 7 nmol/L as shown in Fig. 4.5a. The GSH levels were further experimented using mice liver as sample to investigate the effectiveness of electrochemical and spectrophotometric technique and in real samples. A low relative error of 1.06% between electrochemical and spectrophotometric techniques showed the good functionality of the proposed sensor. Second example was demonstrated by Fallah Shojaei et al. (2016), who designed and developed a modified carbon paste electrode ($ZnFe_2O_4$/MNPs/IL/CPE) via in situ process for

the detection of 5-fluorouracile (5-FU) in drug samples. The presence of $ZnFe_2O_4$/MNPs and IL on CPE enhanced the electron transfer of 5-FU from solution to the electrode surface at 1.187 V and lowers the oxidation peak current and decreased overvoltage as shown in Fig. 4.5b curves c and b, respectively, which was not observed in case of bare CPE (Fig. 4.5b curve d). The final nanomaterial, i.e., $ZnFe_2O_4$/MNPs/IL/CPE, significantly improved the electro-oxidation of 5-FU at lower potential of 1.167 V with a high peak current (19.5 μA) (Fig. 4.5b curve a). The detection of 5-FU was determined using square-wave voltammetry (SWV), linear sweep voltammetry (LSV), chronoamperometry (CA), and EIS. The optimal pH was kept at 7.0 for all experimental conditions. The electro-oxidation current peak for the detection of 5-FU was found to be linearly increasing with the increasing concentrations in the wide range of detection from 0.1 to 1400 μM using SWV. The modified $ZnFe_2O_4$/MNPs/IL/CPE electrode possessed benefits like good detection limits and high sensitivity in selective and trace estimation, along with good reproducibility. After establishing, the modified electrode was applied for the quantification of 5-FU in real urine samples and commercially available pharmaceutical samples.

Similarly, electrochemical sensing of heavy metal ions using magnetic nanoparticles is another demanding area to increase the selectivity of detection even in presence of various interfering agents. Reports are also available where the detection of various heavy metal ions at the same time is demonstrated using magnetic nanoparticles. For example, Baghayeri et al. introduced an electrochemical application for the determination of heavy metal ions like lead (Pb^{2+}) and cadmium (Cd^{2+}) in real samples using GSH-functionalized magnetic nanocomposite (GSH@Fe_3O_4)-modified GCE for the development of an easy, stable, and selective sensor. The detection method is based on electrochemical preconcentration/reduction of lead and cadmium on the surface of the GSH@Fe_3O_4-modified electrode (Fig. 4.5c). The detection limit of Pb^{2+} was found to be 0.182 μgL^{-1} and for Cd^{2+} ions, it was found to be 0.172 μgL^{-1}. The developed sensor was low-cost, user-friendly, and used for the estimation of Pb^{2+} and Cd^{2+} in real water samples. These findings conclude that the GSH@Fe_3O_4-based sensor can be an excellent platform for sensing water samples for the quantification of heavy metal ions using various electrochemical techniques (Baghayeri et al. 2018). Another example which was reported by Lee et al. was a simultaneous electro-analytical assessment of trace metal ions like Zn^{2+}, Cd^{2+}, and Pb^{2+} using an electrochemical sensor modified with iron oxide/graphene nanocomposite and in situ plated bismuth, i.e., Fe_2O_3/G/Bi. The collective effect between G and iron oxide NPs showed enhanced electrocatalytic activity with high sensitivity toward heavy metal ions. The DPV electrochemical technique was applied for the detection of Zn^{2+}, Cd^{2+}, and Pb^{2+} (Fig. 4.5d). A wide linear range was found from 1 to 100 $\mu g\ L^{-1}$ with a good sensitivity of 0.11 $\mu g\ L^{-1}$, 0.08 $\mu g\ L^{-1}$, and 0.07 $\mu g\ L^{-1}$ which were recorded for Zn^{2+}, Cd^{2+}, and Pb^{2+}, respectively. The modified electrode showed good repeatability and reproducibility properties for five different sensor electrodes. The Fe_2O_3/G/Bi sensor electrode was also applied for the sensing and analysis of heavy metal ions in real samples. In

conclusion, the simple and easy synthesis of Fe_2O_3/G/Bi nanomaterial can be a good sensing platform for various heavy metal ion detections (Lee et al. 2016).

4.4 MNPs in Sensing Application

Magnetic nanoparticles (MNPs) are known as sensor materials and have a high significance in providing detection below its lower limit and eliminating nonspecific bindings, which are viable solutions to the enduring challenges of sense. The presence of various functional materials with unique properties on the surface of the MNPs helps them become biocompatible and is highly suitable for biomedical applications and heavy metal ion detection. The rapid expansion in the sensing application of MNPs creates a need to overview the current state of the art of MNPs for sensing applications in clinical analyses and heavy metal ions for environmental safety. This present chapter is focused on the sensing of clinical analyses and heavy metal ions for environmental safety using such magnetic nanoparticle (MNP) materials. Sensing with magnetic nanoparticles (MNPs) is a vast area of application that consists of three categories: colorimetric, fluorescence, and electrochemical sensing. Reported literature states that MNPs have been used in detections of clinical analyses, for example, DNA, miRNA, glucose, leukemia cancer cells, thrombin, nucleic acid and H_2O_2, and heavy metal ions, for example, arsenic, cadmium, zinc, lead, and copper (Ge et al. 2012; Rocha-Santos 2014; Shao et al. 2012; Tang et al. 2020; Yang and Yin 2017).

4.4.1 Magnetic Nanoparticles for Clinical Analyses

Magnetic functionalized nanoparticle-based biosensors represent a significant verge for the future of clinical diagnosis at an early stage. These magnetic functionalized nanoparticle-based biosensors have already gained an important place among various biomedical applications, for example, site-specific imaging in vivo (Veiseh et al. 2010), DNA/RNA detection (Jangpatarapongsa et al. 2011; Wang et al. 2016a), cancer detection (Hosu et al. 2019), sensing important biomolecules, and biomedical cargo delivery (Anderson et al. 2019). Table 4.1 shows the various types of sensors used for the detection of analytes of clinical importance.

Deoxyribonucleic acid (DNA): The development of DNA biosensors is crucial in the research field for gene analysis, tissue matching, identification of genetic disorders, forensic applications, etc. (Zhang et al. 2008). In a report, Donnelly et al. (Donnelly et al. 2014) demonstrated a novel sensing platform based on the combination of functionalized AgNPs and Ag-coated MNPs for the detection of target DNA using SERS. Here, the DNA samples were extracted from *Candida* fungal species, i.e., *C. krusei* and *C. albicans*. The sandwich assay incorporates two DNA sequence probes; each probe was complementary to a particular portion of the target DNA. The first probe was a Raman reporter, i.e., AgNP-conjugated 12-base DNA sequence, and the second probe consisted of Ag@MNPs conjugated to a

Table 4.1 Types of magnetic nanoparticle-based sensors and their applications in clinical analyses

Sensor nomenclature	Application	Sensitivity/capacity toward application	References
Silica-coated Fe_3O_4 nanoparticles	Fluorescence-based detection of biotin	NA	Jang and Lim (2010)
Thiolated sgc8c aptamer-immobilized AuNPs-coated magnetic Fe_3O_4NPs	Electrochemical-based detection of leukemia cancer cells	The linear detection range is 10 to 1×10^6cell mL^{-1}	Khoshfetrat and Mehrgardi (2017)
Fe@Au MNPs	Fluorescence-based detection of DNA	The linear detection range is 0.1 to 100 nM; LOD is 0.1 nM	Liu et al. (2010)
MNPs conjugated with two Antithrombin DNA aptamers	Spectroscopic-based detection of thrombin	The detection limit of thrombin is 4 nM or 2 pmole	Zhang et al. (2013)
Fe_3O_4@Ag MNPs	SERS-based detection of microRNA (miRNA)	The LOD of miRNAs is 0.3 fM (15 zeptomoles, 50 μL)	Pang et al. (2016)
Glucose oxidase-Cu hybrid nanoflowers embedded with Fe_3O_4 MNPs (MNPs-GOx NFs)	Peroxidase-like catalytic activity-based detection of glucose	The linear detection range is 5×10^{-6} M–150×10^{-6} M; LOD is 2.5×10^{-6} M	Cheon et al. (2019)
RGO-Fe_3O_4/GOD modified on a magnetic SPE	The amperometric technique (electrochemical)-based detection of glucose	The linear detection range is 0.05 to 1 mM; LOD is 0.1 μM	Pakapongpan and Poo-arporn (2017)
$ZnFe_2O_4$/MNPs ionic liquids carbon paste electrode	Electrochemical detection of 5-fluorouracil in drugs	The linear detection range is 0.1–1400 μM; the detection limit is 0.07 μM 5-FU	Fallah Shojaei et al. (2016)
DNA/chitosan/Fe_3O_4/HRP/glassy carbon electrode (GCE)	The amperometric technique (electrochemical)-based detection of H_2O_2	The linear detection range of H_2O_2 is 2 μM to 100 μM; LOD is 1 μM	Gu et al. (2014)
SPE/MNP-K2	The DPV, CV, and EIS were used for the detection of JWH 073 (4-butanoic acid metabolite)	The linear detection range is 5–400 ng/mL; LOD is 22 ng/mL	Sanli et al. (2020)

different 12-base DNA sequence. These DNA sequence bases of both probes were complementary to a particular portion of the target DNA. The hybridization of the two probes with the target DNA results in AgNPs aggregation as shown in Fig. 4.6a. The marker used for *C. krusei*-specific probe 1 (Raman reporter) was 4-mercaptopurine (MP) and malachite green isothiocyanate (MG) for *C. albicans*

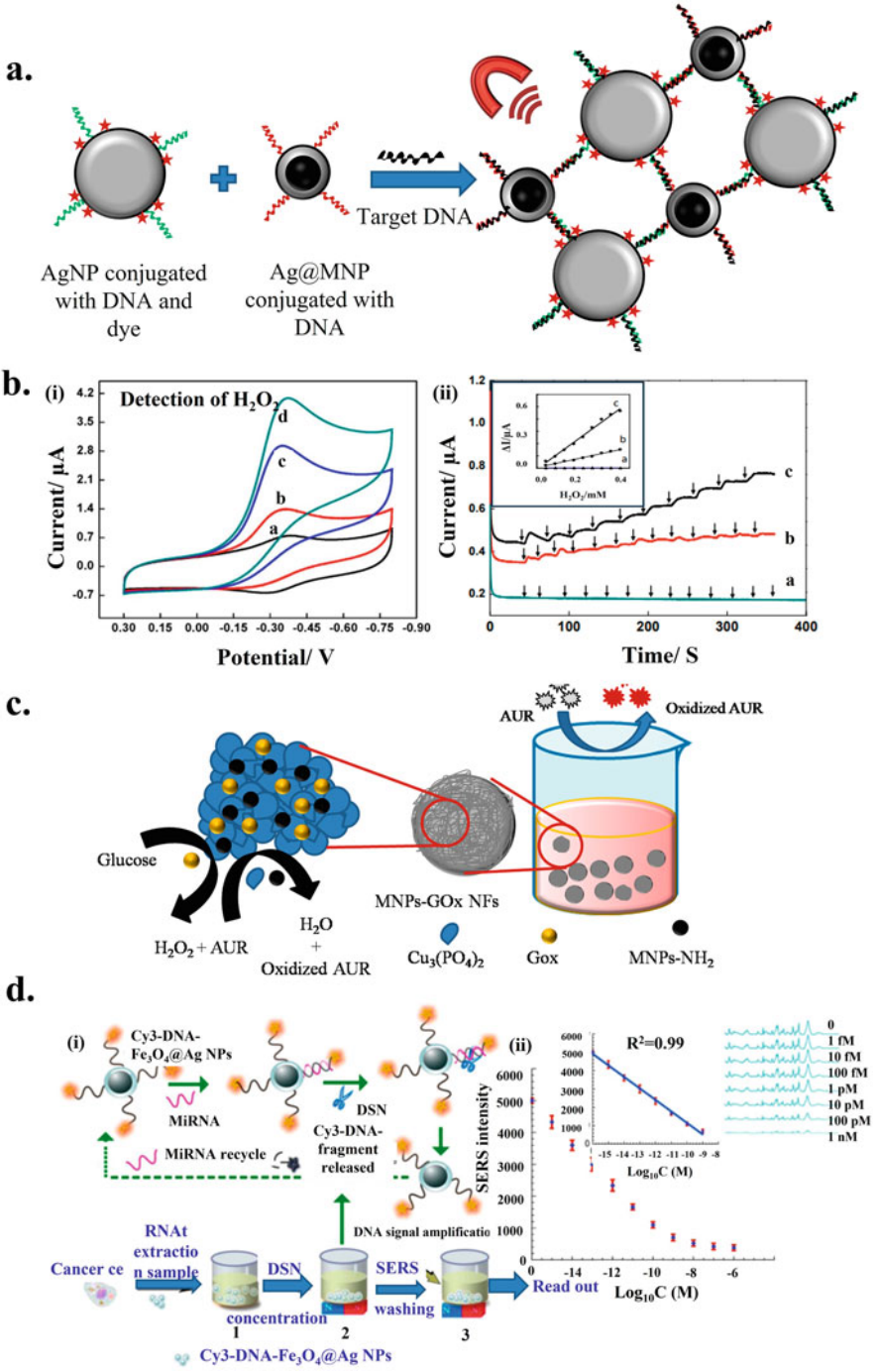

Fig. 4.6 (**a**) (i) Schematic of assay showing AgNP assembly in presence of target DNA; (**b**) (i) CV for H_2O_2 concentrations (**a**–**d**) 0, 1, 3, 4 mM and (ii) current-time response curve for 0.1 mM of H_2O_2 obtained by DNA/chitosan/GCE, DNA/chitosan/HRP/GCE, and DNA/chitosan/Fe_3O_4/HRP/

for the selective detection of target DNA using enhanced Raman signal after aggregation of NPs.

Hydrogen peroxide (H_2O_2): H_2O_2 is a reactive oxygen species that is an important molecule present in the human body. H_2O_2 plays an important role in physiological processes, like apoptosis, cellular dysfunction, signaling, and immune activation when present in the normal range (Kim et al. 2015). However, at high levels, it can be harmful to the system that can cause inflammatory disease, cell damage, and even deadly disease like cancer (Lisanti et al. 2011). High H_2O_2 reactivity and low physiological concentration in the body make it difficult to determine the concentration. To improve understanding of the vital role of H_2O_2 in biological systems, researchers keep on trying and developing sensors to detect and quantify H_2O_2 in the solution phase as well as in real samples under various physiological conditions. Gu et al. developed DNA/chitosan-Fe_3O_4 magnetic nanoparticle-based bio-complex film for horseradish peroxidase (HRP) immobilization on GCE for sensitive and selective determination toward H_2O_2. The modified DNA/chitosan/Fe_3O_4/HRP/GCE showed good selectivity and sensitivity toward H_2O_2 detection, and the linear response was obtained from 2 μM to 100 μM ($R^2 = 0.99$) with a LOD of 1 μM and sensitivity of 20.8 $A{\cdot}cm^{-2}{\cdot}M^{-1}$ (Fig. 4.6b) (Gu et al. 2014).

Glucose: Glucose is a monosaccharide, essential in the life cycle of humans. Glucose level alterations can lead to disorders mainly in the kidneys, heart, and eyes (Cheon et al. 2019). To control their blood glucose level, patients have to regularly check their glucose levels in a simple, affordable, and real-time manner. In this context, we have discussed glucose detection using MNPs. Cheon et al. reported novel glucose oxidase (GOx)-copper hybrid nanoflowers (NFs) embedded with Fe_3O_4 MNPs that exhibit superior peroxidase-like activity for glucose detection. The GOx showed enhanced catalytic activity and generates H_2O_2 in presence of glucose, which is subsequently used by MNPs and crystals of Cu-phosphate, near GOx molecules, to convert to fluorescent product from Amplex UltraRed substrate. Determination of glucose with the MNPs-GOx-NFs was used for obtaining excellent stability, along with good selectivity, sensitivity, and magnetic reusability (Fig. 4.6c). The MNPs-GOx-NFs-based biosensor also exhibits high selectivity and reproducibility with real blood samples (Cheon et al. 2019). Fluorescent biosensors have great potential for the sensitive determination of various clinically important molecules.

MicroRNAs: MicroRNAs are a series of small-sized, noncoding, single-stranded RNA molecules. MicroRNAs could regulate diverse gene expression levels and types, which are directly related to the targeted therapy, early diagnosis, and

Fig. 4.6 (continued) GCE (**a**–**c**) (inset: the plots of amperometric responses (ΔI) vs. H_2O_2 concentration); (**c**) schematic of glucose detection using MNPs-GOx NFs; (**d**) (i) schematic of miRNA assay strategy and SERS intensities versus let-7b concentrations and (ii) linear relationship between the SERS intensity and the miRNA concentration. (Reprinted with permission from references Gu et al. (2014) and Pang et al. (2016))

prognosis of diseases. Hence, accurate determination of miRNAs would lead to enormous progress in the early treatment of numerous deadly diseases (Ouyang et al. 2019). Several methods have been employed by many research groups to improve the sensitivity of miRNA detection. For example, Pang et al. have synthesized Fe_3O_4@Ag MNPs biosensors for miRNA detection as well as ultrasensitive determination in total RNA extract from cancer cells (Fig. 4.6d). Here, Fe_3O_4@AgNPs were modified with Raman tags-DNA probes for SERS and duplex-specific nuclease signal amplification platform. The detection sensitivity of miRNA was recorded up to 0.3 fM (15 zeptomoles, 50 μL). From the above discussion, this is clear that the Fe_3O_4@Ag biosensor is suitable for single-base recognition and signal-amplifying ability of the endonuclease DSN with an affordable SERS strategy for detection of miRNA as point-of-care clinical diagnostics (Pang et al. 2016).

4.4.2 MNPs for Environmental Safety

Rapid industrialization has resulted in increased environmental pollution levels of air and water (Kurshanov et al. 2020; RoyChowdhury et al. 2018). Environmental pollutants can be of several types like organic pollutants, inorganic pollutants, organic volatile compounds (VOC), and bacterial contamination (Akbarzadeh et al. 2012; Govan 2020). Pollutants enter the food chain through drinking water and inhalation and lead to toxic effects on human and animal health. Thus, time-to-time monitoring of water and air quality is important for a clean and safe environment. In this context, MNPs have also been extensively used. The MNPs provide easy surface modification with small molecules, ligands, biomolecules, and other nanostructures like quantum dots and noble metal nanostructures forming nanohybrid for developing the customizable sensing probe. This results in higher specificity for target analyte and enhanced properties due to nanohybrid system. Moreover, the MNPs also provide a common platform for simultaneous sensing and removal of target analyte from sample due to their magnetic property. Under the effect of external magnetic field, the MNP-analyte complex precipitates out of the sample and can be separated out easily (Zhou et al. 2016). Thus, MNPs play an important role in development of environmental quality monitoring methodologies. Table 4.2 shows application of magnetic nanoparticle-based sensors used in various environmental sensing applications.

Organic pollutants: These are the compounds made up of oxygen, and nitrogen, with carbon backbone, which includes phenols, chlorinated phenols, polychlorinated biphenyls, polyaromatic hydrocarbons, phthalates, pesticides, and dyes (Bharagava et al. 2019; Chandra et al. 2011). These pollutants are widely used in industries; in agriculture as pesticides and herbicides, pest control, and disease control; and in plastics (like bottles, containers, and toys). Organic pollutants are persistent and nonbiodegradable, accumulate in the fat cell, and cause toxic effects like damage to a nerve and endocrine systems and cancer (Rusiecki et al. 2008). Magnetic nanoparticle has greatly contributed to the rapid sensing and remediation of these organic toxins. Wang and co-worker prepared Fe_3O_4 MNP-decorated graphene-metal

Table 4.2 Magnetic nanoparticle-based sensors used conjugates in various environmental sensing applications

Sensor nomenclature	Target pollutant	Mode of sensing	LOD	References
Porphyrin-modified Fe_3O_4@SiO_2	Hg^{II}	Fluorescence enhanced in presence of Hg^{II}	-----	Sun et al. (2011)
AuNP-Fe_3O_4	As^{III}	Impedance-based electrochemical	0.00097 mg L^{-1}	Cui et al. (2012)
1,4-Dihydroxyanthraquinone (1,4-DHAQ) and 9-fluorenylmethyl chloroformate (Fmoc-cl) Co-modified Fe_3 O_4	Cu^{II} Cd^{II} Zn^{II} Hg^{II}	Fluorescent detection	8×10^{-9}, M 1.5×10^{-8} M 10^{-8} M 10^{-8} M	Wang et al. (2013)
AuNP-coated $La_{0.67}Sr_{0.33}MnO_3$ MNP	Pb^{II}	Electrochemical Square-wave voltammetry	-----	Kong et al. (2013)
Fe_3O_4 modified with fluorescently labeled DNA	As^{V}	Fluorescence enhanced in presence of As^{V}	300 nM	Liu and Liu (2014)
Oligonucleotide-modified $CuInS_2$ QD@Fe_3O_4	As^{V}	Fluorescence enhanced in presence of As^{V}	0.13 nmol·L	Liu et al. (2015)
Rhodamine-functionalized Fe_3O_4	Hg^{II}	Fluorescence enhanced in presence of Hg^{II}	4.13 x 10^{-5} M	Kim et al. (2016)
L-cysteine (L-Cys)-capped Fe_3 O_4@ZnO	Fe^{III}	Fluorescence quenched in presence of Fe^{III}	3 nmol L^{-1}	Li et al. (2016)
DNA-functionalized AuNP@Fe_3O_4	Ag^{I} Hg^{II}	Electrochemical	0.37 ppb 0.34 ppb	Miao et al. (2017)
Fe_3O_4 modified with fluorescently labeled DNA	As^{V}	Fluorescence enhanced in presence of As^{V}	300 nM	Lopez et al. (2017)
Fe_3O_4	AsV	Colorimetric Fenton-like catalytic reaction (colorless to blue)	0.358 nM	Babu Christus et al. (2018)
Fe_3O_4@ZIF-67 (MNP-MOF composite)	Hg^{II}	Colorimetric peroxidase-like catalytic reaction (colorless to blue)	0.36 nM	Christus et al. (2018)
Fe_3O_4@SiO_2@UiO-67 magnetic-Zr MOF	Glyphosate ($C_3H_8NO_5P$)	Fluorescence enhanced in presence of glyphosate	0.093 mg L^{-1}	Yang et al. (2018)

(continued)

Table 4.2 (continued)

Sensor nomenclature	Target pollutant	Mode of sensing	LOD	References
Fe_3O_4@ZIF-8 nanocomposite	Diazinon ($C_{12}H_{21}N_2O_3PS$)	Colorimetric (blue-to-colorless) peroxidase-like catalytic reaction	0.2 nM	Bagheri et al. (2019)
Fe_3O_4/rGO/MOF nanocomposite	Phenol	Colorimetric (colorless-to-red) peroxidase-like catalytic reaction	6.67×10^{-7} M	Wang et al. (2019)
$AgInS_2$/ZnS QD-Fe_3O_4 embedded on $CaCO_3$ porous matrix	CoII NiII Pb^{II}	Fluorescence quenched in presence of heavy metals	0.01 ppm, 0.1 ppm, 0.01 ppm	Kurshanov et al. (2020)
AuNP-Fe_3O_4	Hg^{II}	Colorimetric Red to colorless	5 μM	Liu et al. (2020)

organic framework (MOF) composite (Fe_3O_4/rGO/ZIF-8) for colorimetric detection of phenol mimicking peroxidase-like enzymatic activity. The Fe_3O_4/rGO/ZIF-8 catalyzes the oxidation of 4-amino antipyrine (4-AAP) in presence of H_2O_2. The solution of 4-AAP − H_2O_2 in presence of phenol is colorless. However, when the reaction is carried in presence of Fe_3O_4/rGO/ZIF-8, the solution color changes to pink, and the intensity of color increases with an increase in phenol concentration (Fig. 4.7a) (Wang et al. 2019). Bagheri et al. synthesized Fe_3O_4@ZIF-8 magnetic nanocomposite for colorimetric sensing of diazinon ($C_{12}H_{21}N_2O_3PS$), an organophosphate insecticide. Fe_3O_4@ZIF-8 nanocomposite catalyzes the oxidation reaction of TMB − H_2O_2 producing blue color. However, addition of diazinon turns solution to colorless (Bagheri et al. 2019). Yang et al. reported Fe_3O_4@SiO_2@UiO-67 magnetic-Zr MOF as a fluorescent sensor for the detection of glyphosate ($C_3H_8NO_5P$), a residue of organophosphorus pesticide. The zr-OH group has a high affinity for the phosphate group and shows a significant change in fluorescence intensity when excited at 315 nm (Yang et al. 2018).

Inorganic pollutants include toxic heavy metals like lead (Pb), arsenic (As), mercury (Hg), chromium (Cr), cadmium (Cd), copper (Cu), etc. Heavy metals are nonbiodegradable and accumulate in soft tissues triggering toxic effects (Bharagava et al. 2019; Nath et al. 2018; Priyadarshni et al. 2020; Priyadarshni et al. 2018; Priyadarshni et al. 2021; Saxena and Bharagava 2017). Li et al. reported L-cysteine (L-Cys)-capped Fe_3O_4@ZnO core-shell nanoparticles as a fluorescence sensor for selective detection of Fe^{III}. The interaction of Fe^{III} to Fe_3O_4@ZnO MNP decreased the fluorescence of the nanosensor. Under the influence of the magnetic field, Fe^{III} can be easily separated leaving Fe^{III}-free water (Fig. 4.7b) (Li et al. 2016). Wang and

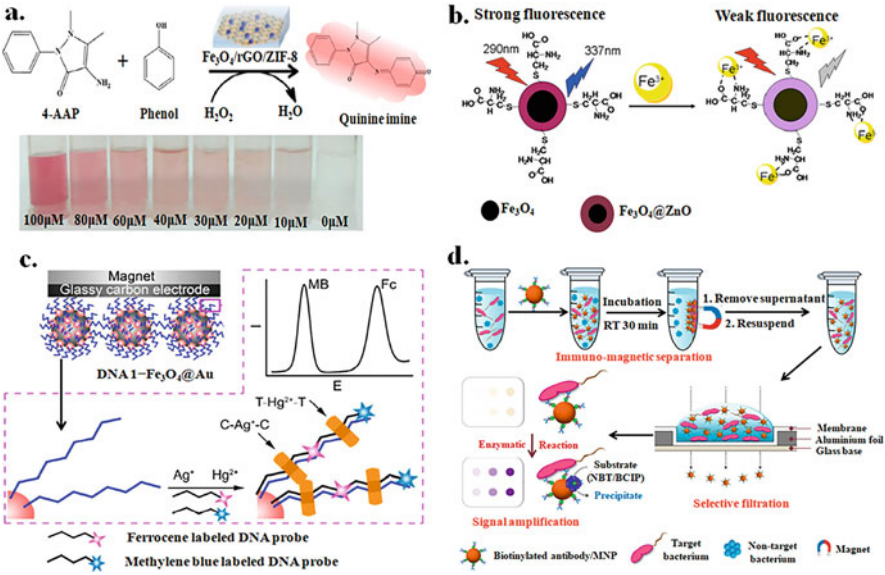

Fig. 4.7 Application of magnetic nanoparticle in detection of environmental toxins and bacterial pathogen. (**a**) Fe_3O_4 MNP-decorated graphene-metal organic framework composite (Fe_3O_4/rGO/ZIF-8) for colorimetric detection of phenol (colorless to pink), mimicking peroxidase-like enzymatic activity; (**b**) L-cysteine (L-Cys)-capped Fe_3O_4@ZnO core-shell nanoparticles as fluorescence sensor for selective detection of Fe^{III}; (**c**) DNA-modified Fe_3O_4MNP and gold nanoparticle-based nano-conjugate (DNA1 − Fe_3O_4@Au) used as electrochemical biosensor for detection of silver (Ag^I) and mercury (Hg^{II}); (**d**) colorimetric detection and simultaneous separation of *E. coli* O157:H7 using MNP modified with biotinylated antibody forming grayish-purple color with NBT/BCIP. (Reprinted with permission from reference a. (Wang et al. 2019), b. reprint with proper citation, no permission required (Li et al. 2016), c. (Miao et al. 2017), d. (Kim et al. 2018))

co-worker synthesized Fe_3O_4 MNPs co-modified with 1,4-dihydroxyanthraquinone (1,4-DHAQ) derivative and 9-fluorenylmethyl chloroformate (Fmoc-Cl) (denoted as 1,4-DHAQ/Fmoc-Cl-Fe_3O_4) and used as fluorescence sensor for rapid qualitative detection of Cu^{II}, Cd^{II}, Zn^{II}, and Hg^{II}. When the 1,4-DHAQ/Fmoc-Cl-Fe_3O_4 is excited with 250 nm, the emission band at 395 nm (Fmoc-Cl) enhances with Hg^{II} and Cu^{II} while decreasing with Zn^{II}. When the 1,4-DHAQ/Fmoc-Cl-Fe_3O_4 is excited with 450 nm, the emission peak decreased at 530 nm (from 1,4-DHAQ) with Cd^{II}, $Cu^{II,}$ and Zn^{II} (Wang et al. 2013). Cui et al. developed an electrochemical sensor for arsenic using Fe_3O_4 MNP and gold nanoparticle-modified glassy carbon electrode (Au NPs/Fe_3O_4/GCE). The working electrode was prepared by dropping Fe_3O_4 on GCE followed by electrodeposition of Au-NP. As(III) was determined by square-wave anodic stripping voltammetry (SWASV) with a detection limit of 0.00097 mg L^{-1} (Cui et al. 2012). Miao et al. developed three DNA-modified Fe_3O_4 MNPs and gold nanoparticle-based nano-conjugate (DNA1 − Fe_3O_4@Au) and used them as an electrochemical biosensor for the detection of silver (Ag^I) and

mercury (Hg^{II}). The first DNA probe (DNA1) was thiolated and used to modify the surface of Fe_3O_4@Au; the other two probes, DNA2 and DNA3, were labeled with two different electrochemical species ferrocene (FC) and methylene blue (MB), respectively. Bare GCE-DNA1 − Fe_3O_4@Au when determined by square-wave voltammetry did not show any current. However, after treatment with Ag^{I} and Hg^{II}, significant current peaks at 0.36 V (FC) and − 0.39 V (MB) appeared suggesting the presence of Ag^{I} and Hg^{II} in samples, respectively. Ag^{I} and Hg^{II} form a stable C − Ag^{I} − C and T − Hg^{II} − T base pair that assists the hybridization of DNA1-DNA2 and DNA1-DNA3 on the surface of DNA1 − Fe_3O_4@Au nano-conjugate (Fig. 4.7c) (Miao et al. 2017).

Sensing bacterial contamination: The clinical diagnosis of pathogenic bacteria often involves culturing a pathogen, enzyme-linked immunoassay, polymerase chain reaction, Western blotting, and genome sequencing (Pazos-Perez et al. 2016; Xu et al. 2018). These traditional methods involve the use of expensive and sophisticated instruments, trained professionals to operate, and time-consuming and laborious procedures (Váradi et al. 2017). The ease of application of MNPs can be employed to overcome the limitations of these techniques and provides a platform for easy and early-stage identification of bacterial contaminants (Ali et al. 2021; Yuan et al. 2018). Kim and a co-worker developed a selective colorimetric detection method for *E. coli* O157:H7 using MNP modified with a biotinylated antibody specific to target bacteria. The biotinylated antibody/MNP binds specifically with the target bacterial strain through target-antibody recognition. The bacteria-biotinylated antibody/MNP complex is separated magnetically, resuspended in fresh solution, and filtered through a nitrocellulose membrane filter. The unbound biotinylated antibody/MNP is smaller than the pore size of membrane filters and passes through. The target bacteria-bound biotinylated antibody/MNP complex is larger and cannot pass through the filter pores. The target-biotinylated antibody/MNP complex that remained on the membrane filter surface is treated with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) forming grayish-purple color. The intensity of color generated depends on the concentration of *E. coli* O157:H7 (Fig. 4.7d). The lowest detection limit (LOD) for *E. coli* O157:H7 was 10 colony-forming units (CFU) per milliliter through the colorimetric technique (Kim et al. 2018).

4.5 Conclusion

The MNP has widely been employed in a variety of applications ranging from targeted drug delivery to analytical techniques. Their properties displaying the responses in both electrical and magnetic stimuli have leveraged extremely unique advantages for preconcentration of the target analytes and the selective detections, which attracts its implication to the development of various clinical, biomedical, and environmental sensing modules for sensitive and selective detection. Here, in this chapter, we have discussed various strategies for the preparation and application of MNPs using representative examples, illustrations, and a comprehensive table. The

future direction in this context relies on the MNP-based nanocomposite synthesis, characterizations, and reproducible sensing of the properties that offer simultaneous multianalyte detections. Since magnetic field-based detectors are used as MNPs labels, future works should involve the precise operation of these in micrometer and nanometer scales to extend the wide range of environmental, clinical, and food safety and security applications. In addition, the works in this field should rely onto addressing the limitations in contemporary diagnostic strategies for better analytical performances.

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Optically Active Nanomaterials for Point-of-Care Diagnosis in Healthcare

5

Merin Joseph, P. P. Muhammed Shafeeque Rahman, and Lakshmi V. Nair

Abstract

Nanomaterial-based point-of-care (POC) diagnostic devices have revolutionized healthcare monitoring by simultaneously miniaturizing the detection module and improving its sensitivity to detect a particular disease. In healthcare, POC testing (POCT) follows ASSURED criteria for diagnosis of different diseases near the place and time of patient care. Among all types of biosensors, optical biosensors offer simplicity and quick detection method, instrument-free naked eye detection, and options of noninvasive imaging. Use of optically active nanomaterial is one of the most promising candidates for the noninvasive POC diagnosis of diseases. In this chapter, the recent advances in applications of optically active nanomaterials for the diagnosis of different diseases like cancer, cardiovascular diseases, neurodegenerative diseases, and multiplexing are discussed.

Keywords

Optically active nanomaterials · Noninvasive point-of-care testing · Detection of biomolecules · Multifunctional nanomaterial

M. Joseph · P. P. M. S. Rahman · L. V. Nair (✉)
School of Materials Science and Engineering, National Institute of Technology Calicut, Calicut, Kerala, India
e-mail: lakshmivnair@nitc.ac.in

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5.1 Introduction

5.1.1 Point-of-Care Diagnosis

The diagnostics test performed near the place and time of the patient care is referred to as point-of-care (POC) diagnosis. Despite tremendous advancements in medical diagnostics in the last few years, a lack of timely and accurate diagnosis leads to mortality and morbidity. However, on-time detection and treatment can minimize the suffering of the patients and even save many lives. Advancement in POC technology is one of the alternatives. An ideal POC diagnostic tool can be considered a small, portable, user-friendly diagnostic device that could detect various parameters without the presence of a doctor or a skilled laboratory technician or sophisticated instrumentations. This could be the future of ideal diagnostics in all parts of the globe, especially for the developing and undeveloped countries where in rural areas, medical infrastructure and skilled practitioners are less significant.

Nowadays, user-friendly point-of-care diagnostics glucose sensors are available in the market for the accurate monitoring of fasting blood sugar (FBS) and post prandial blood sugar (PPBS) levels on a daily basis (Purohit et al. 2022a). These devices are beneficial for those patients whose glucose level needs to be monitored, unlike the needle prick, which is a painful thing in the early morning. A noninvasive point-of-care diagnostic tool will be an ideal option when one thinks of having a point-of-care diagnostic device.

5.1.2 Noninvasive Point-of-Care Diagnosis

In the present era, the point-of-care diagnostics of some of the analytes was clinically established for the early diagnosis and control of diseases. The majority of the diagnostics kits are invasive in nature, where measurement has to be performed in blood. Annoying pain due to finger prick or blood draw restricts those who need continuous monitoring. Due to the abovementioned reason, the POC technology limits its application. In this scenario, a noninvasive and real-time monitoring and diagnosis system for home use is the current trend in point-of-care diagnostics. Apart from this, the noninvasive point-of-care testing should have accuracy and sensitivity comparable to a conventional hospital analysis. Such accurate results help humanity to track their health condition from home or workplace.

The unique properties offered by the nanoparticles due to the nanodimensions provide better performance in the point-of-care diagnostics technologies. Using the nanotechnological approach, one can manipulate the material in the nanosize dimension for the desired application. They possess unique physicochemical properties compared to their bulk counterpart. High surface-to-volume ratio, catalytical activity, high selectivity and sensitivity, biocompatibility, etc., make these materials a better candidate in the field of biosensing and thereby in the POC diagnostics field (Quesada-González and Merkoçi 2018; Sun et al. 2014). They are increasingly used as a biosensor probe for various bioanalytes. Different nanoparticles such as metallic

nanomaterials (gold, silver, etc.) (Xu et al. 2014; Yen et al. 2015), semiconducting nanomaterials (quantum dots) (Yang et al. 2010; Nair et al. 2020), carbon-based (carbon nanotubes, carbon dots, graphene, etc.) (Vilela et al. 2017; Yin et al. 2021) nanomaterials, etc., with varied sizes and shapes were demonstrated as better candidates in the field of biosensing. Apart from these, the nanoparticles were used as electrochemical, optical, and/or magnetic sensors for signal generation, transduction, and amplification. For example, urease-immobilized gold clusters were used for the selective detection of urea in real samples (Nair et al. 2013a). In the presence of immobilized nanoparticles, urea gets converted into ammonium and carbon dioxide. The formed ammonium ion neutralizes the surface charge of the nanomaterial, which in turn results in fluorescence quenching of the nanomaterial (Nair et al. 2013a). This portion of the book chapter mainly focuses on the recent advancement of optically active nanomaterials in the field of point-of-care diagnostics.

5.1.3 Optically Active Nanomaterials

Light-based diagnostics techniques are known to be noninvasive, as light is made to pass through the body, and as a result, the risk factor or side effects on continuous usage are less compared to other methods. For this purpose, visible, near-infrared (NIR), or infrared (IR) rays are commonly employed for medical diagnostics. Ultraviolet (UV) rays are harmful to humans and thus cannot be utilized. Figure 5.1 represents different optically active nanomaterials in point-of-care detection.

Optical/light-based oxygen sensors (oximeters) are widely used in medicine. Such devices work based on the difference in light absorption and emission from the translucent body part of the patients (Purohit et al. 2022b). It generally uses LEDs as the light source. Commercially available oxygen-level-detecting sensors are small, easy to use, and portable. Similarly, in smartwatches, pulses can be detected using light-based sensors. This miniaturization has led to a growing interest in noninvasive methods of POC detection using light sources. Such light-based techniques help the patients for continuous real-time monitoring of analyte in a

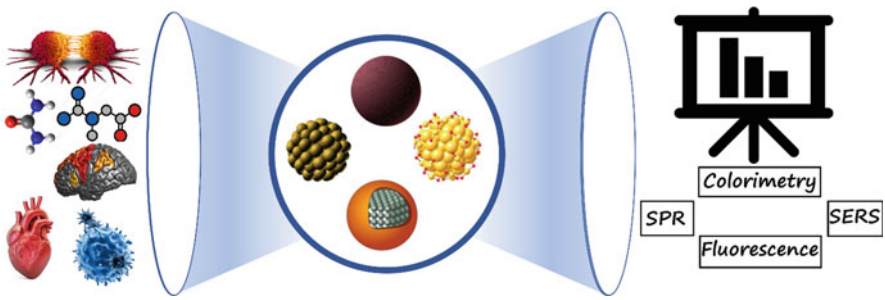

Fig. 5.1 Schematic representation of different optically active nanomaterials in optical point-of-care detection

painless mode. This constant monitoring helps to obtain a better overview of the patient's health. Optically active nanomaterial will be an asset in that sense in a noninvasive way. In this chapter, optically active nanomaterials refer to nanomaterials that respond to light. Optical properties such as surface plasmon resonance (Inci et al. 2013; Park et al. 2012; Yanik et al. 2010; Lathika et al. 2021), two-photon luminescence, fluorescence property (Vilela et al. 2017; Sierra et al. 2020; Qin et al. 2007), etc., are commonly employed in current popular POC techniques. Gold nanoparticles, quantum dots, and gold clusters are widely used candidates in the class of optically active nanoparticles. Three surface proteins of antibody-conjugated gold nanoparticle show a visual change in the color of gold nanoparticle upon interacting with SARS COVID-19 (Ventura et al. 2020). The absorption of gold nanoparticle red-shifts when the sample has COVID-19 virus. The unique photoluminescence property of quantum dots was demonstrated for the POC diagnostics of bioanalyte detection. Syphilis-detecting POC diagnostics lateral flow test was developed using the quantum dot (Yang et al. 2010). This quantum dot detection system offers high sensitivity, is economical, and offers rapid screening toward syphilis.

5.2 Current Point-of-Care Diagnostics Technologies

Pregnancy kit, oximeter, and blood glucose meter are commonly used POC diagnostics at home. Modern POC diagnostics technology can be divided into two broader ways. One is a small handheld device having strips for qualitative and quantitative reading. The second type is more complex bench-top devices commonly used in conventional laboratories. With the advancement of nanotechnology, the miniaturization of devices is possible, making smaller and smaller devices that incorporate all of the critical design features.

Disease diagnosis is possible by employing lateral flow strips from body fluids such as saliva, blood, sweat, urine, etc. (Miočević et al. 2017; Majdinasab et al. 2022) The most common lateral flow strip consists of four parts: sample compartment, conjugate pad, membrane, and absorbent pad. Body fluid is kept in the sample compartment. The conjugate pad is coated with specific ligands such as antibodies specific for the analyte of interest for the generation of the signal. Generally, the membrane consists of a control and test line. The control line represents the accuracy of testing. The liquid sample in the sample compartment moves under the influence of a capillary force along the strip, and the conjugate pad transfers it into the membrane. In the presence of a specific antigen/analyte, the antibody-conjugated material binds with them, followed by the detection antibody on the test line. Only a control line is observed in the absence of a target analyte. Figure 5.2 shows examples of gold nanoparticle-based lateral flow detection systems (Xu et al. 2014; Hwang et al. 2018).

The first generation of lateral flow tests contains gold nanoparticles and latex beads as a label. Due to the inherent color of the label, a qualitative result can be visualized using the naked eye. Strip reading devices capable of detecting the

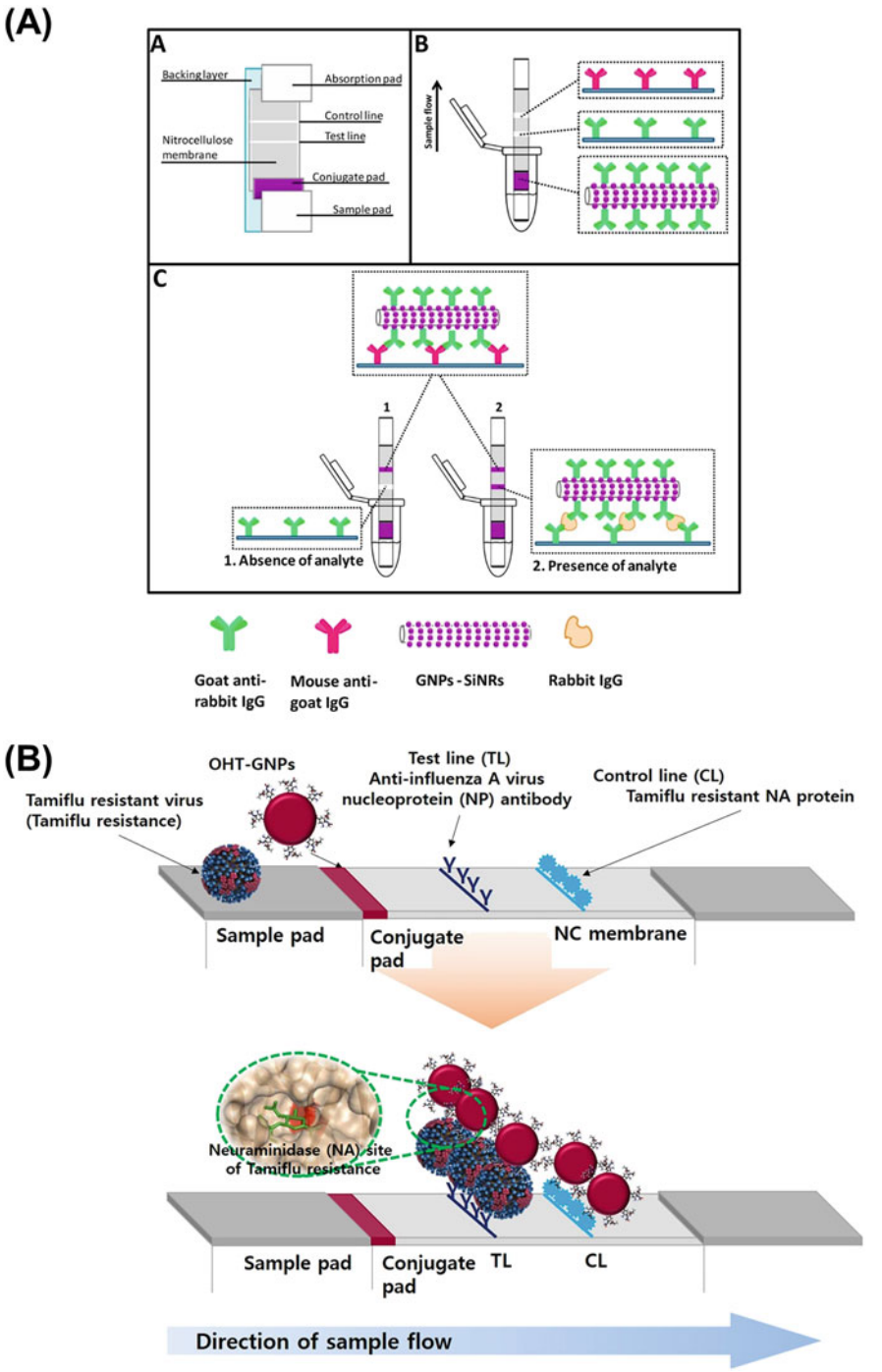

Fig. 5.2 (**a**) Shows schematic representation of lateral flow detection using gold nanoparticles. Adapted from the permission (Xu et al. 2014) (https://pubs.acs.org/doi/10.1021/ac502249f). Copyright@2014 American Chemical Society. (**b**) Gold nanoparticle-based lateral flow assay for

scattering of light from gold nanoparticles give a quantitative measurement. The limitations of first-generation devices were further improved by using different nanoparticles, surface modification, etc. Functionalization strategy is one of the vital components for improving the performance of a POC diagnostic device.

5.3 Functionalization Strategy for Point-of-Care Diagnostics

Nanomaterial possesses unique physical properties and features that make them ideal material in point-of-care diagnostics technology (Kumar et al. 2020). To use these materials for the said application requires controlled interaction with body fluids, tissues, or cells (see Chap. 2). In other words, the material should be stable enough to survive in that environment. Also, the developed nanoparticle should show desired response toward the analyte of interest and not with all components present in the body fluids. The surface functionality of the nanoparticle will determine its stability in the physiological fluids and help in the detection of the analyte of interest.

Molecules rich in –SH, $–COO^-$, –OH, $–NH_2$, etc., functionalities that can easily bind to metals to form complexes and further stabilize them on reduction are often used as ligands (see Chap. 1). Ligands can be of small molecules and large molecules (polymers, dendrimers) that can stabilize nanoparticles through steric and electrostatic means; however, while considering medical applications, biomolecules are often preferred. Furthermore, these ligands can act as targeting ligands also, or additional molecules can be conjugated to render the same. For instance, V. Nair et al. demonstrated an SPR nanobiosensor based on quantum dot in which it is conjugated with picric acid (PAQd) for specifically sensing creatine (Nair et al. 2020). Yen et al. described a colorimetric-based POC to detect and distinguish biomarkers corresponding to dengue, yellow fever, and Ebola viruses based on the color of nanoparticles (AgNPs) that are conjugated with specific antibodies (Yen et al. 2015). In the abovementioned examples, picric acid and antibodies conjugated act as targeting agents.

5.4 Optically Active Nanomaterials for Point-of-Care Diagnosis of Cancer

According to WHO, after cardiovascular disease, cancer stands second for the cause of death rates worldwide and is excepted to be a reason for 19.3 million deaths by 2025 (Gulland 2014). Mutation in a single cell emerges into a tumor after continuous and rapid cell division and eventually spreads to the whole body and becomes this

Fig. 5.2 (continued) colorimetric detection of Tamiflu-resistant virus. Reproduced from (https://www.nature.com/articles/s41598-018-31311-x) (Hwang et al. 2018) (Creative Commons CC BY license)

fatal. Hence, early-stage diagnosis is of great significance. It was reported that detecting and treating cancer at an early stage significantly increases the probability of survival, i.e., >85%; on the other hand, detection at later stages has a comparatively much lesser possibility of survival for all forms of cancer (Gloeckler Ries et al. 2003). Hence, point-of-care (POC) detection becomes very important for early-stage diagnosis and continuous user-friendly monitoring. Nanoparticle-based transducer platforms are widely employed in biosensors for immobilizing biomarkers, thereby detecting and amplifying signals on adsorption of analyte molecules by surface-enhanced Raman scattering (SERS), surface plasmon resonance (SPR), etc. Furthermore, absorption spectroscopy, fluorescence, and color changes (colorimetry) in the presence of specific analytes are also employed. Wang et al. demonstrated a potential point-of-care method for diagnosing oral cancer. Aggregation of rose bengal-functionalized gold nanorods (RB-GNR) in the presence of analyte can be understood from SPR properties (Wang et al. 2013). On comparing to transverse SPR peak of nanoparticles, longitudinal SPR peak of nanorods is more sensitive to surrounding environment, and here it undergoes a red shift in the presence of cancer cell lysate, which was manipulated as the sensing probe. The conjugated rose bengal is specific for oral cancer cells and often used as a staining agent for the detection of the same. Hence, RB-GNR was utilized for quantitative detection of oral cancer cells and NIR absorption imaging with the help of a CCD camera and LED as a light source. Furthermore, Liu et al. developed a noninvasive and rapid POC for cancer screening combining SERS and paper technology with 70% and 60% sensitivity and specificity, respectively (Paper-Based Plasmonic Platform for Sensitive 2014). SERS is a surface-sensitive technique for enhancing signal intensity in Raman spectroscopy. Raman spectroscopy records vibrational transitions from inelastically scattered photons after interaction with matter. This interaction leads to the gain or loss of energy of the photon and arises Raman lines (stoke and anti-stoke). However, these lines are comparatively less intense, and SERS is one such method to increase the intensity. Molecules are adsorbed on a metal surface like gold or silver having a nanoscopic roughness to give LSPR, which increases the polarizability of the molecule through electromagnetic effect (EME), and a chemical effect (CE) in turn increases the intensity (Campion and Kambhampati 1998). Liu et al. coated CTAB-mediated synthesized gold nanorods on commercial filter paper by dipping it in nanorod solution, further removed and dried (Xu et al. 2014). Exfoliated cancer and normal cells were placed on SERS substrate and analyzed spectra to obtain significant differences in Raman peaks. Normal cells showed lipid peaks more pronounced, whereas cancer cells showed more protein and DNA peaks.

5.5 Optically Active Nanomaterials for Point-of-Care Diagnosis of Neurodegenerative Diseases

The development of innovative and extremely sensitive point-of-care technology devices has gotten a lot of interest because of the potential societal implications. Many neurological problems are still unsolved clinical issues. Drugs and

pharmaceuticals are inhibited by the blood-brain barrier (BBB) to enter the central nervous system (CNS). Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD) are neurodegenerative diseases that are characterized by the gradual loss of certain neuronal cell types and are linked to protein aggregation. Nanomedicines can help with targeted therapy and imaging by manipulating the transport of biologically active substances (Gendelman et al. 2015). Thus, optical-based nanomedicine is promising as a POC diagnostic tool for neurodegenerative diseases. Major optical parameters include fluorescence, reflectance, chemiluminescence, absorption, scattering and interference, and a few more. Fluorescence stands as the major point of attraction in manufacturing devices in the biosensing industry (Syahir et al. 2015; MacBeath 2002; Stiles et al. 2008; Chen et al. 2018). As a result, instruments for real-time label-free detection are becoming more appealing as an emerging approach that requires further development, as no major sample preparation is required, like modifying the sample with conventional fluorophores or quantum dots. These nanoparticle bounding are utilized in terms of their optical sensing properties. These label-free optical sensors are also categorized as light scattering sensors and evanescent wave biosensors. The light scattering-induced electromagnetic (EM) scattering of biomolecules and nanodimensional structures on the sensor surface generates transducing signals. Examples are SERS and Raman spectroscopy (Stiles et al. 2008). One of the popular methods among optical detection methods is by noticing fluorescence-dependent conformational variations after laser irradiation. With a femtomolar range limit of detection (LOD) and no photo blinking, these sensors are sensitive, selective, and adaptive for detecting mRNA biomarkers; as a result, they can aid in the early identification of crucial disorder like Alzheimer's disease. Alzheimer's can be treated using gold nanoparticles that bind to L-glutamic acid. Since the size of a nanoparticle is significant, smaller ones limit beta-amyloid protein aggregation more. Iron nanoparticles, carbon nanotube, carbon dots, cerium oxide nanoparticles, nanopolymers, and other metal nanoparticles take part in the optical point-of-care device sensing mechanism (Bilal et al. 2020). A shape-code nanoplasmonic biosensor was developed by Kim et al., capable of detecting biomarkers associated with Alzheimer's disease. A highly selective biosensor was made by stacking AuNPs of varying sizes and antibodies onto a single substrate, which was subsequently analyzed by localized SPR. AuNPs are prepared in a range of shapes and sizes to achieve a variety of optical properties, and hence their diverse characteristics could be analyzed using optical spectrometers to create a barcode (Kim et al. 2018). In the evolution of bioanalytical procedures, quantum dots (QDs) have grown highly important due to their optical and electrical qualities that are unique and outstanding. The wide absorption spectra, size-tunable and limited emission spectra, high photostability, unique photoelectrochemical activity, and distinct electrochemical detection make them increasingly important as labels. Medina et al. designed an apolipoprotein E on-chip device of magneto-immunoassay using electrochemical detection and QD labeling (Stanisavljevic et al. 2015). Figure 5.3 illustrates a fluorescence upconverting nanoparticle-based sensor for the detection of Alzheimer's disease (Vilela et al. 2017).

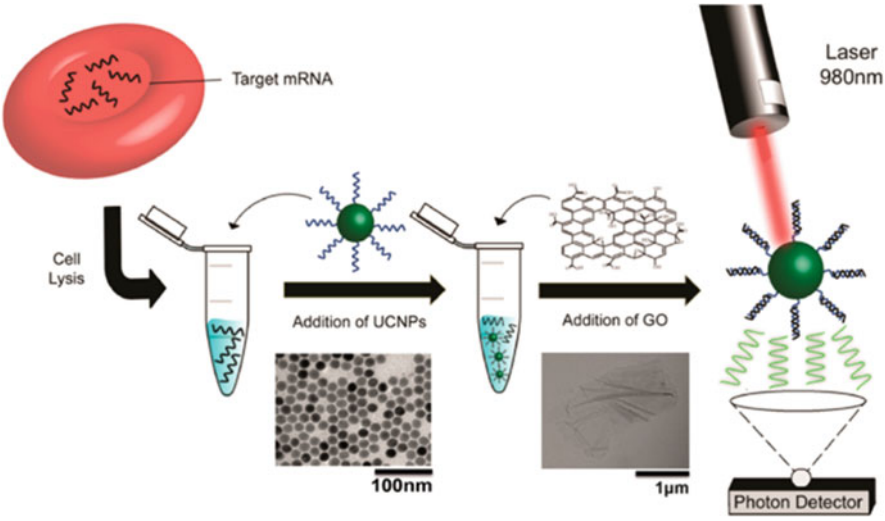

Fig. 5.3 Schematic representation of the detection of Alzheimer's disease using a fluorescence upconverting nanoparticle. Adapted from the permission (Vilela et al. 2017) (https://doi.org/10.1021/acssensors.6b00651). Copyright@2016 American Chemical Society

5.6 Optically Active Nanomaterials for Point-of-Care Diagnosis of Cardiovascular Diseases

Cardiovascular diseases (CVDs) are the primary cause of mortality and anguish worldwide. Most CVDs are preventable and treatable, with interventions that are both timely and effective, such as prognosis, diagnosis, and therapy monitoring that are critical. Several portable analytical devices for detecting cardiovascular diseases at POC have been developed. The most often used detection methods are optical assays. (Borisov and Wolfbeis 2008) Smartphones are now widely employed in optical and electrochemical tests in biomedical research. Colorimetric assays, chemiluminescent assays, fluorescent assays, fluorescence resonance energy transfer (FRET), and SERS are now the common approaches for detecting CVD biomarkers (Dinter et al. 2019). The signals can be observed and evaluated with the naked eye and measured with the help of a reader in colorimetric tests like AuNP-based lateral flow immunoassay (LFIA) and microchip enzyme-linked immunosorbent assay (ELISA). The underlying principle for quantifying relies on the quantity of light in the test region (Hu et al. 2016). The signal of quantum dot barcode in a microwell device was measured using a smartphone-based fluorescent readout method for multiplexed diagnostics. Ultrasound imaging is one of the most extensively utilized modalities for imaging numerous CVDs (Wang et al. 2018). Due to their excellent versatility and signal intensification capabilities, immunoassays and fluorescence assays are so far the most widely used analytical procedures. Nanomaterials have been used in fluorescence assays because of their strong biomolecule binding

affinity, high solubility, and low toxicity; thus they can be used in conjunction with some fluorescence amplification materials (Hicks 1984). Ex vivo laser projection imaging of the heart and aortas of atherogenic and control mice revealed that nanosensor aggregation develops preferentially in atherosclerotic-prone locations in mice, indicating therapeutic relevance of the in vivo method in small animals (Rouleau et al. 2013). Another technique for employing AuNPs in cardiovascular imaging is optical coherence tomography (OCT). The IR signal is steered to the target tissue via a broadband coherent light source in this manner, and a picture is formed from the back-scattered light (Yelbuz et al. 2002). The suggested approach sought to increase the efficiency of fluorophore-mediated biosensors by increasing fluorescence properties. Gold nanoparticles and organic solvents may improve fluorescence signals by mobilizing free electrons and minimizing self-quenching when positioned at a certain distance from fluorophores. In this case, researchers mixed gold nanoparticles-SAMs with solvents to create a nanogold particle reagent (Hong and Kang 2006).

5.7 Optically Active Nanomaterials for Point-of-Care Diagnosis of Glucose, Creatinine, and Urea

5.7.1 Glucose

Regular monitoring of glucose levels for a diabetic patient is essential, and blood sugar control is necessary. Recent advances have replaced finger stick and invasive blood emitting methods by photonic sector and by the advanced optical biomarkers. The powerful data analysis techniques can be designed from comparatively less expensive, painless, reliable, economic, and simple technology optical sources and detectors. The use of nonionizing radiation, quick response, and fewer chemical consumables are major attractions to use optical glucose sensors (McNichols and Coté 2000; Alsunaidi et al. 2021). In the physiological range, the nanoparticle sensors showed outstanding glucose responsiveness, which offers a viable technique for glucose surveillance in real time. The current interest of researchers is to encapsulate this sensing motif on a nanoparticle surface, as well as to optimize the concentration of local surface area for optical glucose sensing. Carbodiimide chemistry was used to immobilize the sensing motif onto silica nanoparticles (Le et al. 2020). An NIR phosphor optical biosensor in an encapsulated 3D gel was formed for the detection of glucose (Lee et al. 2022). For glucose sensing, the developed substrate possesses a high SERS sensitivity and outstanding electrochemical characteristics. To prepare Ag nanodendrites on a Cu mesh substrate, a simple electrochemical deposition process was used (Gao et al. 2021). A single-element focused ultrasonic transducer with an infrared laser (1645 nm) is employed in the ultrasound-modulated optical sensing (UOS) approach to keep track of glucose noninvasively. In a scattering medium, focused ultrasonic waves can acoustically localize scattered photons, allowing for a significantly higher spatial resolution representation of optical contrast (Park et al. 2020). Photo-acoustic spectroscopy

(PAS), fluorescence, and especially NIR spectroscopy are the noninvasive optical approaches suggested as prospective possibilities for accomplishing the aim of getting optimum glucose control.

5.7.2 Urea

Gold clusters act as a novel sensing material for urea detection without separating serum from blood. This Au cluster detection of urease is a highly sensitive and direct detection method via nanomaterial (Nair et al. 2013b). Juanjuan Liu et al. designed a low-cost, electrochemistry-based urea detection on a portable platform by electrodepositing AgNPs in one of the channels (working electrode) after removing enzymes embedded on the strip (Liu et al. 2020). As a fluorescent probe, stable green-emitting carbon dots (CDs) were manufactured in a single-step technique to assess the content of urea in milk (Yin et al. 2021). Botewad et al. developed an intrinsic optical fiber-based urea sensor. The developed sensor exhibited a direct response to urea concentrations in the range of a few nanomolar to 1 M at a wavelength of 250 nm in absorption spectrum with particular selectivity (Botewad et al. 2020). In a separate experiment, zinc oxide nanoparticles were generated at various pH levels to investigate bandgap change upon changing the particle size where the nanoparticles are N-doped. The addition of urea to N-doped ZnO produced an excellent fluorescence intensity response of 2.6–26 mM with an LOD of 4.93–0.02 mM and further confirmed using blood serum (Soundharraj et al. 2021). Another optical biosensor was developed based on urease enzyme for urea, where enzyme immobilized on functionalized calcium carbonate nanoparticles ($CaCO_3$-NPs). The nanoparticle was synthesized from the cockleshell, and the reflectance sensor mechanism was utilized for the study (Zakaria et al. 2022). A novel silica-gel nanosphere (SiO_2 NPs) composition was developed and further functionalized the surface with biomolecules. The color shift of the immobilized chromoionophore dye was used to detect urea concentrations (Alqasaimeh et al. 2014). Surface plasmon resonance-based detection of enzymatic and nonenzymatic urea was facilitated on Au nanoparticle-coated gold thin film (Vikas et al. 2020).

5.7.3 Creatinine

The resultant metabolites of nitrogen in the human body, creatinine (CR), are mostly eliminated from the blood by the kidneys, namely, through glomerular filtration. The failure of filtration might lead to an evident increase in creatinine content in blood and a reduction in urine if there is significant damage to functional nephrons. Hence, there is a link between creatinine concentration in bodily fluids and kidney functioning and related renal disorders (Perrone et al. 1992). CR is a metabolic by-product of muscles that has become more important in the identification of renal disorders due to its sensitivity and selectivity. Hong Du et al. used Ag on an AuNP surface to build a synergistic creatinine coordinating system, which was demonstrated and can be

used to identify creatinine quickly, selectively, and quantitatively. Thus, both colorimetric qualitative and quantitative identification via naked eye and absorbance spectroscopy may be made with a good LOD value when compared to earlier methodologies (Du et al. 2016). Muyang et al. suggested an uncomplicated, sensitive, and portable biosensor based on single-mode fiber-multicore fiber-multimode fiber-single-mode fiber (SMF-MCF-MMF-SMF) for creatinine spotting in the human body. Gold nanoparticles (AuNPs), graphene oxide (GO), creatininase (CA) enzyme, and the molybdenum disulfide nanoparticles (MoS_2-NPs) are used to functionalize the sensor probe. Fiber optic LSPR is used to assess creatinine concentration (Li et al. 2021). In the general testing procedure and practical application, a colorimetric approach based on AuNPs was established avoiding the use of sophisticated instruments. AuNPs stabilized by citrate are used as a platform, while polyethylene glycol (PEG) as a modifier. Mercury ions, which served as a linking unit, synergistically coordinated PEG and creatinine, resulted in AuNP aggregation and apparent solution color change (Xia et al. 2019). Sharma et al. developed a biosensor based on lossy mode resonance (LMR) utilizing optical fiber technology with $MOS_2@SnO_2$ nanocomposite as artificial antibodies and the LMR supporting material. This study was effective in developing a quick and simple method for measuring creatinine levels in an aqueous solution and also in synthetic urine samples, and it is now utilized as a biomarker to evaluate the effective functioning of human kidneys (Sharma et al. 2020). A supermolecular fluorescent dye was used to bind the CR analyte, and the shift of indicator fluorescence was observed for the detection of CR level (Sierra et al. 2020). For sensitive and specific CR identification on fiber optic long-period gratings, a new imprinting approach employing liquid phase deposition (LPD)-based TiO_2 nano-thin films was presented. Polyacrylic acid was included in the film to assist and regulate the shape of the film as well as the TiO_2 activity inside the binding site (Lee et al. 2021).

5.8 Optically Active Nanomaterials for Point-of-Care Diagnosis of Infectious Diseases

Many organisms live in our body, like bacteria, viruses, parasites, and fungi, of which most of them are helpful; however, some of them are harmful and affect the normal functioning of the body. Utmost care must be taken as a society while considering communicable diseases since it can affect the balance of the whole system, as in the case of COVID-19. According to WHO, in the European region, registered deaths due to COVID-19 are 98%, and in the African area, it's 10%. Proper vigilance and immediate actions like ensuring strict safety protocols, steps for vaccine development, etc., are a necessity for preventing or controlling any super spreading diseases.

Many infectious diseases significantly contribute to the death rate worldwide. Viral infections like recent COVID-19, hepatitis B and C virus (HBV and HCV, respectively), and human immunodeficiency virus (HIV), bacterial infections such as tuberculosis and cholera, and parasitic infections like mosquito spreading malaria

are some of the major infectious diseases, which raise the mortality rate. A perfect diagnostic tool should be specific, rapid, robust, sensitive, low-cost, accurate, and user-friendly (Wang et al. 2017a). Many conventional techniques for diagnosis are in practice, such as identification by culturing and further understanding features by microscopy, polymerase chain reaction (PCR), and immunology. However, more user-friendly, low-cost, and efficient POCs are significant for controlling infectious diseases at the initial stage itself. Being time-consuming (e.g., for diagnosis of tuberculosis, it may take weeks) and the requirement of a certain threshold level of pathogens in initial serum samples adds to the flaws of current diagnostic tools. Furthermore, certain bacteria life cycles involve a dormancy state, leading to negative results. Also, many of the conventional techniques require skilled labor and high cost, which again brings difficulties in resource-poor and developing countries, especially in developing nations.

Fluorescence properties, localized SPR, colorimetric assays, and SERS are exploited to develop POC devices for infectious diseases. The widely used prevailing conventional techniques, considered gold standard methods, like real-time polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), are fluorescence-based. For these, quantum dots or organic dyes are used for labeling specific target bacteria or viruses. However, fluorescence-based technologies are not fast and precise since it often suffers from limitations of sensitivity and multiplex detection of biomarkers (Chen et al. 2020).

Colorimetric techniques using nanoparticles have been established owing to their simplicity. Peng and Chen demonstrated colorimetric detection of bacterial pathogen by observing color change via the naked eye due to the aggregation of gold nanoparticles on thiolated phages, which could recognize its corresponding bacteria (Peng and Chen 2019). The principle behind LSPR-based biosensors is that LSPR from nanoparticles undergoes a wavelength shift of the absorbed light as a result of change in refractive index on the association of analyte molecules. Inci et al. realized diagnosis and estimation of HIV subtypes, namely, panels A, B, C, D, E, and G, without any further preparation of whole blood based on LSPR of gold nanoparticle. They developed a fast, label, and fluorescence-free quantification assay utilizing a nanoplasmonic platform for virus detection (Inci et al. 2013).

SERS finds helpful in developing fast and easy-to-use biosensors and hence can be considered as most promising in point-of-care devices. For developing SERS technology-alone-based POC, we have to go much more forward; however, incorporating SERS technology to current technologies has been explored (Li et al. 2019). Xiuli Fu et al. designed SERS-based lateral flow (LF) assay for addressing the drawbacks of LF strips used in POC (Fu et al. 2016). Raman reporter-labeled gold nanoparticles were positioned on the conjugate pad of LF strip so that HIV-1 DNA marker gives rise to a sandwich assay of AuNPs, target, and conjugate DNA to obtain a red line even at low concentrations due to the surface enhancement mechanism. The lower LOD was found to be <0.3 pg/mL, whose detection capability is ten times greater than the commercially available HIV-1 assay kit (Fu et al. 2016). A silver nanorice/DNA/patterned gold triangle nanoarray chip for detection of hepatitis B based on SERS was realized (Li et al. 2013). Concentration-dependent

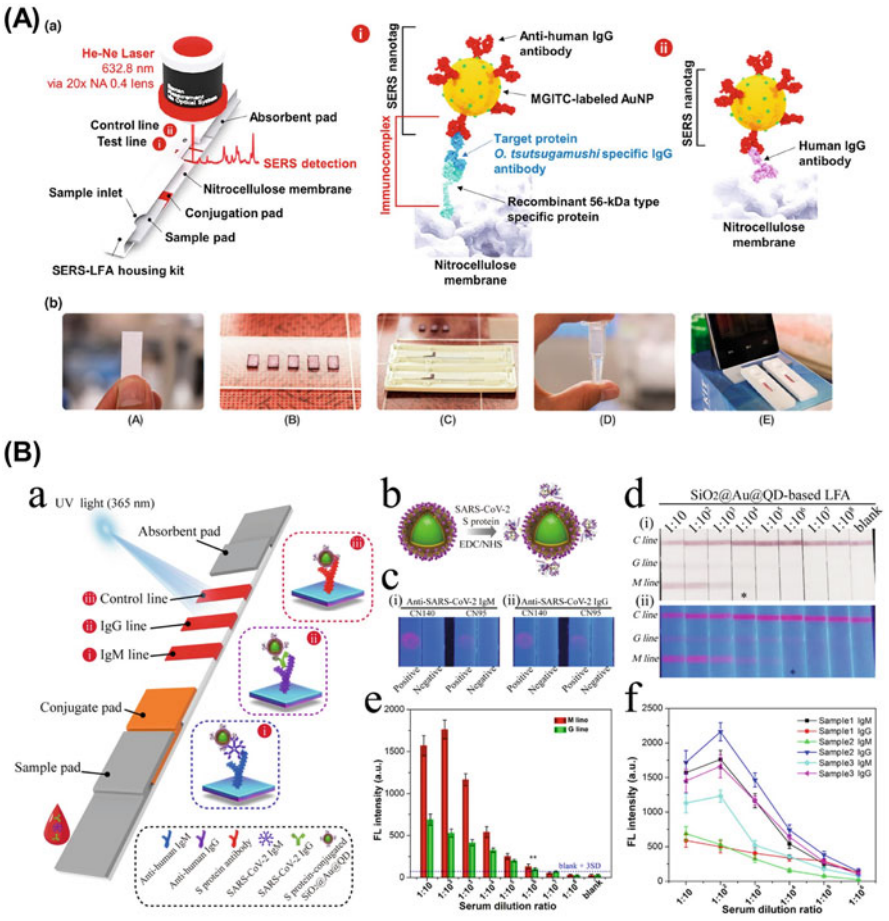

Fig. 5.4 (**a**) Schematic representation for the SERS-LFA process for the detection of *O. tsutsugamushi* in the human serum. Adapted from the permission (Lee et al. 2019) (https://doi.org/10.1021/acs.analchem.9b02363). Copyright@2019 American Chemical Society. (**b**) SiO_2@Au@QD nanobead-based lateral flow immunoassay POC for detection of SARS-CoV-2. Adapted from the permission (Wang et al. 2020) (https://doi.org/10.1021/acs.analchem.0c03484)

SERS intensity allowed sensing up to mutation in a single base of the target DNA. Furthermore, the SERS technique was utilized in the detection of mycobacteria responsible for tuberculosis by Mühli et al. (Mühlig et al. 2016) This lab-on-a-chip device recognizes silver nanoparticle-mediated SERS spectra of mycolic acid, which forms the cell wall (Mühlig et al. 2016). Figure 5.4 represents SERS-LFA process for the detection of *O. tsutsugamushi* in the human serum designed by Hi Lee et al. (Lee et al. 2019) and SiO_2@Au@QD nanobead-based lateral flow immunoassay POC for detection of SARS-CoV-2 developed by Wang et al. (Wang et al. 2020) Some of the representative works on optical assays are summarized in Table 5.1.

Table 5.1 Some of the POC methods utilizing optical activity of nanomaterials for the detection of infectious diseases are listed below

Detection method	Target pathogen	Limit of detection	Assay time	Reference
Colorimetric	Hepatitis B and C viruses	$\approx 4 \times 10^{-11}$ M and 4×10^{-10} M	3 h	Wang et al. (2003)
	Influenza	3×10^{8} particles	NR	Le et al. (2014)
	Mycobacterium tuberculosis	NR	5 min	Gonzalez et al. (2014)
	E. faecalis and *S. aureus*	710 and 680 CFU/mL	75 min	Wang et al. (2017b)
	Leptospira	$\approx 4 \times 10^{-1}$ genomic equivalent ml^{-1}	1 h	Najian et al. (2016)
	Yellow fever, Ebola, and dengue viruses	150 ng/mL	NR	Yen et al. (2015)
	Shigella spp.	≈ 6 CFU per tube	1 h	Wang et al. (1834)
	L. Monocytogenes	10 femtogram of genomic templates	1 h	Wang et al. (2017c)
	Mycobacterium tuberculosis	<1 μg of DNA	2 h	Baptista et al. (2006)
	COVID-19	10 copies/μL	40 min	Alafeef et al. (2021)
	SARS-CoV-2 and *enterococcus faecium*	SARS-CoV-2, 42 fg μL^{-1}; *E. faecium* spp., 1000 CFU mL^{-1}	<40 min	Sivakumar et al. (2021)
Fluorescence	H1N1 (influenza A)	3.45 nM	NR	Zhang et al. (2013)
	Mycobacterium tuberculosis	36×10^{4} cells/mL	4 h	Qin et al. (2007)
	H3N2 (influenza)	10 PFU/mL	5 min	Takemura et al. (2017)
	H9N2 (avian influenza)	8.94 ng/mL	NR	Xiong et al. (2014)
	Zika virus	<2 copy/ml	3 min	Adegoke et al. (2017)
	S. aureus	10 CFU/mL	0.5 h	Yu et al. (2017)
SPR	HIV subtypes, namely, panels A, B, C, D, E, and G	98 ± 39 copies/ml for D	1.17 h for capture and analysis	Inci et al. (2013)
	H5N1 and H9N2 (avian influenza)	1 pg/mL	NR	Park et al. (2012)
	Ebola	10^{6} PFU/ml	> 90 min	Yanik et al. (2010)
	Dengue NS1 antigen	0.047 μg mL^{-1}	30 min	Lathika et al. (2021)
SERS	Hepatitis B	50 aM	NR	Li et al. (2013)
	Anti-SARS-CoV-2 IgM/IgG	1 pg/mL	25 min	Liu et al. (2021)
	HIV-1	≈ 0.2 pg/mL	NR	Fu et al. (2016)

5.9 Optically Active Nanomaterials for Point-of-Care Diagnosis of Multi-Analyte Detection

There are many progresses in the area of biosensors facilitating multi-analyte detection. However, it has to go much forward in terms of point-of-care diagnosis. Developments in POC methods for multi-analyte detection can significantly contribute to medical diagnostics, pharmaceutical research, food safety, and security (Homola et al. 2005; Akhtar et al. 2018; Chandra et al. 2010; Chandra and Prakash 2020; Mahato et al. 2016).

Yen et al. described a colorimetric-based POC to detect and distinguish biomarkers corresponding to dengue, yellow fever, and Ebola viruses from the color of the test lines utilizing the size-tunable absorption property of silver nanoparticles (AgNPs) (Yen et al. 2015). Different colored AgNPs were prepared and each conjugated with antibodies that recognize the abovementioned pathogens while passing through a lateral flow strip. With antibody-conjugated AgNPs on recognizing the corresponding biomarker present on the test line of the lateral flow strip, test line becomes colored. The LOD was quantified as 150 ng ml^{-1} for each (Yen et al. 2015). Another colorimetric-based portable POC was designed by Sivakumar et al. to detect SARS-CoV-2 and *Enterococcus faecium* by in situ AuNP formation due to the affinity of Au with nitrogenous bases (Sivakumar et al. 2021). V. Nair et al. demonstrated an SPR nanobiosensor to exhibit independent dual emissions on detecting creatinine and copper based on quantum dot (Nair et al. 2020). Quantum dot was conjugated with picric acid (PAQd) for specifically sensing creatine. In the presence of creatine, PAQd shows a fluorescence enhancement which is concentration-dependent (turn-on) due to the formation of the creatine-picric acid complex, while in the presence of copper, fluorescence quenching (turn-off) is observed due to aggregation. They also attempted to examine the presence of creatinine via a mobile camera utilizing sensor strips and demonstrated with human blood samples (Nair et al. 2020).

5.10 Challenges, Future Scope, and Conclusion

Point-of-care diagnosis technology in the healthcare industry is one of the growing markets in the present scenario. Especially in this pandemic situation, a POC portable diagnostics kit at home will be the most feasible solution to avoid hospital visits for diagnosis, especially for aged people and kids. The limitations suffered by the traditional biosensors can be resolved by utilizing nanomaterials. Optically active nanomaterial offers promising advantages in noninvasive point-of-care diagnostics as a future of clinical diagnosis. Such material is expected to have several advantages, such as cost-effectiveness, portability, and easy-to-use devices. Most of these techniques are in the laboratory set up with smaller sample sizes and need to be evaluated in great detail in a broader sample size with multiple permutations and combinations. Still, some issues may limit large-scale applications of these biosensors and devices in clinical diagnostics, such as lack of multiplex ability,

relatively low reproducibility, and moderate sensitivity and specificity. The future of the optically active nanomaterial-based biosensors is to reduce the cost, analysis time, multiplex ability, etc. Furthermore, the future scope of the optically active nanomaterial in POC diagnostics is in developing and undeveloped countries where the sophisticated, costly instruments can't be afforded for the effective and early diagnosis of diseases.

As a conclusion the present chapter highlighted the recent advancement in optically active nanomaterials for point-of-care diagnostics in healthcare. The fast and growing development of nanotechnology has shown significant advancement in point-of-care diagnostics. Optically active nanomaterial is one of the promising materials in POC technology by utilizing the interaction of nonradiative, visible, and NIR light sources with the body fluids/tissues. The optically active nanomaterial-based POC diagnostic devices will be a great choice and can be utilized in clinical trials, particularly in low-resource and laboratory-free settings.

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Aptamer-Based Optical Sensors for Food Safety

6

Surbhi Goel, Sanjay Singh, and Neeti Kalyani

Abstract

Food safety is a worldwide problem and ensuring food safety requires strict monitoring of toxins, antibiotics, and bacteria. Thus, sensitive methods of analysis to determine the types and residues of harmful components in food are needed. Point-of-care sensors have been abundantly developed over the last two decades and are proven to be innovative to analyze the contaminants present in food samples both quantitatively and qualitatively. In this book chapter, an overview of aptamer-based optical methods for monitoring food safety is provided. This chapter will focus on optical biosensing techniques such as UV-visible spectroscopy, fluorescence spectroscopy, surface-enhanced Raman spectroscopy, and photonic crystals. The principle, mechanism, advantages, and limitations of each technique are described with an ample number of examples.

Keywords

Food safety · SERS · Toxins · Pesticides · Antibiotics · Biosensors

S. Goel
Indian Institute of Technology Delhi, New Delhi, India

S. Singh
Jackson State University, Jackson, MS, USA

N. Kalyani (✉)
Department of Biotechnology and Biomedicine, Technical University of Denmark, Copenhagen, Denmark

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6.1 Introduction

Food safety is extremely important for better nutrition, good health, and good quality of life. There is a general propensity to link food and health, to boost well-being and prevent diseases. Any spoilage, deterioration, contamination, pathogenic infestation, or adulteration of food not just leads to degrading its nutritional value, but also consuming these contaminated foods steers foodborne diseases. Foodborne illnesses are a global health problem that contributes to social and economic burden of many countries. According to the World Health Organization (WHO), there are more than 200 food-related diseases that are caused by ingesting contaminated or spoiled food. Approximately 600 million people get sick by contaminated food, of which 4.2 lakhs die every year. Children under 5 years of age are worse affected with 40% of all illnesses and 1.25 lakh deaths every year (World Health Organization 2022). Besides this, foodborne diseases account for economic losses worth billions of dollars around the world.

In the last 30 years, there is an enormous growth in the food sector to fulfill the need of big population and to adapt to changes in lifestyles. Increasing consumption of ready-to-eat food items leads to many socioeconomic and health-related impacts. Many microbiological and chemical changes occur throughout the food processing, distribution, and storage that affects the shelf life and quality of the food, which in turn negatively affects consumer health. Escalating regulatory requirements to control the presence of unwanted or harmful molecules in food leads to increasing food safety concerns. Scientific groups working in food science are constantly required to provide the sufficient answers to the users, whose awareness and concerns for food safety are accelerating (Arduini et al. 2016). Existing methods to assess food safety are chromatographic techniques like gas chromatography, thin-layer chromatography, or high-performance liquid chromatography and advanced techniques such as quantitative real-time polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). These techniques are costly, time-consuming, laborious, and complex and need heavy instruments and experienced personnel. All these limitations make them difficult to apply in rural areas or resource-limited places where the foodborne diseases are prevalent (Choi et al. 2019). To overcome these problems, biosensors can be a solution (Akhtar et al. 2018, Chandra et al. 2010, and Chandra and Prakash 2020). Over the last two decades, biosensors have emerged as a crucial tool for the analysis of food, and researchers are working on many analytical strategies and technologies for the easy, fast, reliable, sensitive, and economic detection of contaminants, toxins, pesticides, carcinogens, and foodborne pathogens.

Most common biorecognition elements for the development of biosensors are antibodies, enzymes, molecularly imprinted polymers (MIP), and aptamers. Morales and Halpern (2018) have very well explained different types of biorecognition molecules that have been used for the development of biosensors and how to select the biorecognition element for a biosensor. Aptamers are short single-stranded oligonucleotides that are identified by an iterative process called SELEX (selective evolution of ligand by exponential enrichment). They are known as chemical

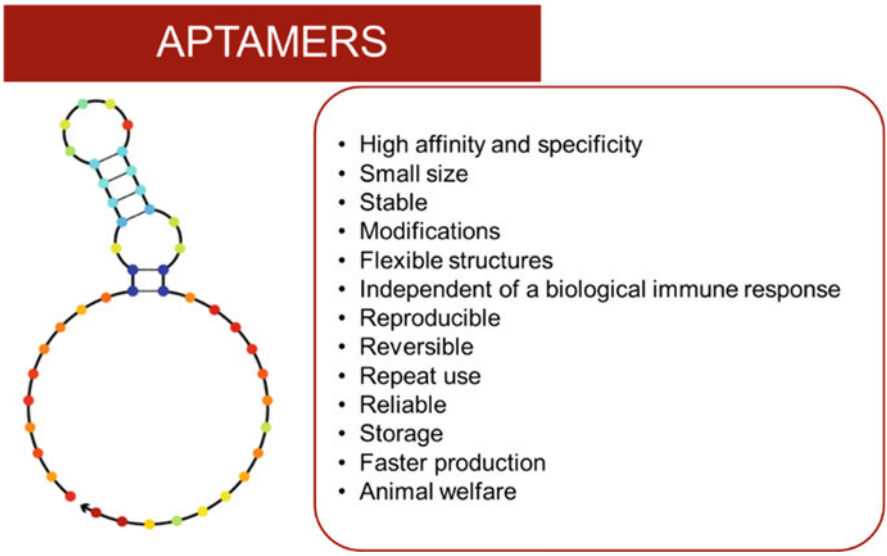

Fig. 6.1 Advantages of aptamers

antibodies, but are easier to produce without any batch difference. Chemical modifications of aptamers are also relatively easy, and therefore, various types of distinct tags can be used for sensing applications (Groff et al. 2015; Kalyani et al. 2021a). In addition to this, they can be extended at both 3′ and 5′ ends for tagging and binding or to incorporate enzymatic activity (Setlem et al. 2020). Aptamers are being used in many biosensors as they have many advantages such as high affinity, small size, no batch variation, easy chemical modifications, and faster production (Fig. 6.1). They have been exploited in conjugation with various detection techniques, viz., fluorescence, SERS, electrochemical, lateral flow assays, and quartz crystal microbalance (QCM), to fabricate biosensors (Dhiman et al. 2017; Arroyo-Currás et al. 2020; Kalyani et al. 2021a; Bayramoglu et al. 2022).

Over the last two decades, there has been exponential growth in the development of optical biosensors as they provide various advantages over other detection techniques. They have been applied for simple, cheap, rapid, and label-free sensing of several chemical and biological molecules in real time. Optical biosensors exploit the interaction of optical field with a biorecognition element that could be an antibody, enzyme, receptor, MIP, or aptamer. They were implemented for clinical diagnosis, food industries, environmental sensing, and medicine. The most commonly used aptamer-based optical methods for food safety applications are colorimetric assays, fluorescence spectroscopy, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), and photonic crystals (Chen and Wang 2020; Asghari et al. 2021; Kaur et al. 2022). The principle, mechanism, advantages, and limitations of each of these techniques are explained with examples in further sections.

6.2 Colorimetric Sensors

It is one of the most widely employed biosensing techniques where target detection can be achieved via observing a change in the color of reaction mixture. The color change is visible to the naked eyes and can be easily quantified using a spectrophotometer (Kalyani et al. 2021b). Aptamer-based colorimetric biosensors employ target-specific aptamers, selected often using SELEX method, and involve conjugation of these aptamers with various nanostructures or enzymes for producing color changes visible to naked eyes. Gold nanoparticles (AuNPs) are most commonly used nanostructures in conjugation with target-specific aptamers where binding of target and aptamer results in salt-induced aggregation of AuNPs and color change. In a study, a highly specific ssDNA aptamer for oxytetracycline in conjugation with gold nanoparticles has been designed where binding of aptamer and target causes release of AuNPs followed by salt-induced aggregation, thereby inducing color change from red to purple (Kim et al. 2010b). Similarly, AuNP-based colorimetric sensors have been devised for detection of mercuric ions (Li et al. 2009), ochratoxin A (Yang et al. 2022), and bisphenol A (Zhang et al. 2016).

In addition to using AuNPs, other novel mechanisms have also been employed for colorimetric assays. In a study a structure-switchable aptasensor has been devised for the detection of aflatoxin B1. Here an aflatoxin B1-specific aptamer and two split halves of DNAzymes having complementary sequence are made to hybridize, resulting in the formation of functional G-quadruplex structure capable of carrying out color change reactions (Fig. 6.2). In the presence of aflatoxin B1, aptamer-DNAzyme complex undergoes structural changes causing release of halves of DNAzymes rendering it incapable of catalyzing color change reaction, thus causing decrease in colorimetric signal (Seok et al. 2015). In the absence of target, the peroxidase-mimicking split DNAzyme is in right configuration and converts light green ABTS to dark green-colored radical anion. When target is present, the DNAzyme remains in split form and no reaction occurs. Table 6.1 includes the list of the colorimetric sensors developed for food safety.

6.3 Fluorescence Sensors

Another type of widely used optical sensor is fluorescence biosensor and used to quantify a number of pollutants and contaminants like heavy metals, antibiotics, food allergens, and toxins. A typical fluorescence-based aptasensor involves the use of a fluorophore and a quencher. Fluorescence-based aptasensors are divided into two groups: (1) "turn-on" fluorescence, i.e., increase in fluorescence can be observed when target molecule binds with aptamer, and (2) "turn-off" fluorescence, where binding leads to decrease in fluorescence (Nsibande and Forbes 2016; Kiruba Daniel et al. 2019; Kalyani et al. 2020). In turn-on fluorescence biosensors, initially fluorophore compound is positioned very close to quencher, and in the presence of target molecule, aptamer-target binding leads to change in its conformation, thus separating the fluorophore from quencher, and increase in fluorescence can be

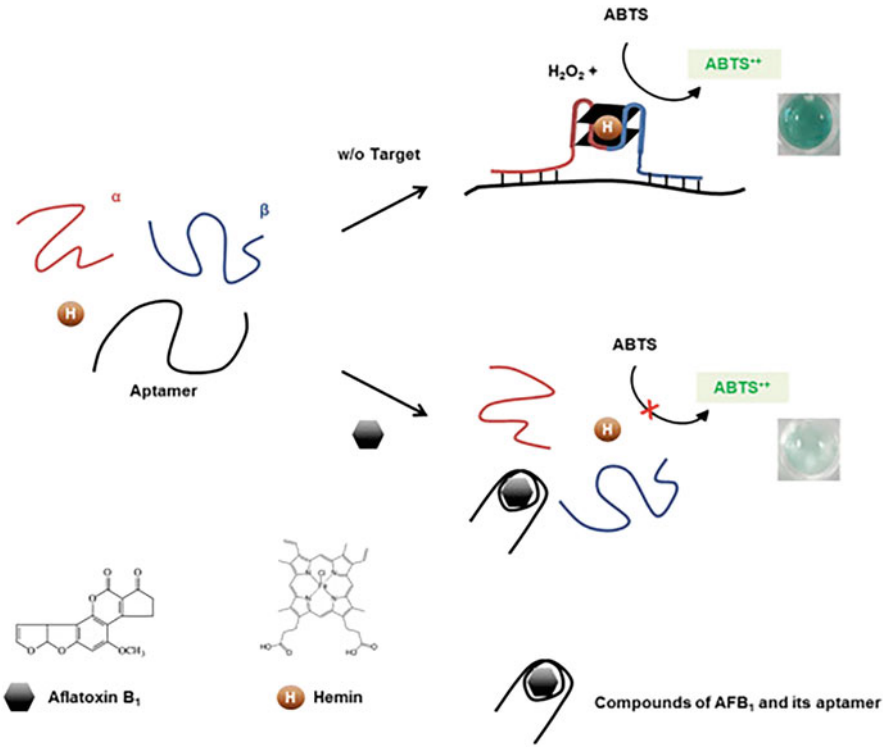

Fig. 6.2 Colorimetric detection of aflatoxin B1 using structure-switching aptasensor. Reused with permission from Seok et al. (2015). Elsevier © 2015

observed. For instance, graphene oxide (GO) sheets have been used to quench the fluorescence of quantum dots in the absence of target molecule (Lu et al. 2015). For the detection of lead (II), the aptamer-QD conjugates are bound to GO sheets, and the resulting energy transfer between QDs and GO leads to quenching. In the presence of lead ions, aptamer-Pb^{2+} binding causes conformational change in aptamer causing the release of aptamer-QD-Pb^{2+} complex from GO sheets, and restoration of fluorescence can be observed (Li et al. 2013). Similarly, using turn-on fluorescence-based technique, CdTe quantum dots-aptamer-GO complex was employed for the detection of aflatoxin B1 (AFB1).

In turn-off sensors, the presence of target molecule brings fluorophore and quencher in close proximity, thus reducing the fluorescence signal, which was otherwise powerful in the absence of target (Akki and Werth 2018). There are other mechanisms which are based on photoinduced electron transfer (PIET) mechanism between fluorescent molecules and guanine residues. For instance, for the rapid detection of ochratoxin A (OTA), carboxyfluorescein (FAM)-labeled aptamer, when hybridized to its complementary strand containing guanine residue at its 3″ end, results in the quenching of fluorescence (Fig. 6.3). In the absence of

Table 6.1 Aptamer-based colorimetric sensors utilized for food safety

Target	Detection mechanism	Linear dynamic range (LDR)	Limit of detection (LOD)	References
Staphylococcus aureus	Utilizes aptamer and dsDNA-SYBR green 1 (SG1) complex	10^2 to 10^7 CFU/mL	81 CFU/mL	Yu et al. (2020)
Vibrio parahaemolyticus	Enzyme-based detection system employing magnetic nanoparticles, gold nanoparticles, and specific aptamers	10 to 10^6 CFU/mL	10 CFU/mL	Wu et al. (2015)
Salmonella enteritidis	Aptamer-based sandwich-type capillary detection platform	10^3 to 10^6 CFU/mL	10^3 CFU/mL	Bayraç et al. (2017)
Salmonella enterica serovar Typhimurium	Aptamer conjugated with gold nanoparticles	10 to 10^6 CFU/mL	10^2 CFU/mL	Sudha et al. (2016)
Aflatoxin B1	Utilizes assembly of aptamer and peroxidase mimicking DNAzyme split probes	0 to 1 ng/mL	0.1 ng/mL	Seok et al. (2015)
Ochratoxin A (OTA)	OTA binding induces conformational changes on OTA aptamer and salt-induced aggregation of AuNPs	20 to 625 nM	20 nM	Yang et al. (2011)
	Aptamer-cross-linked hydrogel which upon interaction with OTA causes hydrogel disruption and AuNPs aggregation	0 to 100 nM	1.25 nM	Liu et al. (2015)
	Gold-conjugated OTC aptamer	25 nM to 1 μM	25 nM	Kim et al. (2010b)
	Lateral flow-based aptasensor employing AuNPs-aptamer conjugates	5 to 50,000 ng/mL	0.254 ± 1.62 ng/mL	Birader et al. (2021)
Cd^{2+}	T- and G-rich DNA aptamer conjugated with gold nanoparticles	10 nM to 4 μM	4.6 nM	Wu et al. (2014)
Bisphenol A	Detection based on interaction among aptamer and bisphenol A followed by AuNPs aggregation	1.50 nM–500 nM	1.50 nM	Zhang et al. (2016)
	Aptamer-bisphenol A binding induced AuNPs aggregation	35 to 140 ng/mL	0.11 ng/mL	Xu et al. (2015)

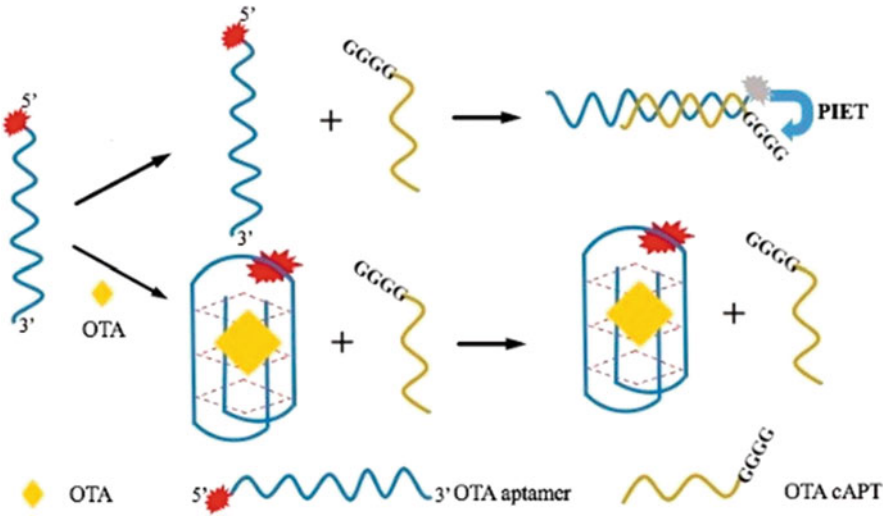

Fig. 6.3 Fluorescence-based sensor for the detection of ochratoxin A. (Zhao et al. 2019)

ochratoxin A, the aptamer is bound to complementary strand that leads to fluorescence quenching. In the presence of target, the complementary strand leaves the aptamer resulting in fluorescence. OTA presence causes the binding of OTA with FAM-labeled aptamer, releasing complementary strand and restoration of fluorescence (Zhao et al. 2019). Table 6.2 highlights some of the aptamer based fluorescensce sensors developed for food safety.

6.4 Surface Plasmon Resonance-Based Sensors

Surface plasmon resonance (SPR)-based sensors rely on the strong oscillation of electromagnetic field at the interface of dielectric medium and nanometal film via p-polarized incident light. At specific incident angle and wavelength of light, this results in a dark band profile of light reflectivity (Prabowo et al. 2018). In SPR, the recognition molecule can be immobilized on chip to capture the target. Various nanostructures have been studied with different SPR properties (Mahato et al. 2018). It provides real-time signals of recognition molecule and target interactions by closely monitoring the change in refractive index. SPR sensors are very sensitive to the external solution refractive index, and the resonant angle and wavelength alter the external solution refractive index. SPR offer advantages of label-free and real-time detection of molecules (Wang et al. 2018). As they are highly sensitive and label-free, they have been extensively used for biological sensing. SPR biosensors have been shown to detect various biomolecules such as virus, proteins, bacteria, and small molecules (Chinowsky et al. 2007; Wang and Zhao 2018; Akgönüllü et al. 2020).

Table 6.2 Aptamer-based fluorescence sensors for food safety

Target	Detection mechanism	LDR	LOD	References
Tropomyosin (food allergen in shellfish)	Aptamer-magnetic nanoparticles complex-based detection	0.4–5 μg/mL	77 ng/mL	Zhang et al. (2018)
Aflatoxin B1	Aptamers are linked to CdTe quantum dots, the fluorescence of which is quenched by graphene oxide	In PBS: 3.2 nM–320 μM In peanut oil: 1.6 nM–160 μM	PBS: 1 nM Peanut oil: 1.4 nM	Lu et al. (2015)
Aflatoxin M1	FAM-labeled aptamer and tetramethylrhodamine (quencher)-modified complementary DNA to aptamer	1–100 ng/mL	0.5 ng/mL	Qiao et al. (2021)
	Carboxyfluorescein-labeled aptamer conjugated to graphene oxide to quench the fluorescence and protect aptamer from DNase I cleavage	0.2–10 μg/kg	0.05 μg/kg	Guo et al. (2019)
Zearalenone (ZEN)	Based on upconverting nanoparticles	0.05–100 μg/L	0.126 μg/kg for corn 0.007 μg/L for beer	Wu et al. (2017)
Ochratoxin A (OTA)	FAM-labeled quencher-free aptamer (F1 and F2) utilizing quenching abilities of guanine for FAM	F1: 0.69–8.0 nmol/L F2: 0.36–4.0 nmol/L	0.69 nmol/L 0.36 nmol/L	Yang et al. (2022)
	RNase H-assisted fluorescence aptasensor	0.4–20 ng/mL	0.08 ng/mL	Wu et al. (2019)
	Quencher-free method based on photoinduced electron transfer between guanine and fluorophore	3 nM–300 nM	1.3 nM	Zhao et al. (2019)
Kanamycin	Reduced graphene oxide-based fluorescent aptasensor	1 pM–20 pM	1 pM	Ha et al. (2017)
Fipronil	FAM-labeled aptamer and tetramethylrhodamine (quencher)-labeled cDNA	25–300 ppb	53.8 ppb	Kim et al. (2020)
Cd^{2+}	Label-free fluorescent aptasensors utilizing PicoGreen as dsDNA-specific dye	0.10–100 μg/mL	0.038 ng/mL	Luan et al. (2016)
Hg^{2+}	Aptamer-templated ZnO quantum dots	0.1–10,000 ppb	0.1 ppb	Kiruba Daniel et al. (2019)

(continued)

Table 6.2 (continued)

Target	Detection mechanism	LDR	LOD	References
Neomycin	DNA	0.003 to 0.72 µg/mL	1.55 ng/mL	Caglayan (2020)
Aflatoxin B1	DNA-chitosan using an N-terminal histidine	0.19 to 200 ng/mL	0.19 ng/mL	Wu et al. (2018)
Aflatoxin B1	Streptavidin attached to CM5 sensor and afterward coated with biotinylated aptamer	0.4 to 200 nM	0.4 nM	Sun et al. (2017)
Tetracycline	DNA tetrahedron-immobilized gold surface	0.01 to 1000 µg/kg	0.0069 µg/kg	Wang et al. (2018)
Kanamycin	CVD-graphene- and rGO-coated gold surface	5.88 to 100 µM	1.79 µM	Écija-Arenas et al. (2021)
OTA	Aptamer nanoparticles Gold nanorods	3.8 to 9 ng/mL NM	3 ng/mL <1 nM	Wei et al. (2018), Rehmat et al. (2019), and Park et al. (2014)

Wang et al. have developed an SPR-based tetrahedron-assisted aptasensor to detect tetracycline (Wang et al. 2018). To reduce steric hindrance between aptamer molecules and improve the accessibility of the aptamer to tetracycline, DNA tetrahedron was used (Fig. 6.4). Using this strategy, aptamer can be oriented in both directions: lateral and vertical, with precisely 6 nm distance. The pyramid structure of the tetrahedron can act as spacer and provide enough space for aptamer to fold properly. It is reported that there was tenfold improvement when DNA tetrahedron is used. In the absence of tetrahedron, the LOD was 0.0183 µg/kg, whereas when aptamer was properly oriented with the help of DNA tetrahedron, the LOD was 0.0069 µg/kg. The specificity of the sensor was tested with close analogues of tetracycline, viz., oxytetracycline and chlortetracycline. For real sample, different types of honey samples were spiked with tetracycline and recovery of 86–114% was observed.

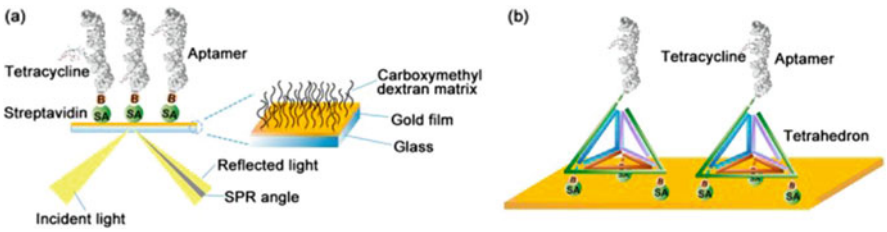

Fig. 6.4 (**a**) SPR aptasensor scheme for detection of tetracycline, (**b**) DNA tetrahedron-assisted oriented immobilization of aptamer

A novel triple-amplification SPR electro-chemiluminescence (ECL) approach was developed by Miao et al. for detecting chloramphenicol (Miao et al. 2016). It is based on horseradish peroxidase enzyme-linked polymer (EV) and single-stranded DNA-binding protein (SSB) attached to gold nanoparticles (EV-Au-SSB) as nanotracer and exonuclease-assisted target recycling. Au nanoparticles in the EV-Au-SSB system efficiently enhance the intensity of ECL of nanocrystals by −1.35 V using SPR technique.

6.5 Surface-Enhanced Raman Scattering (SERS) Sensors

In recent years, SERS has been used for its unique molecular sensitivity and spectral resolution [18] in biomolecular analysis (Lee et al. 2015; Hanif et al. 2017), food industry (Deneva et al. 2019; Muhammad et al. 2020), antibiotics response (Moritz et al. 2010; Kumar et al. 2020), and detection of water pollutants. SERS is an enhancement in the Raman signal from molecules adsorbed on the surface of the SERS substrate. However, this phenomenon can be explained by two general mechanism that are accepted but have distinct factors: electromagnetic enhancement mechanism and chemical enhancement mechanism (Stiles et al. 2008; Ding et al. 2017). The mechanism of electromagnetic factor is mainly based on electric field enhancement caused by LSPR (localized surface plasmon resonance), while chemical enhancement mechanism is based on the charge transfer state between the SERS substrate and molecule absorbed on the nanoparticles (chemisorbed molecules) (Stiles et al. 2008). Therefore, the production of such substrates or platforms is very important, and it's still a huge task to improve the specificity and sensitivity of Raman signals in complex environments like biological samples.

The specificity and sensitivity factor of aptamer-based SERS assays is due to the specific interaction of sample target molecules with the aptamer. There are generally two types of aptamer-based SERS tests: with Raman-labeled molecule (label-based) and without Raman-labeled molecule (label-free). In the label-free strategy, the aptamer can interact with the target molecule and induce conformal changes that alter the aptamer's Raman signal. On the other hand, in Raman labeling method, externally sensitive Raman molecules attached to the detected target help with the measurement. In both the cases, specificity is a main key characteristic of aptamer-based detection, which depends on the target molecule interaction and the capturing DNA sequences of aptamer, and sensitivity depends on the Raman signal generated by various mechanisms of amplification, including the SERS effect, which can be significantly improved.

Since the first application of aptamers in SERS (Barhoumi et al. 2008a, 2008b; Kim et al. 2010a), many SERS biosensors based on diversified aptamers have been developed. It is expected for these sensors to be sensitive and targeted to facilitate quick diagnosis in various fields and applications. Wang et al. review the detection of various substances using aptamer-based SERS sensors (Wang et al. 2019). Similarly, the free use of aptamer labels in Raman spectroscopy is also discussed by Scatena et al. (2019). Table 6.3 lists the SERS based sensors developed for food safety.

Table 6.3 SERS-based sensors for food safety

Target	Detection mechanism	LDR	LOD	References
Acetamiprid	AgNPs@Si-CS as substrate	1 pM to 1 μM	0.30 pM	Shi et al. (2020)
Acetamiprid	AuNP-MMBN aptamer as Raman probe	25 to 250 nM	6.8 nM	Sun et al. (2019b)
Malathion	AgNPs@Si and Ag nanosol substrate	0.5 to 10 μM	1.8 nM	Nie et al. (2018)
Aflatoxin B1	Aptamer-conjugated magnetic-bead and gold nanotriangles	0.001 to 10 ng/mL	0.54 pM	Li et al. (2017)
Oxytetracycline	AuNP tetramer-based aptasensor	0.01 to 250 ng/mL	0.003 g/mL	Wu (2019)
Kanamycin	Au@ag CS NPs	10 μg/mL to 100 ng/mL	0.90 pg/mL	Jiang et al. (2019)
Pb^{2+} Hg^{2+}	Aptamer regulated the production of AuNP Based on the formation of $THg^{2+} - T$ pairs for detection of Hg^{2+}	0.006 to 0.46 mM 10 nM to 1 mM	0.0032 mM 10 nM	Wang et al. (2019) and Lu et al. (2018)

An interesting strategy for using SERS-aptamer-based sensors is to combine them with catalytic reactions (Li et al. 2018a; Sun et al. 2019a; Yang et al. 2020). The fundamental idea is to replace the detection target molecule, which can be more easily detected or enhanced by a catalytic reaction. When encountering biological enzymes, the combination of catalytic amplification and SERS becomes especially important. Basically, the reaction product is enhanced by the introduction of the molecule or the catalytic enhancement of the catalytic activity of the process. The design of target-specific enzymatic reactions is very useful for the development of aptamer-SERS biosensors, especially when enzymes start acting on DNA/RNA.

Fang et al. reported a dual mode of aptamer-attached and enzyme-assisted SERS module for the detection of chloramphenicol (CAP) antibiotic trace at very low section limit of 15 fM (Fang et al. 2019). In this study, a special aptamer was designed against CAP (anti-CAP) which can recognize the alteration in the conformation of the analyte, which initiates the de-hybridization of the target aptamer's DNA (Fig. 6.5a). The addition of a DNA probe labeled with the reporter gene and the exonuclease (III) increases the SERS signal when hybridized with the captured DNA due to the creation of high number of surplus DNA molecules during the amplification loop.

In addition, the catalytic reaction approach can facilitate the generation of SERS-active nanoparticles that can enhance the SERS signal. Li et al. presented redox-GO reactions for the synthesis of enhanced SERS nanoparticles (Fig. 6.5b) that can

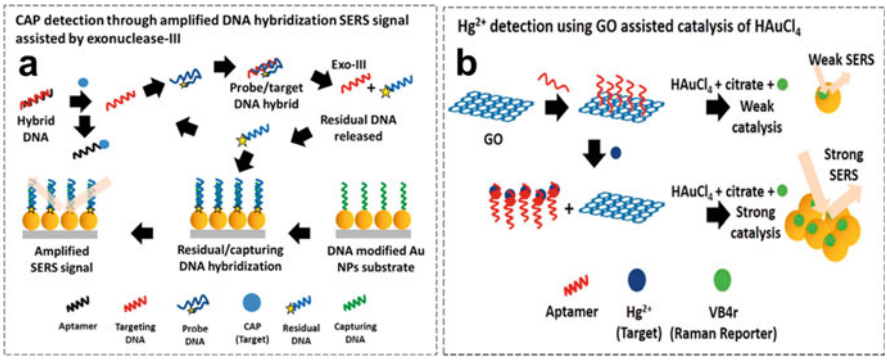

Fig. 6.5 (**a**) Chloramphenicol (CAP), the target molecule recognition process induces a structural change in biorecognition aptamer that is further enhanced by multiple rounds of DNA hybridization with Exo III. (**b**) An aptamer-functionalized sensor for mercuric ions detection uses GO to assist the $HAuCl_4$ reduction reaction. Adapted from reference Fang et al. (2019)

amplify the SERS signaling reporter molecules for indirect identification of mercury ions (Li et al. 2018b). The aptamer binds to the target Hg^{2+} specifically in their presence which leads to the inhibition of their further adsorption onto the GO surface. Due to this process, catalysis reaction of citrate and $HAuCl_4$ took place on GO surface that leads to production of AuNPOs that can be detected via SERS.

The sensitive detection of contaminants in food requires pretreatment to inhibit interference from nontarget molecules. Using gold nanoparticle conjugated with Raman tag 4-(mercaptomethyl) benzonitrile aptamers as Raman probes and AgNPs tagged cDNA-as signal enhancers, a new SERS-based aptameric sensor has been developed to minimize interference from nontarget species. When food samples contain acetamiprid, the target aptamer complex prevents the appearance of MMBN-AuNPs-aptamer-cDNA-AgNPs@Si, and the intensity of the Raman signal MMBN in Au-AgNPs@Si decreases. The real sample testing of the sensor was done to sense acetamiprid in apple juice with a detection limit of only 6.8 nm, which is expected to be used to detect traces of pesticides in complex food matrices.

6.6 Photonic Crystal-Based Sensors

Photonic crystals can be defined as the highly ordered nanostructures with varying dielectric constant and periodic scale of visible light wavelengths. Photonic crystals are nanomaterials and dielectrics having optical sensing features. Their nanostructures affect the movement of photons via periodic spatial modulation of refractive index. The repeated array of refractive index and Bragg reflections on

photonic crystals lattice structure lead to impeded propagation of light and culminate in the formation of photonic bandgap (Fathi et al. 2021). Analogous to flow of electrons in semiconductors, photons move in photonic crystals, and the hindrance in the propagation of photons in all directions led to full photonic bandgap. A pseudogap prohibits photon propagation in only some directions and results in incomplete photonic bandgap. The capability of photonic crystals to alter the spectral position at certain frequencies and the presence of photonic bandgap makes them really interesting and useful material for application in biosensors. Notably, there is no absorption of light in the process, and the photonic bandgap corresponds to the reflection of light via periodic arrays. In the simplest way, the optical sensing can be performed by monitoring the photonic crystal reflectivity shift or transmission spectra (Zhang et al. 2008). Photonic crystals such as liquid crystals, inverse opals, and fibers have been widely exploited for biosensing applications. Photonic crystal-incorporated biosensors have been used to detect a number of molecules like proteins, nucleic acids, pathogens, viruses, and cancer (Shafiee et al. 2014; Panda and Puspa Devi 2020).

Photonic crystal structures comprised of spatially organized periodic dielectric material that uniquely interacts with light, which results in high-efficiency reflection at specific wavelengths. Naturally, there are many photonic crystal-type nanostructures that exist (Vukusic and Sambles 2003). The most common examples are peacock, and *Morpho rhetenor* butterfly (Kinoshita et al. 2002; Zi et al. 2003). Apart from this, sea mouse, opals, and *Eupholus magnificus* also have geometrical patterns on the surface like photonic crystals through which light illuminates and reflects (McPhedran et al. 2003; Marlow et al. 2009; Pouya et al. 2011). On the basis of their geometry, the periodicity can be iterated in one, two, and three directions that implies the different dielectric constants. Thus, the fabrication of the photonic crystals can be done in one-dimensional (1D), two-dimensional (2D), or three-dimensional (3D) orientation (Fig. 6.6). Also, different types of materials have been used for their fabrication such as glass, polymer, silicon, colloids, and silk (Colvin 2001; Edrington et al. 2001; Jamois et al. 2003; Meseguer 2005; Freeman et al. 2008; González-Urbina et al. 2011; Kim et al. 2012; Han et al. 2012; MacLeod and Rosei 2013; Diao et al. 2013). Various top-down (electron beam lithography, thin film deposition, nanoimprint lithography, and electrochemical etching) and bottom-up (self-assembly) approaches have been utilized for the fabrication of photonic structures (López 2003; Kouba et al. 2006). Photonic crystals can be fabricated via various economic fabrication methods like colloidal self-assembly, mold-based replica printing, and hydrogels (Choi and Cunningham 2007; Yan et al. 2011; Fenzl et al. 2013). The advantages of photonic crystal-based biosensors over other techniques are cost-efficient fabrication and shorter assay time. Table 6.4 constitutes the faad safety sensors reported using photonic crystals.

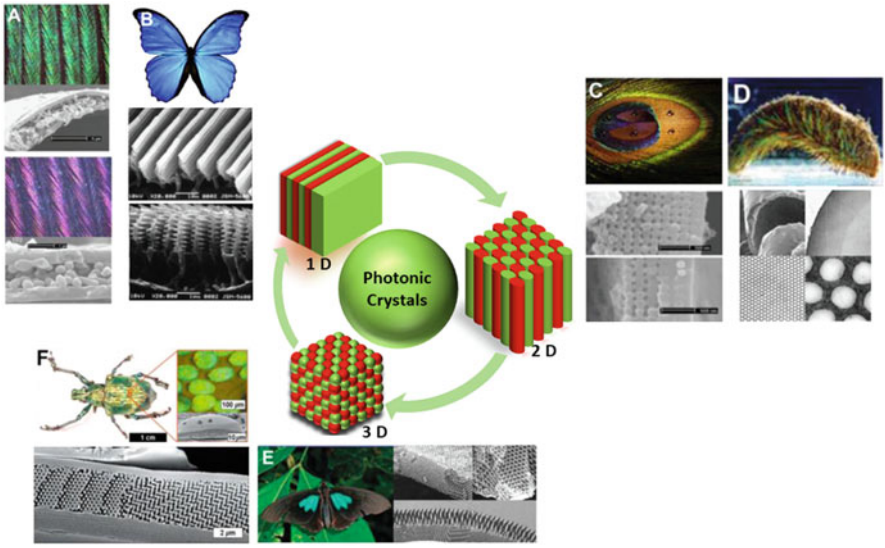

Fig. 6.6 Various examples of photonic crystals in nature constituting different colors: (**a**) one-dimensional neck feathers of domestic pigeons; (**b**) 1D, wings of ***Morpho*** butterflies; (**c**) two-dimensional, barbules of male peacocks; (**d**) 2D, iridescent setae from polychaete worms; (**e**) three-dimensional green spots in the wings of *Parides sesostris* butterfly; (**f**) 3D – *Lamprocyphus augustus* beetle (Chiappini et al. 2020)

6.7 Future Perspective and Conclusion

Optical biosensors are common analytical tools that can be utilized for point-of-care applications. Their small size enables their use in high-throughput applications to test wide array of samples in different conditions. Many optical biosensors are integrated with nanoparticles to enhance sensitivity or specificity. While developing an optical sensor for real-time detection, crucial factors to consider are simplicity, selectivity, sensitivity, robustness, and ease of use. All the discussed techniques have the potential to be practically employed in POC applications for food safety. Various optical sensors have been reported for food safety to detect various contaminants like toxins, pathogens, pesticides, and heavy metals. However, many sensors are in development stage and require substantial efforts before applying in real applications. The major challenge in the field of optical sensing for food safety is to develop the point-of-care sensors that are accessible and commercially available for resource-limited settings or remote areas so that illnesses or harmful effects due to adulterated and contaminated food products can be prevented.

Table 6.4 List of PC-based sensors for food safety

Target	PC used for detection	Technique	LDR and LOD	Real sample	Ref
Oxytetracycline	g-C_3N_4 nanosheet-modified tungsten trioxide film with inverse opal photonic crystals	Photoelectrochemical	1 nM to 230 nM; 0.12 nM	River water	Dang et al. (2019)
Mercury ion	1DPCs consisting of TiO2 and poly (N-isopropylacrylamide-acrylic acid), P(NIPAM-AA)	Fiber optic spectrometer	0 nM to 5 μM and 1 nM	Water	Xuan et al. (2016)
Lead and mercury	Colloidal photonic crystal hydrogel	Colorimetric	Lead: 1 nM to 1 mM; 1 nM Mercury: 10 nM to 0.1 mM and 10 nM	Tap water and lake water	Ye et al. (2012)
Mercury	Angle-independent photonic crystal	Colorimetric	1 nM to 10 μM; 1 nM	Tap and lake water	Ye et al. (2013)
Mercury and silver	Silica colloidal crystal beads and ssDNA-functionalized hydrogel	Fluorescence	10^{-9} to 10^{-2} M; 10^{-2} M	Tap water	Yan et al. (2017)
Ochratoxin A	Silica photonic crystal microspheres	Fluorescence	1 to 100 ng/mL; 0.02 ng/mL	Cereal	Li et al. (2021)
Ochratoxin A and fumonisin B1	Silica photonic crystal microsphere	Fluorescence	Ochratoxin A: 0.01 to 1 ng/mL; 0.25 pg/mL Fumonisin B1: 0.001 to 1 ng/mL; 0.16 pg/mL	Cereal	Yue et al. (2014)
Staphylococcal enterotoxin B	SiO_2 inverse opal photonic crystal	Reflectance spectroscopy	10^{-2} to 10^{3} pg/mL; 2.820 fg/mL	Milk and water	Shen et al. (2022)

(continued)

Table 6.4 (continued)

Target	PC used for detection	Technique	LDR and LOD	Real sample	Ref
Aflatoxin B1, ochratoxin A, and fumonisin B1	High-throughput photonic crystal microspheres	Fluorescence	Aflatoxin B1 and ochratoxin A, 0.1 pg/mL to 0.1 ng/mL;15.96 fg/mL and 3.96 fg/mL; fumonisin B1, 0.1 ng/mL to 10 ng/mL; 11.04 pg/mL	Wheat, rice, and corn	Yang et al. (2017)
Staphylococcus aureus and *Pseudomonas aeruginosa*	Gelatinase-responsive photonic crystal membrane	Reflectance	10 to 10^7 CFU/mL; 10 CFU/mL	Urine, lake water, and milk	Lu et al. (2022)

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Nano-Bio-Analytical Systems for the Detection of Emerging Infectious Diseases

7

Dehong Yang, Ying Fang, Jinbiao Ma, Jiahao Xu, Zetao Chen, Chengyun Yan, and Fenni Zhang

Abstract

The global damage caused by the spreading microbial infection is evident from the devastating COVID-19 pandemic. The infectious diseases caused by emerging viral and resistant bacterial pathogens have been a worldwide medical threat and economic burden. To combat this threat, a technology able to rapidly identify pathogen infection and determine the pathogen resistance profile is needed. The current methods for emerging infectious disease detection are mainly molecular methods based on polymerase chain reaction (PCR) for the detection of the specific pathogenic gene or resistant gene mutations. While sensitive, it requires prior knowledge of the pathogenic cells, which fail to output a negative result when a new pathogen or new resistant strain occurs and wrongly output a positive result when resistance genes are simply present but are not expressed or are not contributing to resistant phenotypes. Considering the life-threatening condition of an emerging infectious disease and the increasing prevalence of emerging pathogens and bacteria with antibiotic resistance in hospitals, automated and fast diagnostic facilities are required. This chapter summarizes the emerging nano-bio-analytical systems for the rapid detection of pathogen infectious diseases and antibiotic susceptibility testing for antibiotic resistance determination that will empower humans to win the epic war between human wits and microbial genes. In viral infection detection, we discussed nano-bio-analytical systems for the detection of viral infectious diseases including point-of-care immunoassay systems, electrochemical detection systems, and plasmonic-based

D. Yang · Y. Fang · J. Ma · J. Xu · Z. Chen · C. Yan · F. Zhang (✉)
Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, People's Republic of China
e-mail: fennizhang@zju.edu.cn

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systems. In resistant bacterial infection detection, we reviewed the emerging optical imaging systems for rapid phenotypic antibiotic resistance determination.

Keywords

Emerging infectious diseases · Pathogen detection · Point of care · Biosensor · Optical imaging system

7.1 Introduction

Following the severe acute respiratory syndrome (SARS) in 2003 and the Middle East respiratory syndrome (MERS) in 2012, the COVID-19, which is the third large-scale pandemic caused by coronavirus in the last 20 years, has been declared as a public health emergency of international concern. COVID-19 has a remarkable efficiency in human-to-human transmission, with relatively high morbidity and mortality, especially among the aged and those with underlying comorbidities (Morens and Fauci 2020; Morens et al. 2020). Unlike other conventional diseases, infectious diseases are caused by infectious pathogens, which can impair the normal functioning of the host, and often spread from person to person or sometimes from animals to humans. Researchers have sorted the infectious agents into categories of bacteria, viruses, fungi, parasites, and prions. The incidence of emerging infectious diseases (EIDs) has increased over the past two decades and is likely to increase in the near future (Wilson 1999). Emerging diseases have been classified as newly emerging, re-emerging, "intentionally emerging", and "accidentally emerging" infection diseases (Table 7.1). Despite the differences, these four categories share something in common: newly emerging diseases can persist and then re-emerge through intentional or accidental release (Morens and Fauci 2020; Morens and Fauci 2012; Morens et al. 2008; Morens et al. 2004; Satcher 1995; Excler et al. 2021).

Global health, economic boom, social stability, and security of human society have been threatened by all EIDs, especially caused by viruses and bacteria. Therefore, it is incredibly important to develop precise and timely diagnostic systems to

Table 7.1 Major categories of emerging infectious diseases (Morens and Fauci 2020)

The category of EIDs	Comments
Newly emerging infectious diseases	Diseases first discovered in humans, e.g., HIV/AIDS (1981), Nipah virus (1999), SARS (2003), MERS (2012), COVID-19 (2019)
Re-emerging infectious diseases	Diseases that have historically infected humans but continue to re-emerge in new locations (e.g., West Nile in the United States and Russia in 1999) or in drug-resistant forms (e.g., methicillin-resistant *Staphylococcus aureus*)
Intentionally emerging infectious diseases	Diseases related to intent to harm, including mass bioterrorism
Accidentally emerging infectious diseases	Diseases unintentionally released by humans, e.g., epizootic vaccinia and transmissible vaccine-derived polioviruses

facilitate the recognition and intervention of EIDs for the prevention of infectious diseases into epidemics and to improve public health (Ozer et al. 2019).

Currently, the major strategies for the clinical diagnosis of EIDs are to cultivate pathogens and to identify or detect specific antigens, antibodies, or nucleic acids. The most common and efficient methods for identifying and diagnosing infectious disease pathogens are nucleic acid-based assays (Koo et al. 2018; Sin et al. 2014). Generally, RT-PCR is regarded as the gold standard of viral nucleic acid detection. The highly sensitive PCR-based methods have disadvantages such as being time-consuming, labor-intensive, and expensive. Biosensors facilitate the rapid detection of targeted biomarkers, enabling real-time disease diagnosis (Mujawar et al. 2020). The combination of nanotechnology and biosensors holds the potential to improve the accuracy, speed, and sensitivity of devices detecting bacterial and viral. At the same time, great advances have been made in deeper understanding and comprehension of the genome and proteome of pathogens and their interactions with hosts (Wrapp et al. 2020).

Therefore, this chapter described the nano-bio-analytical systems for emerging infectious diseases from two aspects: (1) viral infection detection system and (2) resistant bacterial infection detection system. In the field of the viral infection detection system, point-of-care immunoassay, electrochemical, and plasmonic-based platforms were introduced with system design and sensing mechanism. As for the resistant bacterial infection detection system, real-time microscopy system, microfluidic imaging system, surface plasmon resonance imaging system, and light scattering imaging system were introduced with system design and their application in rapid bacterial resistance determination.

7.2 Nano-Bio-Analytical Systems for Emerging Viral Infection Detection

The SARS-CoV-2 event is widely and rapidly spreading around the world, and because of the terrifying contagiousness of this virus, the development of point-of-care testing (POCT) diagnosis assays to detect and manage the disease is urgently needed in the afflicted area, even though RT-PCR test has become the gold standard to recognize SARS-CoV-2 disease (Orooji et al. 2020).

7.2.1 Point-of-Care Immunoassay Systems

Immunoassay is a type of bio-analytical method in which the interaction of an analyte (i.e., antigen) and an antibody is the basis to measure a specific analyte (Huang et al. 2020; Byrnes et al. 2020). Immunological assays, particularly the enzyme-linked immunosorbent assay (ELISA) and lateral flow assay, assess viral infection rates or vaccine efficiency by detecting viral antigens or antibodies against viral antigens (Orooji et al. 2020). Immunosensing assays are commonly used to detect various sorts of viruses with high sensitivity, for instance, SARS

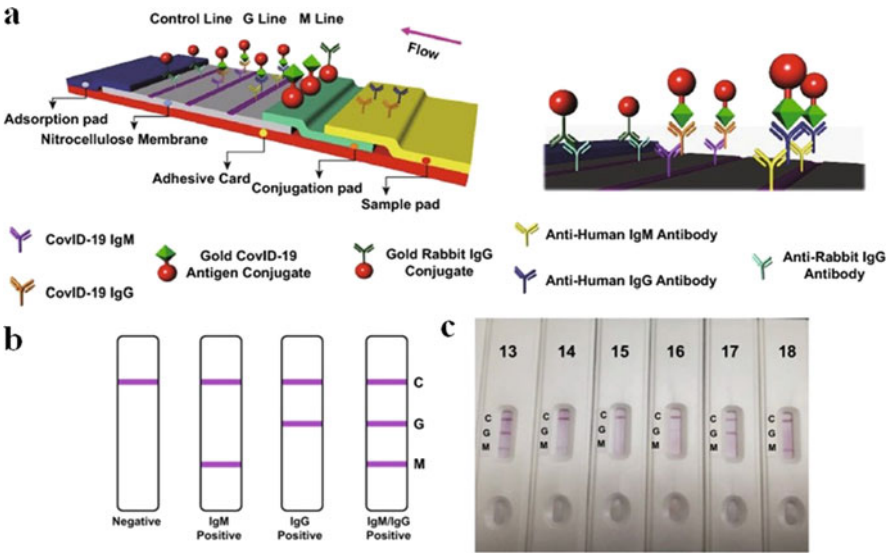

Fig. 7.1 Schematic explanation of rapid SARS-CoV-2 IgM-IgG combined antibody test. (**a**) Schematic diagram of the identification device. (**b**) Interpretation of several experimental results. (**c**) Relates to the control line, G means IgG line, M means IgM line. Reproduced from reference (Orooji et al. 2020)

CoV-2 (Padoan et al. 2020; Chen et al. 2020), HIV (Sevenler et al. 2020; Stalter et al. 2020), HCV (Eshetu et al. 2020; Patel and Sharma 2020), and so on.

ELISA is the most used immunoassay for viral detection in clinical laboratories, for example, ELISA is the gold standard for HIV diagnosis. In the ELISA test, the chromogenic substrate is converted into a colored molecule by using capture antibodies and detection antibodies modified with enzymatic tags. ELISA can test multiple samples and can be automated to increase throughput, but the sensitivity may vary (Carter et al. 2020).

Aiming to detect EIDs, the lateral flow immunoassay (LFIA)-based POCT assay has been developed. Lateral flow tests, also known as lateral flow immunochromatographic assessments, are simple and direct paper-based devices designed to identify the presence of target analytes in a fluid sample without the need for any specific and exorbitant hardware. Figure 7.1 shows a diagram of a quick SARS-CoV-2 IgM-IgG combined antibody test (Orooji et al. 2020).

Yu et al. (Yu et al. 2017) have designed a nanostructured microfluidic immunoassay pathogen detection platform with high sensitivity and selectivity. By utilizing the three-dimensional morphology as well as the unique optical property of the ZnO nanorods, the detection limit of H5N2 AIV can be improved to as low as 3.6×10^3 EID50/mL (EID50, 50% embryo infectious dose), which is approximately 22 times more sensitive compared to conventional ELISA. In Chan's paper, a microfluidic integrated rGO transistor chip was established to detect the gene of H5N1 influenza in a flowing environment (Chan et al. 2017). Lu et al. (Lu et al. 2020) present a novel

digital microfluidic H1N1 virus detection platform using a one-aptamer/two-antibody assay on magnetic beads, and the limit of detection (LOD) is 0.032 hemagglutination units/reaction. This is the first demonstration of a digital microfluidic platform capable of performing the whole diagnostic process for influenza A H1N1 viruses by using electromagnetic force. Iswardy et al. (Iswardy et al. 2017) used a microfluidic dielectrophoresis (DEP) chip with mouse anti-flavivirus monoclonal antibody-coated beads for rapid detection of dengue virus (DENV) in vitro. The platform is capable of accelerating immune response time, with an on-chip assay time of 5 minutes and DENV detection capability down to 10 (Morens and Fauci 2012) PFU/mL.

7.2.2 Electrochemical Detection Systems

Among various approaches used to distinguish viral pathogens, including Zika (Cecchetto et al. 2017), influenza (Hushegyi et al. 2016), HIV (Shafiee et al. 2013), and so on, electrochemical biosensor technology is leading the way in the development of POCT devices owing to the advantages of high selectivity, sensitivity, quick response, and ease of miniaturization (Wang et al. 2021; Huang et al. 2016).

Enzyme-catalyzed reactions between immobilized biomolecules and target analytes generate electrons that affect the electrical properties of the solution, which is what electrochemical biosensors typically rely on to detect. An electrochemical system containing an electrode and a pathogen solution can convert the chemical energy derived from the target pathogen and biorecognition elements to the electrical signals captured by electrodes.

Li et al. (2021) showed that for the detection of HIV p24 antigen, the ZnO-NW-enhanced EIS biosensors proved their high sensitivity with LOD down to 0.4 pg/mL. Li et al. (2013) presented an EV71-specific nanogold-modified working electrode for electrochemical impedance spectroscopy and reached a LOD of 1 copy number/50 μL reaction volume in the detection of EV71, and the interval from sample preparation to detection was 11 min. Navakul et al. (Navakul et al. 2017) present a technique of DENV based on EIS aiming to detect, classify, and screen antibodies (Fig. 7.2). In this research, DENV was used as a component to functionalize a graphene oxide (GO)-polymer surface and make the polymer surface more selective and sensitive to the virus by inducing a self-assembly process. The EIS sensor can detect DENV ranges from 1 to 2×10^3 pfu/mL (LOD down to 0.12 pfu/mL) and classify the virus serotypes accurately.

7.2.3 Plasmonic-Based Systems

Plasmonic-based platforms, which consist of surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR), have become indispensable tools for

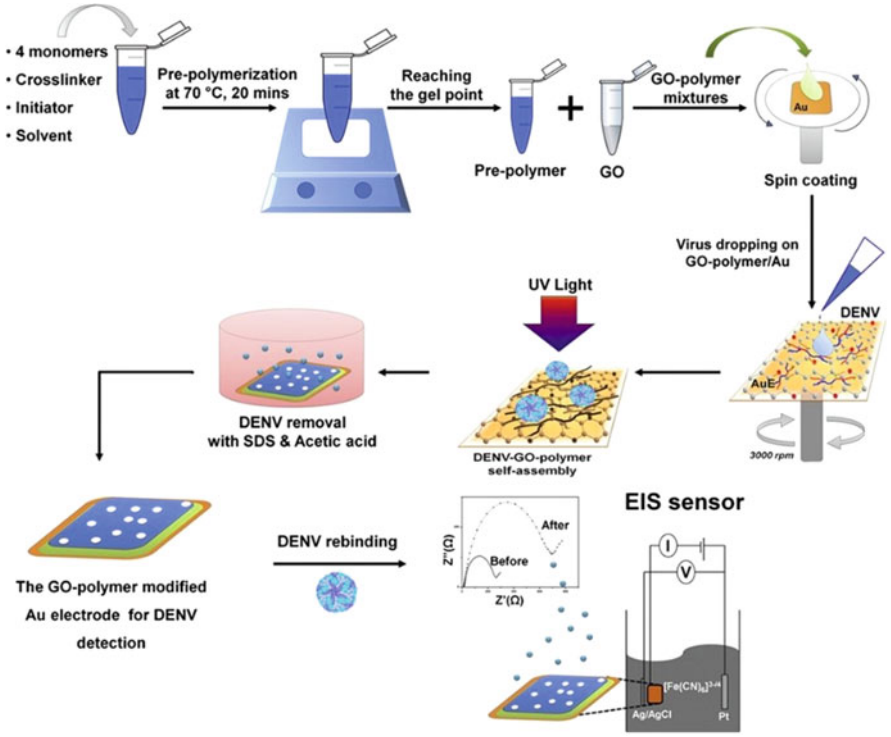

Fig. 7.2 Schematic illustration of the preparation of GO-polymer for DENV detection on a gold electrode. Reproduced from reference (Navakul et al. 2017)

POCT diagnostic applications (Table 7.2) for their advantages of stable, real-time, highly sensitive, and label-free. Plasmonic-based platforms are regarded as a critical candidate for next-generation diagnostics (Li et al. 2019a).

So far, SPR-based sensor has rapidly emerged as the most powerful detection type of optical biosensors. The SPR-based sensor could detect the binding events between the capture probes and analyte molecules by monitoring the refractive index change at the metal-dielectric interface. Inherently as a refractometric device applied in biology, SPR biosensors realize the detection and quantification of analytes by measuring parameters of incident light such as angle, intensity, phase, or wavelength.

As a promising one-step and label-free virus detection method, several SPR-based biosensors have already been adapted into POC devices of EIDs (Li et al. 2019a; Farzin et al. 2020), such as HIV (Kosaka et al. 2017), H1N1 (Su et al. 2012), H7N9 (Chang et al. 2018), etc. Detecting, identifying, and understanding viruses are of great importance for EIDs prevention, diagnosis, and control. When it comes to the detection as well as quantification of the antigens by plasmonic platforms, the work of Kosaka et al. (Kosaka et al. 2017) can detect HIV-1 p24 antigens in human serum less than a week after infection with the LOD of 10^{-17} g/

Table 7.2 Various optical techniques to diagnose different infectious diseases

Detection method	Analyte	LOD	Detection time	Reference
SPR aptasensor	H5N1 AIV	0.128 HAU	1.5 h	Bai et al. (2012)
Intensity-modulated surface plasmon resonance (IM-SPR) biosensor	H7N9	144 copies/mL	<10 min	Chang et al. (2018)
4-MBA/Au SPR chip	Recombinant nucleoprotein of Ebola (EBOV-rNP)	0.5 pg/mL	~ 1 h	Sharma et al. 2020)
Au/DSU/NH2rGO-PAMAM/IgM thin film-integrated SPR sensor	DENV-2 E-proteins	0.08 pM	8 min	Omar (et al. 2020)
GBP-E-SCVme-coated SPR biosensor	SARS coronavirus surface antigen (SCVme)	200 ng/mL	10 min	Park et al. (2009)
LSPR-induced Qdot-MB biosensors	ZIKV RNA	1.7 copies/mL	3 min	Adegoke et al. (2017)
Affinity peptide-guided plasmonic biosensor	Human norovirus	9.9 copies/mL	10 min	Heo et al. (2019)
LSPR-amplified immunofluorescence biosensor	Nonstructural protein 1 (NS1) of the ZIKV	1.28 fg/mL	–	Takemura et al. (2019)
LSPR biosensors based on thermally annealed silver nanostructures	Dengue NS1 antigen	9 nm/(μg/mL)	~30 min	Austin Suthanthiraraj and Sen (2019)

mL, which equals to one virion in 10 mL of plasma. Recent reports demonstrate that the SARS-CoV spike and SARS-CoV-2 spike share the same functional host cell receptor, angiotensin-converting enzyme 2 (ACE2) (Zhou et al. 2020; Wan et al. 2020). Furthermore, Wrapp et al. (Wrapp et al. 2020) provide evidence through SPR both biophysically and structurally to point out the fact that SARS-CoV-2 spike protein has a better affinity than SARS-CoV when bound to ACE2. By integrating IM-SPR biosensor with a newly generated monoclonal antibody, Chang et al. (Chang et al. 2018) developed a simple but reliable platform, being capable of sensitive and quick detection of H7N9 virus with a detection limit at 402 copies/mL in mimic solution, which dwarfs commercial RIDT, homemade target-captured ELISA, and even qRT-PCR. Almost excluding any sample preparation, Huang et al. (Huang et al. 2021) use a spike protein-specific plasmon nanoarray SPR chip to detect SARS-CoV-2 virus particles in one step using a spike protein-specific plasmon nanoarray SPR chip.

Wang et al. (Wang et al. 2010) use high-resolution surface plasmon resonance microscopy (SPRM) and successfully demonstrate label-free imaging, detection, and

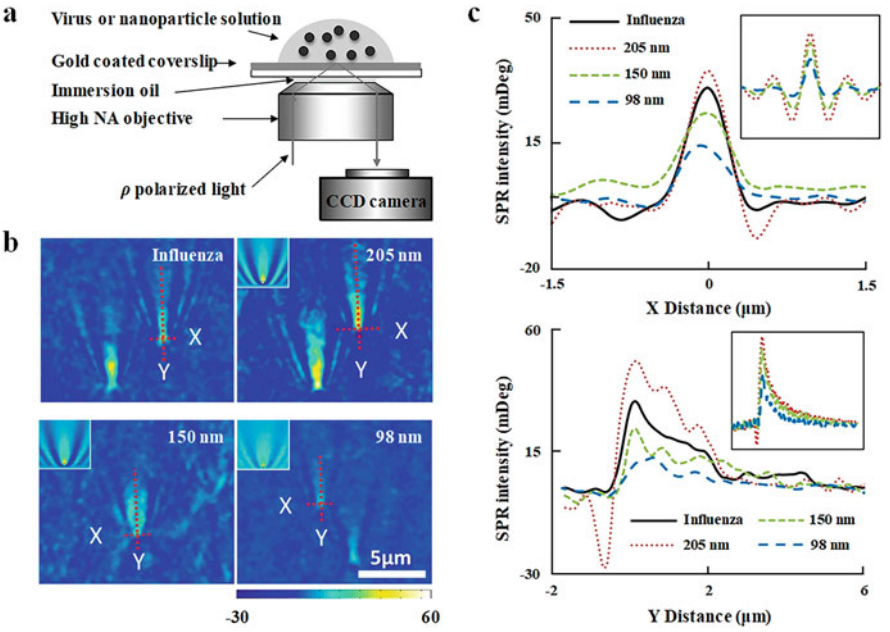

Fig. 7.3 Label-free imaging, detection, and mass measurement of single viral particles by surface plasmon resonance. (**a**) Schematic of the SPRM experiment setup (drawing not to scale). (**b**) SPRM images of the H1N1 influenza A virus and silica nanoparticles of three different sizes in PBS buffer. (**c**) The SPR intensity distributions along X and Y directions for selected particles (respectively, indicated by the dashed lines in b). (Insets) Corresponding profiles from simulated images. Reproduced from reference (Wang et al. 2010)

mass/size measurement of individual virus particles in solution. Based on the Kretschmann configuration, a high numerical aperture objective and an inverted microscope were used in the SPRM (Fig. 7.3a). For the SPRM setting, the sensing area is the whole image area with a size of 0.08×0.06 mm^2. Figure 7.3b is an image showing the discrepancy between silica nanoparticles of three different sizes and the H1N1 virus in PBS buffer, which is obtained by SPRM. For the two viruses studied in this work, the mass and diameter are found to be 6.5 ± 0.8 fg and 218 ± 10 nm for HCMV and 0.80 ± 0.35 fg and 109 ± 13 nm for H1N1 influenza A/PR/8/34, respectively (Wang et al. 2010). The sensing area can detect as small mass as 1 ag of the binding of a single particle, with a corresponding mass detection limit of about 0.2 fg/mm^2 per unit area, while the typical detection limit of the conventional SPR is nearly four orders of magnitude worse than this work (Homola 2008).

When measuring short-range changes in the refractive index owing to a molecular adsorption layer, the response of SPR spectroscopy and LSPR spectroscopy become similar, even though the former is much more sensitive to changes in bulk refractive index (Qiu et al. 2018; Qiu et al. 2019). The enhanced plasmonic field near the nanostructures increases the sensitivity of LSPR sensing systems to local refractive

index changes during molecular binding. Among various biosensing technologies, LSPR biosensing systems are capable of detecting various categories of analytes of clinical interest (Haes et al. 2005).

The LSPR technique may have the potential to substitute to detect SARS-CoV-2 and diagnose COVID-19. The overall performance could be improved by combining the plasma detection and amplification process. Meanwhile, the energy loss of the plasma and the related nanoscale heat generation, also known as the plasmonic photothermal (PPT) effector thermoplasmonics, could bring benefits to a wide range of research and innovation topics. For example, Qiu et al. (Qiu et al. 2020) developed a dual-functional LSPR biosensor using LSPR sensing transduction and PPT effect (Fig. 7.4a), combining both functions on a cost-effective two-dimensional gold nanoislands (AuNI) chip for the detection of SARS-CoV-2 viral nucleic acid. Aiming to improve the stability, sensitivity, and reliability of sensing, plasmonic resonances in LSPR and PPT are excited with two different light sources, i.e., 532 nm laser (normal incident angle) and 580 nm laser (attenuated total reflection mode). The biosensor can sensitively detect the RdRp gene with a LOD value as low as 0.22 pM. Based on previous work, Qiu et al. (Qiu et al. 2021) further expand the use of the photothermal-assisted plasmonic sensing (PTAPS) system and introduce the concept of thermoplasmonic-assisted dual-mode transducing (TP-DMT) (Fig. 7.4b), which allows direct detection of LOD up to 0.1 ± 0.04 pM. Cyclic

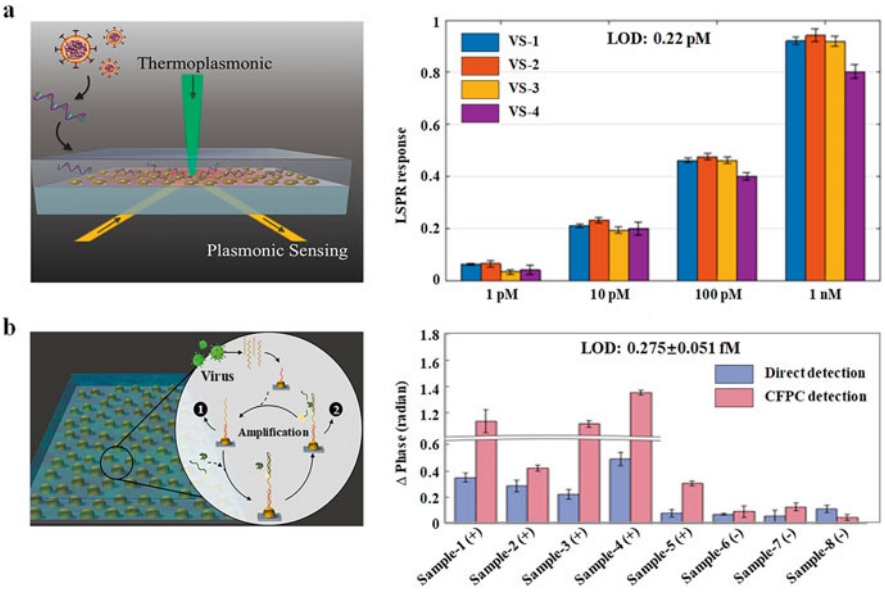

Fig. 7.4 (**a**) Dual-functional plasmonic photothermal biosensors for highly accurate detection of SARS-CoV-2. Reprinted with permission obtained from (Qiu et al. 2020). (**b**) Thermoplasmonic-assisted cyclic cleavage amplification for self-validating plasmonic detection of SARS-CoV-2. Reproduced from Ref. (Qiu et al. 2021)

fluorescence probe cleavage (CFPC) improves the sensitivity of quantitative detection by stimulating transient and cumulative LSPR responses, and the LOD of the second LSPR biosensing can be improved to 0.275 ± 0.051 fM.

7.3 Nano-Bio-Analytical Systems for Emerging Resistant Bacterial Infection Detection

Antibiotic-resistant bacterial pathogens are an emerging health threat, spreading rapidly and widely around the world (CDC 2019; Solomon and Oliver 2014). A critical reason for this worldwide concern is the overuse and misuse of antimicrobials (Laxminarayan et al. 2013; Tacconelli et al. 2018). A technique that could rapidly identify pathogen infection and determine antibiotic resistance profiles is needed to combat this threat. Antibiotic susceptibility testing (AST) is extensively used in the clinic to detect antibiotic susceptibility of isolated bacteria, guide more potent antibiotic treatment regimens, and evaluate therapeutic results. However, aiming to realize AST, the golden standard method often require microorganism culture, separation, and further subculture steps, which depend on overnight cell culture (Jorgensen and Ferraro 2009; Bauer et al. 1966). Therefore, rapid POCT bacterial resistance infection detection methods are necessary to avoid empirical prescription of antimicrobials and improve antibiotic stewardship.

Numerous emerging AST methods generally can be divided into two classes: genotypic and phenotypic approaches (Bauer et al. 2014; Khan et al. 2019), which recognize antibiotic resistance genes and directly analyze the phenotypic features, respectively. The genotypic approaches, which detect antibiotic resistance genes (Khan et al. 2019; Fluit et al. 2001; Frye et al. 2010; Park et al. 2011; Athamanolap et al. 2017), are highly desirable for the AST owing to the outstanding sensitivity (Machowski and Kana 2017). But the methods are still challenged for requiring enough prior knowledge of the bacteria; furthermore, the methods may obtain false-negative results when new resistant strains come into sight, and false-positive results when the existing resistance genes are not expressed or do not promote a resistant phenotype. Aiming to directly monitor bacterial cell growth or reproduction, phenotypic AST technologies generally focus on phenotypic characteristics (e.g., number, morphology, size, and length) (Syal et al. 2016; van den Broek et al. 2008; Cermak et al. 2016; Pancholi et al. 2018; Lissandrello et al. 2014; Pantel et al. 2018; Choi et al. 2014a; Longo et al. 2013; Besant et al. 2015; Schoepp et al. 2017). Optical inspection detection techniques, including real-time microscopy, microfluidic imaging, surface plasmon resonance imaging, and light scattering imaging, have been leading the way in rapid phenotypic AST.

7.3.1 Real-Time Microscopy System

Optical microscopy systems are the leading method used in rapid phenotypic AST detection, which can image the phenotypic characteristics (e.g., number,

morphology, size, and length) for direct bacterial cell growth or reproduction measurements (Syal et al. 2016; van den Broek et al. 2008; Cermak et al. 2016; Pancholi et al. 2018; Lissandrello et al. 2014; Pantel et al. 2018; Choi et al. 2014a; Longo et al. 2013; Besant et al. 2015). Multiplexed automated digital microscopy (Metzger et al. 2014; Chantell 2015) was established to achieve rapid identification and drug-resistance analysis of clinical specimens, which isolates bacterial cells from coexisting or interfering species within the clinical specimens (e.g., urine or blood) utilizing gel channels, attaches purified bacterial cells to the sensing surface based on electrokinetic loading, and identifies bacterial cells with fluorescence imaging within 1 h (Chantell 2015). In order to enable quick AST in individual cells morphological analysis (Choi et al. 2014b), an emerging imaging tool uses bright-field microscopy and an automated image-processing and analysis algorithm to facilitate accelerated AST to ascertain antibiotic-induced morphological changes in single bacterial cells. oCelloScope (Fredborg et al. 2015; Fredborg et al. 2013), another optical imaging technique, images the growth of bacterial cell populations in liquid samples and quantifies the changes in the area occupied by the growing cells for rapid AST. The Accelerate Pheno™ system uses microscopic morphogenetic cellular technology to analyze individual cells and colonies and measure growth, providing AST results of bloodstream infections within 7 h (Pancholi et al. 2018; Pantel et al. 2018; Marschal et al. 2017; Burnham et al. 2014; Schneider et al. 2019; Ehren et al. 2019).

Electrical impedance, which detects the current response of a sample under applied potential, is highly desirable for the label-free biosensing platform. The technology can be employed in various applications, including tissue, protein, and cell studies (Ng et al. 2010; Shamsipur et al. 2018; Nwankire et al. 2015; Lei 2014). As an intrinsic characteristic, the sensitive current response of the target bacterial cells to an applied electric field is connected with subtle differences and variations of parameters such as the shape and size of the target bacterial cell, the internal structure of the bacteria, and the dielectric constant and conductivity of the diversified bacterial components. This enables the impedance analysis for studying bacterial external stimulus-response determining the bacterial viability, studying the principles of antibiotic action, and bacterial identification and bacterial isolation (Mannoor et al. 2010; Yang and Bashir 2008; Xu et al. 2017; David et al. 2012). Based on the intrinsic electrical properties of single bacterial cells, Zhang et al. (2021a) reported label-free electro-optical impedance microscopy (EIM) by imaging the cell responses to low-frequency potential modulations to quantify single bacteria impedance. *E. coli* O157:H7 is immobilized on the surface of an indium tin oxide (ITO) electrode, and the impedance response of potentially modulated bacteria is imaged to complete the detection. EIM can map bacterial viability at subcellular resolution by impedance response, which was used to monitor the impedance changes under two distinctive types of drug for comprehensive bacterial viability detection and antibiotic mechanisms study. Figure 7.5a shows the schematic illumination of the EIM system with an inverted microscope. The ITO electrode surface is modified with antibodies for both *E. coli* potential modulation and immobilization. Figure 7.5b is the representative impedance responses of the single bacterial cells

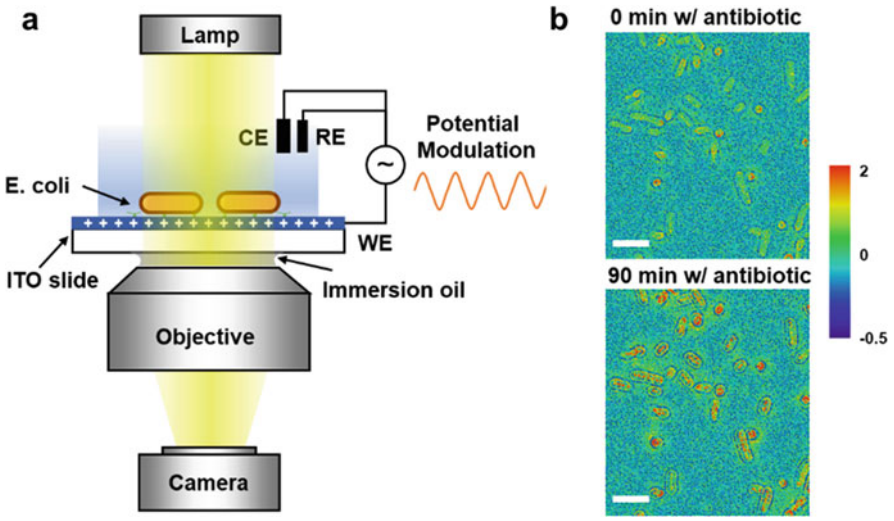

Fig. 7.5 (**a**) Structure diagram of the impedance imaging setup with a standard three-electrode. (**b**) The representative impedance results of the bacterial cells under 90-min antibiotic treatment. Reprinted with permission from reference (Zhang et al. 2021a). Copyright 2021 American Chemical Society

with subcellular resolution under antibiotic treatment, which revealed the heterogeneity response of single bacterial cells. The simple imaging approach can be used to identify resistant bacteria associated with the infectious disease, delivering accurate results more rapidly at a lower cost.

7.3.2 Microfluidic Imaging System

Microfluidics has revolutionized single-cell manipulation and analysis methods with reduced sample/reagent volumes and the associated costs for sensitivity and POCT. It has been found that the combination of imaging-based tools and microfluidics allows for rapid AST (Park et al. 2011; Choi et al. 2013; Baltekin et al. 2017; Li et al. 2019b). Bacterial cells were sequentially captured in microfluidic chambers (Kim et al. 2015), microchannels (Lu et al. 2013), or droplets (Chen et al. 2010; Boedicker et al. 2008) and imaged to detect changes in the cell number (Metzger et al. 2014; Mohan et al. 2015), size (Choi et al. 2014b), morphology (Quach et al. 2016), and viability (Boedicker et al. 2008) in the presence of antibiotics to perform AST. The Astrego Captiver system is composed of 2000 single bacteria-sized channels, and the longitudinal growth of a single bacterial cell was monitored with a time-lapse phase contrast microscopy for bacterial susceptibility determination within an hour (Baltekin et al. 2017). The droplet-based platform dropFAST (Kaushik et al. 2017) allows single-cell encapsulation, incubation, and drug resistance in less than 60 min. Co-encapsulation of bacterial cells with an active probe (e.g., Alamar blue) has been

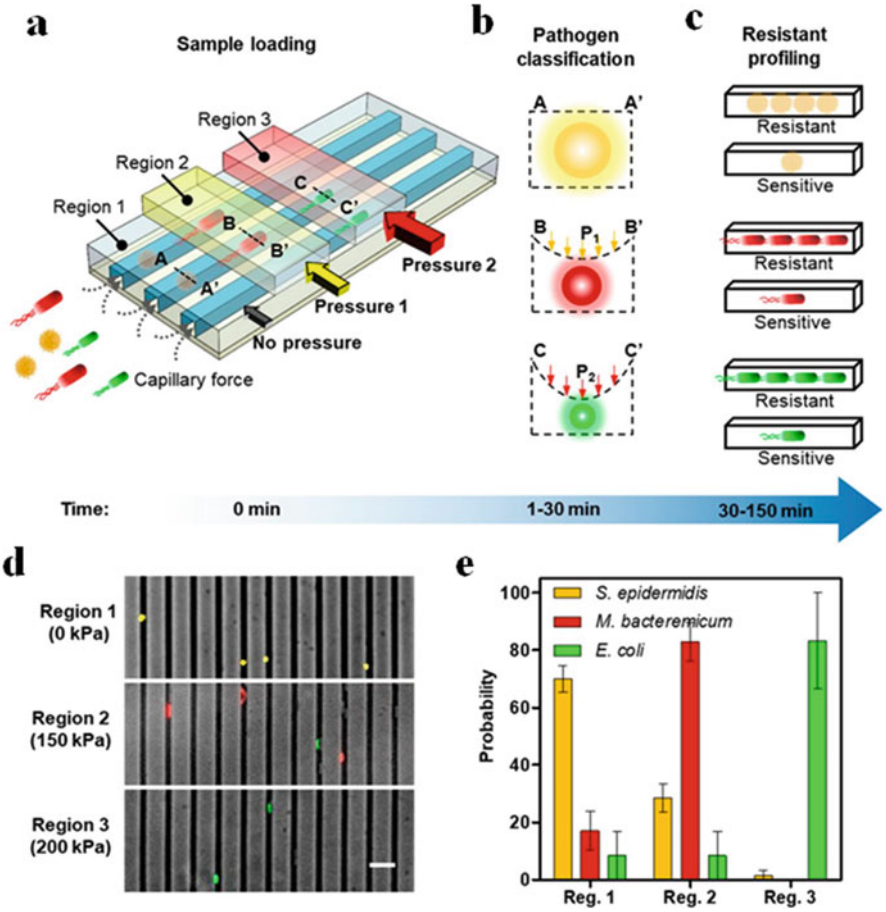

Fig. 7.6 (**a**) Microfluidic device adapted for AST that can be used to classify pathogens at the level of individual bacterial cells. (**b**) Cross-sectional profile of the channel at different air pressures. (**c**) Antimicrobial susceptibility was identified by tracking variances of the bacteria phenotypic growth in the presence of antibiotics. (**d**) Microfluidic separation of three bacterial species (*S. epidermidis*, *M. bacteremicum*, and *E. coli*) by the tunable microfluidic device. (Scale bar, 10 μm). (**e**) Distributions of the bacteria in regions with 0, 150, and 200 kPa applied pressure in the microchannels. Data represent mean ± SEM ($n = 3$). Reproduced from reference (Lu et al. 2013)

used to distinguish AST bacterial subpopulations in not more than 4 hours (Lyu et al. 2018). In another droplet-based method, bacterial cells are captured with antibody-conjugated beads, Hoechst staining is followed by microscopic analysis of cell division and morphology, and drug resistance is determined within 120 min (Sabhachandani et al. 2017).

For direct AST on clinical samples, an adaptable microfluidic system was reported to process clinical specimens (including urine and blood) without any preliminary treatment (Li et al. 2019b). By applying different pressures, the main

pathogens can be captured and classified depending on the shape and size of the polymicrobial sample. A clinical sample was flowed along the microfluidic channel. Once a bacterial cell is noticed with a high-resolution optical microscope, it applies pressure to the channels to trap the cell and then monitors cellular length in the presence of an antibiotic over time. Figure 7.6a shows the adaptive microfluidic device that can be used to classify pathogens at the single bacterial cell level, while Fig. 7.6b shows cross-sectional profiles of the channels at different air pressures. Bacteria remain under different areas of the channel and are sorted according to the applied pressure, which dynamically changes the height of the channel. Antimicrobial susceptibility was identified by tracking variances of the bacterial phenotypic growth in the presence of antibiotics (Fig. 7.6c). Figure 7.6d shows the isolation of three bacteria utilizing a microfluidic device. *S. epidermidis*, *M. bacteremicum*, and *E. coli* were fluorescently stained, mixed, and loaded into the microfluidic system to detect the effectiveness of pathogen isolation ($n = 3$). Figure 7.6e was used to test the effectiveness of bacterial identification by plotting the distribution of bacteria in areas of the microchannel where 0, 150, and 200 kPa pressure was applied.

7.3.3 Surface Plasmon Resonance Imaging (SPRi) System

Surface plasmon resonance (SPR) is an outstanding sensing technique that has many advantages for molecule detection, including in situ, real-time, and fast response. SPR can detect weak refractive index changes on the surface of the sensing chip with high sensitivity. SPR imaging (SPRi) technique can realize real-time imaging and visualization of the local refractive index change near or on the sensing surface, providing spatial information for local mass density sensing. Chiang et al. (Chiang et al. 2009) tested the antimicrobial susceptibility of susceptible and resistant bacteria within a single channel adhered to a gold chip. This is the first report of an antimicrobial test using the SPR system to detect resistant or susceptible strains. To improve the throughput, Ozkaya et al. (Ozkaya et al. 2019) constructed a SPR system with multichannel microfluidic for the multiple detections of methicillin-susceptible *S. aureus*, methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and vancomycin-susceptible *Enterococcus*.

For more sensitive AST detection, Syal et al. (Syal et al. 2016; Syal et al. 2015) introduced the plasmonic imaging and tracking (PIT) technique to monitor and analyze the multidimensional movement of individual bacterial cells, which are closely related to metabolic viability. The PIT equipment is based on an inverted optical microscope, where light from a luminescence diode is directed onto the sensor chip made of gold-coated glass film with immobilized bacterial cells (Fig. 7.7a). Figure 7.7b shows a few snapshots of the plasmonic image, which reveal large fluctuations in the image contrast of a live bacterial cell (*Escherichia coli* O157:H7). The image contrast changes are due to the bacterial cell movement normal to the sensor surface (Z-direction), due to the bacterial metabolism of live cells (Fig. 7.7c), while the dead cell showed very minimal motion (Fig. 7.7d) (Yang et al. 2015; Shan et al. 2014). PIT has been reported to detect the multidimensional

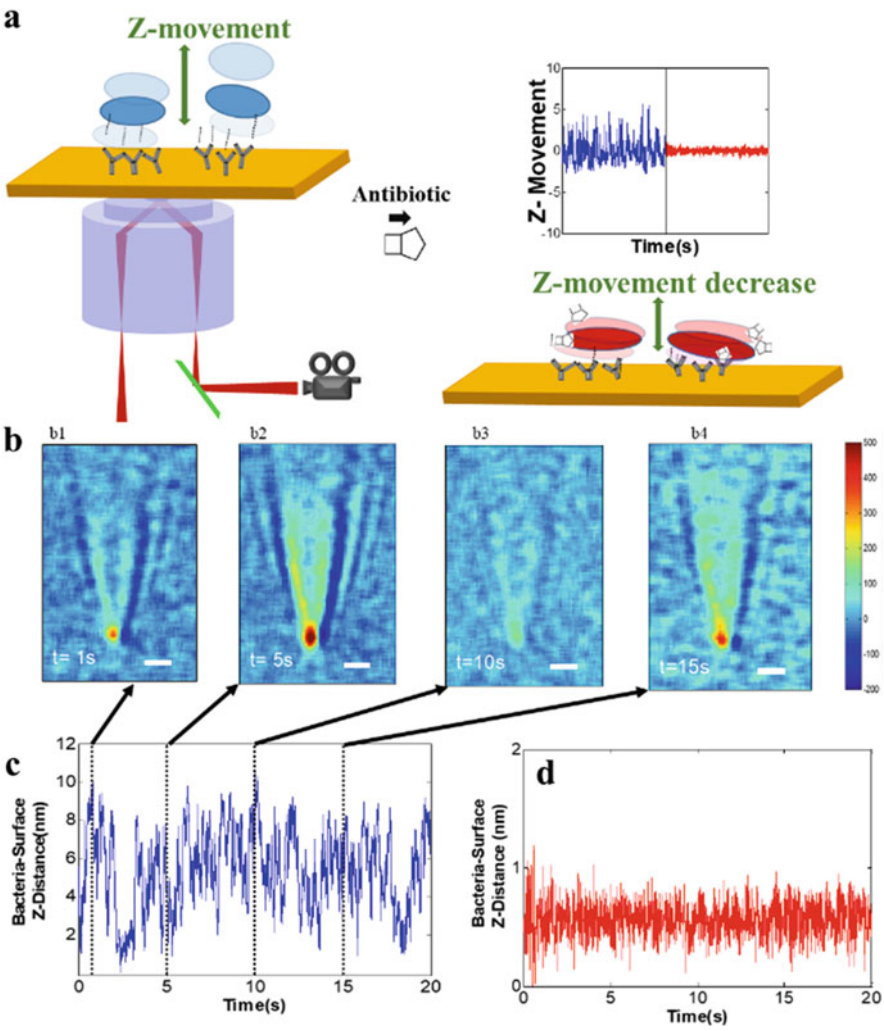

Fig. 7.7 (**a**) Schematic of the plasmonic imaging and tracking of bacterial cell metabolic activity-related 3D movement (referred to here as 3D movement) with nanometer resolution. (**b**) Snapshots of bacteria z-micro-motion. (**c**) z-distance between bacterium and plasmon surface vs. time of an alive (~6 nm). (**d**) Z-displacement plot of a dead bacterial cell showing average motion ~0.50 nm. Reprinted with permission from reference (Syal et al. 2016). Copyright 2019 American Chemical Society

motility of individual bacterial cells and subcellular organelles at spatial resolution <5 nm and temporal resolution <1 millisecond (ms). This detection technique allows rapid monitoring of the multidimensional motility of multiple bacterial cells simultaneously, enabling high-throughput quantitative analysis of single-cell ASTs. The work also demonstrates the feasibility of this technique for the detection of

clinical samples. In addition, PIT has the potential to be used to discriminate and identify bacterial cells in the complex matrix of urine, serum, and other body fluid samples. This will facilitate the real application of PIT and its development into a practical solution for testing real patient samples.

7.3.4 Light Scattering Imaging System

As a label-free and noninvasive analytical tool, light scattering can be widely used to detect and classify small particle analytes such as bacteria and cells in real time (Broeren et al. 2011; Fouchet et al. 1993; Steen 1990). A laser beam is normally used to irradiate a liquid sample for both turbidity and scattered intensity measurement. Generally speaking, measuring light scattered by bacterial cells is the principle of most light scattering-based bacterial growth detection protocols (e.g., BacterioScan (Roberts et al. 2017)). The relative growth of bacteria in culture solutions is commonly characterized by the results of optical density at 600 nm. The principle of this technique is because different concentrations of bacteria scatter different amounts of light randomly in solution. However, this indirect assessment of bacterial growth and cell concentration is often a complex pre-process (requiring inoculation of pure samples and suspension of cells to the appropriate density level) (Sutton 2011). With recent advances in optical detection technology, other light scattering methods have been newly developed to improve detection sensitivity (Roberts et al. 2017; Hayden et al. 2016; Boland et al. 2019). As an important optical detection system in recent years, flow cytometry systems (such as the FDA-approved UF 1000i) integrate light scattering and fluorescence technologies for rapid screening of bacterial cells in urine (Broeren et al. 2011).

The light scattering method can further measure the angular variation in the intensity of the light, with this variation being proportional to the number, size, and even shape of the particles, allowing detection limits up to 2 orders of magnitude lower compared to standard methods. Based on the above approach, Zhang et al. developed a rapid AST in the single-cell scattering intensity tracking for direct AST on clinical urine specimens within 60–90 min with a large-volume solution scattering imaging (LVSi) (Mo et al. 2019) by monitoring and tracking individual cell division (Zhang et al. 2020) and by object scattering intensity detection (Zhang et al. 2021b). Traditional optical microscopy can image bacterial cells but requires immobilization of the bacteria on a surface. These limitations, combined with the small field of view of high-resolution optical microscopy, necessitate bacterial enrichment in low-concentration samples. LVSi overcomes this difficulty by illuminating and imaging a large volume, such that the presence of a few bacterial cells in a clinical sample can be tracked continuously, which can image and count low bacterial concentration urine samples, e.g., 10^4 cells/mL for rapid AST (Mo et al. 2019). To accurately track and quantify single division events of bacterial cells in complex clinical samples, a forward scattering optical imaging configuration (Fig. 7.8a) was introduced with a single-cell division tracking imaging processing algorithm. Figure 7.8b shows the representative example of two division events for two cells

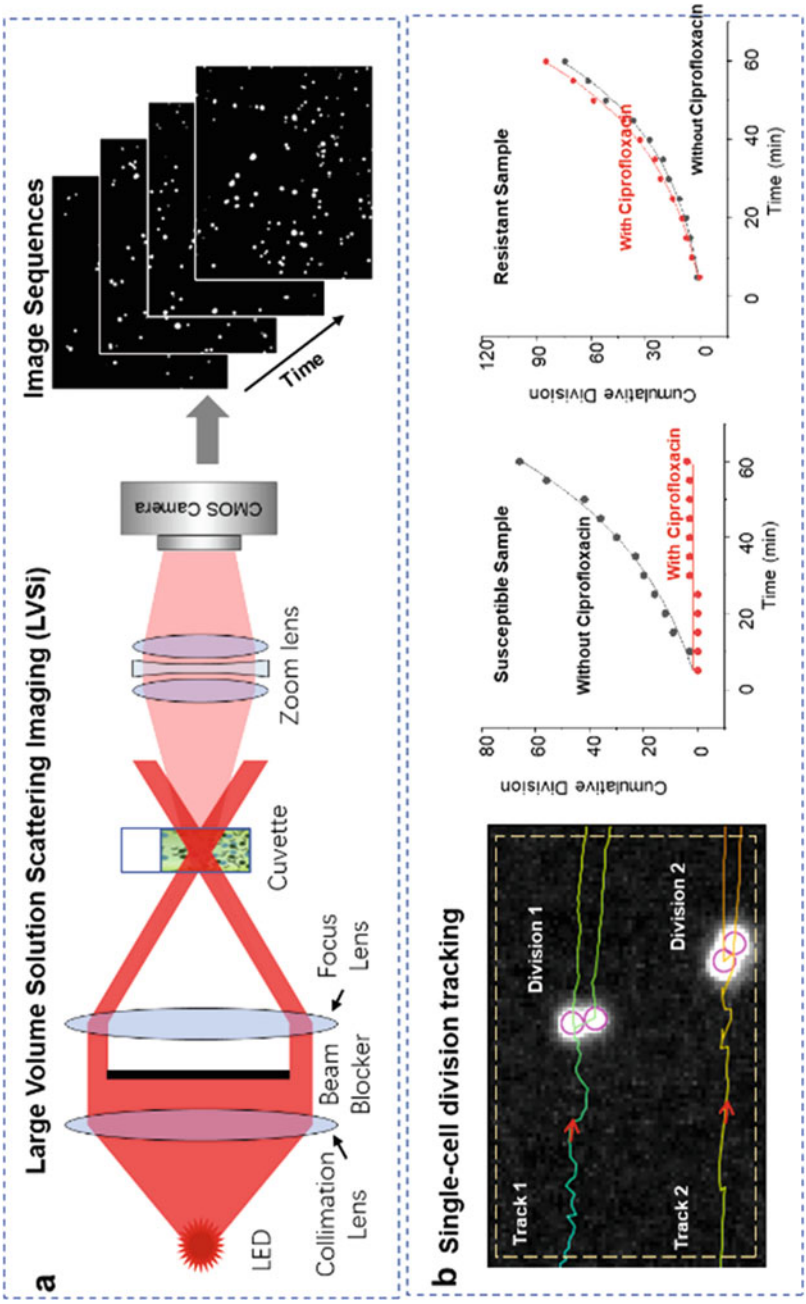

Fig. 7.8 (**a**) Schematic illustration of the LVSi setup which could detect single bacterial cells in urine. (**b**) Demonstration of two-split event detection with processing algorithm and two representative growth curves of susceptible and resistant samples detected by the LVSi-AST tool. Reproduced from reference (Zhang et al. 2020)

tracked using the imaging processing algorithm and two representative growth curves of susceptible and resistant samples detected by the LVSi-AST method. The LVSi-AST method has excellent performance, achieving rapid detection of bacterial infections in 60 clinical samples within 1 h. The digital ASTs of these 30 positive samples were in 100% complete agreement with clinical culture results and field agar coating validation results. This technology will pave the way for more accurate antibiotic prescriptions and prompts proper treatment of the patient within a single clinic visit.

7.4 Conclusion and Future Direction

Current manual and automated techniques for emerging disease detection have become vital tools in today's clinical microbiology labs. In the near future, burgeoning and future innovative technologies, for instance, plasmonic-based biosensing systems, microfluidic-based systems, and light scattering imaging systems, will lead the way in the development of clinical tools for rapid infectious disease diagnosis. These tools have dramatically reduced the time to detect infectious diseases and allow for POCT diagnoses to be made on an outpatient basis. Such rapid and real-time detection tools will not only help save patients' lives but also enable physicians to implement accurate treatments at disease onset, potentially preventing the spreading of EIDs, slowing the evolution of antibiotic resistance, and improving antibiotic stewardship. In summary, given the ever-increasing spread of EIDs, there is an urgent need to develop innovative technologies for the rapid detection of infections, both for real samples collected directly from the patient and for slow-growing or non-cultivable microorganisms.

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Surface Engineered Nanobiosensor for Disease Biomarker Identification

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Hussaini Adam, Yeng Chen, Subash C. B. Gopinath, and Tijjani Adam

Abstract

In clinical context, robust, accurate identification of biomarkers at low concentrations are important. Nanobiosensors with easy manufacturing processes, label-free operation, and fast sensing could be the key to point-of-care diagnostics for clinical diseases. Nanomaterials hold a lot of potential for a new generation of energy storage materials, could recharge fast, and are more stable than their bulk material counterparts. However, increased surface area and enhanced reactivity of active materials overcome these to be practical, and

H. Adam
Institute of Nano Electronic Engineering, Universiti Malaysia Perlis (UniMAP), Kangar, Perlis, Malaysia

Y. Chen
Department of Oral & Craniofacial Sciences, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

S. C. B. Gopinath (✉)
Institute of Nano Electronic Engineering, Universiti Malaysia Perlis (UniMAP), Kangar, Perlis, Malaysia

Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Arau, Perlis, Malaysia

Micro System Technology, Centre of Excellence (CoE), Universiti Malaysia Perlis (UniMAP), Pauh Campus, Arau, Perlis, Malaysia
e-mail: subash@unimap.edu.my

T. Adam
Faculty of Electronic Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Arau, Perlis, Malaysia

Micro System Technology, Centre of Excellence (CoE), Universiti Malaysia Perlis (UniMAP), Pauh Campus, Arau, Perlis, Malaysia

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often overshadow this promise. Several materials that show promise in bulk form for increased capacity or energy density become unstable or reactive in electrochemical conditions when downscaled, especially for energy storage devices. As a result, surface engineering can be a useful technique for decoupling bulk material properties from surface characteristics, which can hinder nanomaterial energy storage applications. Surface engineering may make it easier to develop biosensors, improve the interaction between analyte biorecognition elements, and lessen fouling in biological fluids. Thanks to surface engineering based on nanomaterials, biosensors are now as sensitive as lab-based cutting-edge technology in detecting a number of clinically significant molecules. This chapter covers nanobiosensors, nanobiosensor fabrication, nanobiosensor surface modification, nanobiosensor operating principles, and biomarker detection using nanobiosensors. The functionalisation and characterisation of several types of nanomaterials have also been explored.

Keywords

Nanomaterial · Biosensor · Clinical marker · Surface chemistry

8.1 Introduction

High-sensitivity biomolecule identification is essential for clinical diagnostics. For instance, early identification of Parkinson's disease biomarkers may improve prognosis and survival rates (Blesa et al. 2017). However, the concentration of clinical biomolecules is very low at the early stages of disease progression. Furthermore, it takes time to identify an analyte using ultrasensitive detection techniques including microscopy, cell culture, and proteomics (Purohit et al. 2020). These methods are complex, and the equipment is costly and difficult to transfer to remote areas, making it unreliable in emergency situations. Therefore, portable technologies with fast and accurate detection methods are needed, such as nanobiosensors. They are most commonly employed in hospitals to detect clinical pathogens. Nanobiosensors are bioanalytical devices that detect extremely tiny amounts of analyte using nanomaterials and surface engineering (Naresh and Lee 2021). Via isolated enzymes, tissues, immune systems, organelles, or complete cells as the mediators of certain biochemical events, a nanobiosensor is a device that can identify chemical molecules, typically using thermal, electrical, or optical signals (Noah and Ndangili 2019). An amplifier, a biorecognition element, and a transducer are the three parts that make up a biosensor (Malekzad et al. 2017). The biorecognition element recognises the analyte of interest, which is then converted into measurable signals by the transducer. The generated signal outputs are then processed by a processor and an amplifier. Biosensors are analytical tools used to investigate a variety of phenomena, including cell mechanics, physiology, and the effect of drugs on cells (Zhu et al. 2015). As a result, nanobiosensors based on the target and transduction process are being developed in order to get a measurable signal with a high

resolution capable of distinguishing even the smallest change in target analyte concentration. Biosensors must be capable of distinguishing between target and non-target molecules in the detecting fluid. Nanobiosensors are developed utilising a number of techniques. Nanobiosensors integrated with higher affinity biomolecules may detect a wide range of analytes effectively. Disease detection, biomolecule identification, and disease progression are some of the applications of biosensors. Nanobiosensors also employ electrical and electrochemical sensors for a variety of applications. These nanobiosensors have a stronger impact on the biosensor system's overall function and performance. A biorecognition matrix is also used to help biosensors detect sensitive analytes. When creating nanobiosensors, parameters such as sensitivity, selectivity, detection limit, detection range, reusable capacity, and others are taken into account. In addition, the effect of the sensing matrix and co-existing molecules in nanobiosensors may produce signals in biosensor sensing systems (Purohit et al. 2020). Therefore, to enhance biosensor performance and lessen interference from the sensing matrix, researchers have used polymers, metallic nanoparticles, quantum dots, carbon nanomaterials, and other nanomaterials of varied sizes, shapes, and properties (Naresh and Lee 2021). Point-of-care diagnostics, a portable diagnostic kit for the precise and sensitive on-site detection of therapeutically relevant chemicals, has been created using surface engineered nanobiosensors based on nanomaterials (Kour et al. 2020). As a result, nanomaterials have become essential components in biosensor design because they increase signal amplification and sensitivity whilst also having a larger surface area, size-dependent electrical conductivity, optoelectronic properties, and high catalytic activity (Cho et al. 2020a). A nanomaterial-modified surface may adsorb and detect more biorecognition elements and analytes than a non-modified surface (Purohit et al. 2020). Nanomaterials help in the miniaturisation of sensing devices by providing a portable nanoscale platform with improved sensitivity. Biorecognition elements are attached to nanomaterial-modified nanosensor surface using suitable immobilisation procedures to achieve the selectivity. By increasing the loading of biorecognition elements on nanomaterials, functionalisation of nanobiosensors helps to enhance the detection range of biosensors (Putzbach and Ronkainen 2013). These approaches can involve covalent or non-covalent interactions. Non-covalent interactions prevent the conjugated skeletons from being destroyed, which minimises the loss of electrical characteristics in nanoparticles (Wang et al. 2017a). Covalent interactions, on the other hand, are better for the stability and reproducibility of the conjugation surface (Wang 2017). Nanoscale gaps between electrode arrays can be used to create nanobiosensors. A number of sensing applications, including bio and chemo sensing, now highly depend on nanogap biosensors. Nanomaterials and surface chemistry are required in nanobiosensors for the detection and stability of extremely low analyte concentrations of biological materials on the sensor's sensing surface (Khan et al. 2019). Nanobiosensors are progressively becoming more reliable in various applications. Nanobiosensors have the ability to detect even the smallest signal. Nanobiosensors are also easy to fabricate, miniaturised, and they are cost-effective. Therefore, this chapter provides a brief overview of the fabrication of nanobiosensors and application of

nanomaterials and surface engineering to improve biosensor efficiency for biomarkers identification.

8.2 Biosensor

Biosensors have been effectively employed in many areas of current research, including clinical detection, environmental studies, cell physiology, and studying the effect of space on astronauts (Pan et al. 2017). Nanobiosensors are improving in terms of reliability, response time, and efficiency. When developing biosensors, both the target analyte and the transducing mechanism are taken into account. Depending on how labels are utilised, biosensors can be labelled or label-free (Naresh and Lee 2021). In labelled biosensors, the analyte, such as enzymes, electroactive compounds, or fluorescent molecules, is detected using a reporter or label (Lei et al. 2020). The label-based method increases overall sensing cost and time whilst improving signal amplification and selectivity for sensing applications. Label-free biosensors employ biorecognition components to identify the target, and their simple design makes them portable and easier to develop. Biosensor technology has seen an increase in popularity and advancement over the past 20 years. The performance and effectiveness of the resulting biosensor system can be more significantly influenced by electrical and electrochemical systems, which are used in biosensors (Rocchitta et al. 2016). A biosensor is a standalone integrated receptor transducer that uses a biological recognition component to deliver accurate quantitative or semi-quantitative data (Tamayo 2018). A biosensor is a device that uses a biologically sensitive recognition element immobilised in a physicochemical transducer connected to a detector to quantify the concentration and kinetics of species in a sample (Bhalla et al. 2016). The catalytic or affinity characteristics of the biological element determine the specificity and selectivity of the biosensor. The digit width and gap of the sensing surface are two essential factors to be taken into account for developing interdigitated electrodes. Generally, interdigitated electrodes with a smaller spacing enable improved sensitivity. For instance, electrodes with a diameter of less than a micron are effective for detecting DNA coupling, whereas electrodes with a size in the micrometre range are useful for detecting bacteria and cells (Munteanu et al. 2018). Biosensors are more advantageous, effective, precise, economical, and simple to use than other conventional laboratory-based detection techniques due to their portability, reusability, real-time detection, high specificity, sensitivity, and selectivity (Campuzano et al. 2017) The operation of biosensors is determined by the signal transduction principle. The interaction is detected by the transducer, which generates a signal. The signal output strength and analyte concentration are proportional. The signal is subsequently processed and amplified by the electrical system (Fig. 8.1).

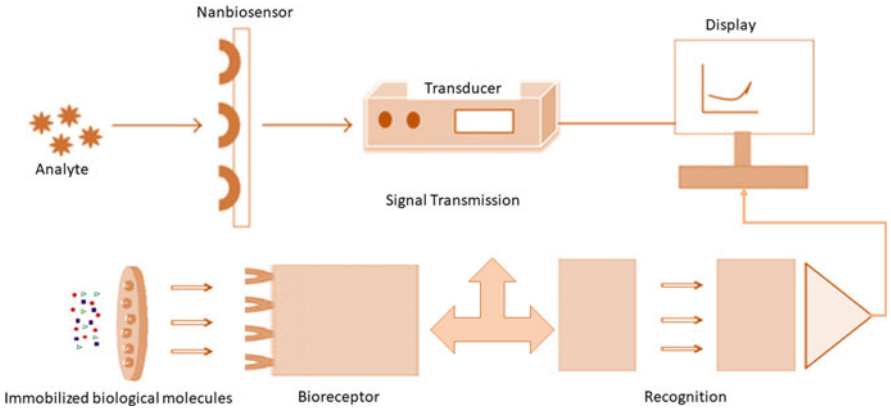

Fig. 8.1 Operating principle of nanobiosensor: Signal transduction is the basis for how biosensors work, and the transducer measures this interaction and outputs a signal

8.2.1 Resistive and Capacitive Nanosensors

Capacitive or resistive sensors are both often used in nanosensors. Both of these enhance the sensor system's and the sensor material's surface area in contact. The surface advantage of the resistive sensor enhances the detecting process (Karuthedath and Schwesinger 2018). The conductivity and resistance of the thin film alter over its whole length when the sensor region detects analyte. As a result, electricity discharges as it travels from one electrode to the other across the gap of the nanosensors.

8.2.2 Fabrication of Nanobiosensor

Numerous sensor-related applications use nanobiosensors. The sensing surfaces of nanobiosensors are used to identify electrical signals generated by sensing materials, and nanobiosensors are widely employed as a component for detecting functions (Zhu 2017). Photolithography is widely used to create nanobiosensors and microstructures (Fig. 8.2). Biosensor devices are made using a variety of techniques, including inkjet printing, hydrothermal growth, solvent casting, classic photolithography, traditional wet etching, and molecular beam epitaxy, amongst others (Tortorich et al. 2018). Biosensors are analytical devices that combine biological detecting elements such as a sensor system and a transducer into a single device. When compared to any other currently available diagnostic device, these sensors are superior in terms of selectivity and sensitivity (Mehrotra 2016).

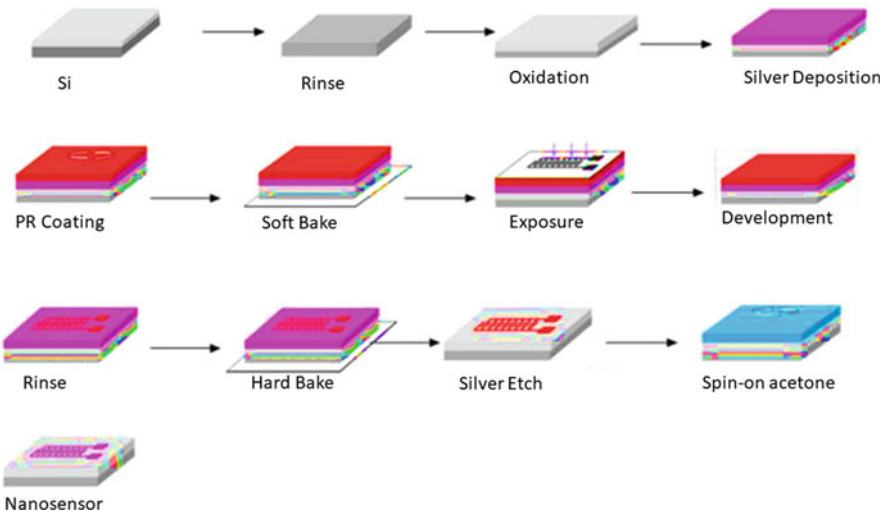

Fig. 8.2 Steps for conducting photolithography technique and aluminium interdigitated electrode development. The photolithographic process includes the steps of wafer cleaning, barrier layer generation, photoresist application, soft baking, mask alignment, exposure and development, and hard baking. Aluminium etching and acetone dipping are two steps in the development of aluminium interdigitated electrodes

8.2.3 Sensor Matrix

The foundation of biosensors is a functionalised support matrix that makes it possible to couple biorecognition components for sensitive analyte detection (Naresh and Lee 2021). The ultimate detecting capacity of the sensor is more heavily influenced by the type of material used, the fabrication process, and the sensor matrix designs. Due to its porosity and the availability of natural capillary action, which allows sample fluids to self-pump, paper has proven effective in the development of optical, electrochemical, and microfluidic sensing platforms. To achieve the required sensitivity and stability, the paper must have a high composition and porosity. The bulk of biosensors on the market today were developed using lateral flow assays and paper-based dipsticks. Au is used as electrodes in the development of nanobiosensors as a result of its durability and inertness. Indium tin oxide is also employed as a ceramic electrode in the development of nanobiosensors due to its superior electrical conductivity, large working window, strong substrate adhesion abilities, low capacitive current, robust electrochemical and physical qualities, and optical transparency (Wu et al. 2017). In addition, unlike indium tin oxide, which is unstable in acidic conditions, glassy carbon electrodes, and noble metal electrodes have faster electron transport kinetics (Purohit et al. 2020). Glassy carbon electrodes have a low oxidation rate, are chemically inert, and have a broad potential window, which are all required for effective biosensing (Afrasiabi et al. 2016). Due to its reputation as a chemically inert material, bare glassy carbon electrodes may

efficiently catalyse specific sensing processes in analyte concentrations without the need for nanofabrication (Sharma 2018). Edge plane sites/defects are routinely inserted on the glassy carbon electrode surface, leading to faster electron transfer. Disposable electrodes with tunable shape, composition, pattern, and biorecognition components are made by screen-printing various types of inks on ceramic or plastic substrates (Hayat and Marty 2014). Working with screen-printed electrodes offers several advantages, such as mobility, low cost, ease of functionalisation, and the ability to integrate all electrodes into a single electrochemical system (Martínez-Periñán et al. 2020). Therefore, sensor matrix should be chosen with caution, as each has its unique pattern and behaves differently.

8.2.4 Interdigitated Nanobiosensors

Interdigitated nanobiosensors are becoming more and more common because of their high sensitivity and simplicity of fabrication. Parallel electrodes are often coupled with interconnected comb-shaped electrodes to create interdigitated nanobiosensors (Yoo et al. 2021). Because of their structural designs, interdigitated-based nanobiosensors offer various benefits in the production of biosensors. An electrical field will be produced if voltage is placed between the electrodes (Muaz et al. 2014). When a biomolecule or cell is present on the surface of an interdigitated nanobiosensor, the electric field may change, and the interdigitated nanobiosensors may then be able to detect the changes. The interdigitated nanobiosensors are easier to build using affordable materials than other biosensors and have bigger surface surfaces for sensing mechanisms that use minimal energy to assess biological changes (Gu et al. 2021). To create interdigitated nanobiosensors, a mask can be developed using AutoCAD software and then printed on a chrome mask. Following the creation process, the interdigitated nanobiosensor may be characterised using a high-power microscope, atomic force microscope, scanning electron microscopy, 3D nano-profiler, and others to examine the effectiveness of the interdigitated nanobiosensor fabrication technique. One of the most critical steps in ensuring that the surface of the interdigitated nanosensors is not faulty is physical characterisation (Wang et al. 2014). Characterisations of nanobiosensors are also done to check current flow through the sensor. For example, when the working electrode is connected to the potential difference, current can flow through the nanobiosensor's surface, and the current is then measured as an output (Fig. 8.3). Interdigitated nanobiosensors are made up of two-digit electrodes that are linked yet independent of one another. In developing a nanobiosensor with interdigitated electrodes, the digit width and gap must be considered. Sensitivity is generally higher in interdigitated nanobiosensors with narrower gaps. The geometries of interdigitated nanobiosensors have a significant impact on their capacity to respond to variations in current applied by surface activities. Because of their smaller gap sizes, interdigitated nanobiosensors provide a faster electrical response in applications such as biological, optical, and environmental sensing. The high surface area to volume ratio of interdigitated nanobiosensors improves their electrical

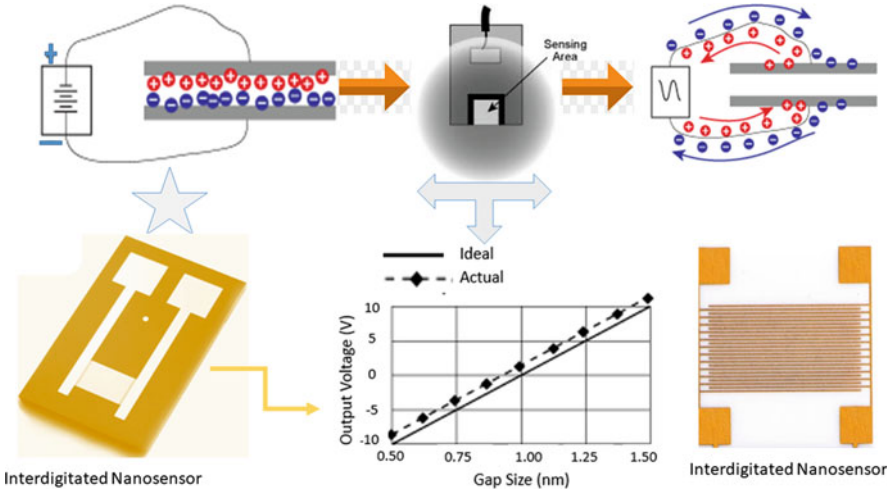

Fig. 8.3 Interdigitated nanobiosensor: Interdigitated nanobiosensors typically consists of two conducting electrodes separated by an insulating gap/layer of nanoscale dimension. These sensors primarily rely on the regulation of conductance or capacitance upon the introduction of biological species

sensitivity. Also, interdigitated nanobiosensors have been used in a variety of applications, including biomolecular sensing.

8.3 Biorecognition Elements

In biosensors, the biorecognition elements are immobilised on the transducer surface so that they can selectively interact with analyte molecules and these interactions are then measured (Bhalla et al. 2016). Biorecognition elements are classified as biocatalytic recognition elements (enzymes) and biocomplexity/bioaffinity recognition elements (nucleic acid sequences, antibodies, and aptamers) (Table of Contents/Содержание 2019). Because electrochemical biosensors detect changes in conductivity, potential, resistivity, and current, the signal generated by the interaction of a bioelement with analyte and transmitted to the transducer is crucial in biosensor systems. Due to their catalytic behaviours, a nanomaterial-modified surface sensors are also used to detect analytes, however, they lack the selectivity of biorecognition elements. Biorecognition elements come in a variety of forms. Antibodies and enzymes are two of the most common biorecognition components that occur naturally. Synthetic biorecognition elements, on the other hand, are biologically derived biorecognition elements that achieve analyte specificity through physiological interactions (Denmark et al. 2020). Morales and Halpern developed a decision map for biorecognition elements that may be used to select biorecognition elements during biosensor development (Using and Neural 2019). The following are the most widely utilised biorecognition elements in biosensor design. The substrate selectivity

of enzymes, also known as biocatalysts, may be characterised using a lock-and-key or induced fit model (Bucur et al. 2021). By establishing an antigen–antibody immunological complex, the antibody, a protein-based biorecognition element with a unique 3D recognition pattern on its Fabarm, may recognise a specific analyte (Mollarasouli et al. 2019). The immunological complex of antibodies and antigens can be detected using an electrochemical, optical, or piezoelectric sensor. Recombinant antibodies, monoclonal antibodies, and polyclonal antibodies are used to enhance the sensitivity of biosensors (Sharma et al. 2016). An artificial oligonucleotide known as an aptamer is created by exponentially increasing ligands. It can detect a wide range of target analytes, such as ions, proteins, drug compounds, and cells (Sharma et al. 2016). Aptamers are chosen over antibodies in biosensors because they are exceedingly selective and unaffected by changes in temperature, pH, and ionic strength (Yoo et al. 2020). Aptamers are single-stranded oligonucleotides that fold into specialised shapes and bind to specific targets such as proteins. They often alter binding proteins' protein–protein interactions, which can have both positive and negative consequences. Biosensors detect and monitor a variety of analytes as well as cell physiology and mechanics using cells and tissues (Ning et al. 2020). Using bacteriophages and protein-based phage displays to detect analytes based on protein–protein interactions has lately gained popularity. Non-catalytic transmembrane or soluble protein receptors are biorecognition elements that bind to particular molecules with excellent specificity and affinity (D'Agata et al. 2017). Synthetic polymers known as molecularly imprinted polymers have a target-specific binding site. Using a molecular template, the molecularly imprinted polymers form a cavity into which the analyte may be collected. At present, molecularly imprinted polymers are only used to create small compounds (Huertas et al. 2019). The transducer of a biosensor translates a biorecognition event into a quantifiable signal. A device that detects changes in phase, polarisation, or frequency in the optical field of a biorecognition element as a result of analyte interaction is known as an optical biosensor (Ligler and Taitt 2002). Adsorption-based biosensors may detect a change in amplitude when light strikes a bare sensing surface with analytes present. The analyte concentration in a solution may be estimated using this amplitude variation (Scholz 2015). This amplitude variation may be utilised to calculate the concentration of an analyte in a solution. This type of biosensor is referred to as a colorimetric biosensor since it operates using visible light. In low-resource situations, unaided optical detection colorimetric biosensors (typically labelled) are used instead of an apparatus to detect analytes (Dyussembayev et al. 2021). For example, Mahato et al. created a colorimetric optical biosensor that detects the analyte alkaline phosphatase in milk samples by using a colour reagent to catalyse the change in colour of the sample solution (Banoub 2014). They monitored the variations in the solution's alkaline phosphate content whilst studying the colour changes using a smartphone. They discovered that the analyte may be detected using the RED colour code in the image RGB profile. They created a paper-based assay for detecting alkaline phosphatase in milk based on the plot they formed (Ratajczak and Stobiecka 2020). For the purpose of drug development and diagnostics, a variety of analytes, such as inflammatory mediators, neurotransmitters, small metabolites, enzymes, and

hormones, require prompt and precise detection (Heaster et al. 2021). Fluorescence-based biosensors provide sensitive, fast, and highly selective detection. A fluorophore-tagged aptamer-based biosensor for thrombin detection was proposed by Furukawa et al. Fluorescence was formed as a result of the interaction between the aptamer and the thrombin (Mcconnell et al. 2020). There are a number of biorecognition elements, both natural and man-made. Naturally occurring biorecognition components include antibodies and enzymes. These biologically generated structures provide analyte specificity through spontaneous physiological interactions.

8.4 Nanomaterials in Biosensors

Nanomaterials are used in the development of biosensors to allow charged ions to flow rapidly between the target and electrode. Nanomaterials also improve the electro-catalytic functions, analyte diffusion rate, analyte pre-concentration on the electrode surface, and anti-fouling properties of biosensors (Huang et al. 2021). Nanomaterials with a variety of sizes, shapes, properties, and architectures, utilised for a variety of applications (Chavali and Nikolova 2019). Due to their optical, electromagnetic, and electrochemical capabilities, metallic nanoparticles are used in the development of biosensors (Hu et al. 2020). Gold nanoparticles are integrated with other nanomaterials, antibodies, or aptamers to create bioaffinity-based biosensors. Metal oxide nanoparticles derived from Ce, Zn, Mn, and Fe are employed in a variety of processes, including catalysis, adsorption, and biocompatibility (Shaba et al. 2021). Carbon-based nanomaterials are employed in biosensing applications because of their conductivity, catalytic activity, and biocompatibility. Compared to carbon-based nanomaterials, metallic nanoparticles are utilised in biosensing applications less frequently. Carbon-based nanomaterials in one, two, and three dimensions have been used in the creation of optical and electrochemical biosensors (Yang et al. 2019). Due to its outstanding electrical characteristics and simple functionalisation, graphene is another nanomaterial employed in biosensing applications (Lee et al. 2019). Quantum dots, luminescent semiconducting nanocrystals, are another type of nanomaterial utilised in optical and electrochemical biosensors for photo-electrochemical behaviour (Holzinger et al. 2014). Because of their tremendous functionalisation potential and low toxicity, polymers are employed in biosensing applications. Additionally, they are flexible, inexpensive, and biocompatible nanomaterials (Gómez et al. 2021). Despite being organic, conducting polymers are electrically conductive and are extensively employed in electrochemical biosensors. Due to its distinct characteristics and high molar extinction, gold nanorods are utilised in biosensor applications (Namsheer and Rout 2021). When combined with aptasensors, gold nanoparticles can be used in methods such as colorimetry, fluorometry, electrochemistry, and electrophoresis. Surface-enhanced Raman scattering, surface plasmon resonance, and calorimetric measurements are all possible using gold nanoparticles (Amina and Guo 2020). To enhance signal quality, gold nanoparticles are utilised instead of functionalised aptamers as an amplifier.

Also, due to their high conductivity and substantial surface area per mass, they improve selectivity and sensitivity (Mao et al. 2020). Through layer-by-layer construction or self-assembly using thiolate chemical bonding, an aptamer can be immobilised on a sensor (Oberhaus et al. 2020). Additionally, zinc oxide (ZnO) nanoparticles have made great progress in recent advances in nanoscience and nanotechnology due to their numerous special features and adaptability (Sharma 2018). ZnO has received a lot of attention as a remarkable and practical material due to its features like high specific surface area, electrochemical activities, biocompatibility, chemical and photochemical stability, non-toxicity, high-electron communicating features, and ease of synthesis (Chaudhary et al. 2018). ZnO nanoparticles have been employed in a variety of applications, including electronics, optoelectronics and sensing, biomedical, and environmental, due to their unique properties.

8.5 Nanomaterials Surface Engineering and Functionalisation

Surface engineering is a subfield of material science and engineering that focuses on improving the surfaces of solids for a variety of applications (Darband et al. 2020). Enhancing the surface's electrical and thermal properties is the primary goal of surface engineering for nanomaterials (for more details, see Chaps. 1 and 2). Furthermore, surface engineering could improve the compatibility of nanomaterials with some matrices (Rajak et al. 2019). The EU Commission and the US Food and Drug Administration define nanoparticles as substances with feature sizes and dimensions between 1 and 100 nm. The British Standards Institution, on the other hand, describes nanoparticles as having feature sizes between 1 and 1000 nm (Jeevanandam et al. 2018). Nanomaterials are used in many scientific fields, including drug transport, treatments, photothermal therapy, and biosensing applications (Yetisgin et al. 2020). As it directly impacts the integrity of the sensing layers, the nano-surface engineering of the sensing probe is essential. Biorecognition elements are immobilised on the detecting surface of nanobiosensors in sensing probes with a nano-biointerface layer, where they interact with analyte molecules to generate a signal (Patra et al. 2018). The biorecognition elements must be uniformly and tightly attached to the nanomaterial surface in order to interact with the analyte and generate a homogeneous signal (Peltomaa et al. 2018). A certain number of biomolecules per nanoparticle attachment on the surfaces of the nanomaterials is maintained by the exact alignment of the biorecognition elements on the nanomaterials and a suitable functionalisation method for nanoparticles. To accomplish these goals, biosensors must have surface engineering (Lee et al. 2019). Covalent and non-covalent attachments are the two types of attachments that can take place between nanomaterials and biorecognition components (Fig. 8.4). Proteins can attach to the surface of nanomaterials by interactions such as electrostatic, hydrophobic, van der Waals, and hydrogen bonding. On the other hand, these interactions are influenced by variations in ionic strength, temperature, pH, and surface properties (Poncin-Epaillard et al. 2012). The levels of adhesion for covalent and non-covalent bonding are different. When gold nanoparticles interact with thiol-containing compounds like

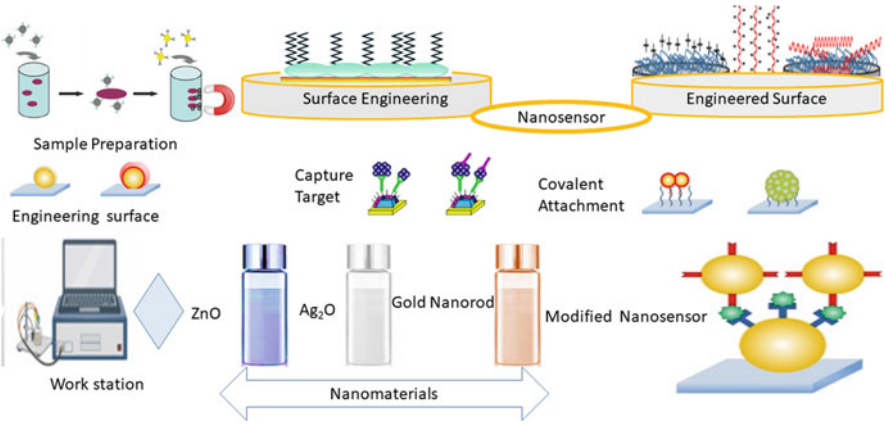

Fig. 8.4 Surface engineering of nanobiosensor: To increase the sensitivity of the gadget, the electrodes can be coated with nanoparticles, which are incredibly conductive. Electron transport is improved by increased surface area and conductivity

11-mercaptoundecanoic acid, a monolayer layer of self-assembly occurs (Guerrini et al. 2018). Methods of covalent attachment are often used in research. A water-soluble carbodiimide process may be used to covalently bind a number of extracellular matrices-derived components. For instance, Chou et al. demonstrated that 1-ethyl-3 carbodiimide could be immobilised onto a chondroitin-6-sulphate scaffold by acting as a crosslinking agent (Zhou et al. 2019). The difference between covalent and non-covalent attachments is, when two atoms share their electrons, this is known as a covalent attachment. Non-covalent attachment is formed when two atoms fully exchange electrons or when no electrons are exchanged at all between two atoms (Wickramathilaka and Tao 2019). The main advantage of this approach is that the reaction may occur even in physiological pH water, resulting in a strong covalent bond. One of the strongest covalent bonds is biotin-avidin, which is used in affinity-based biosensing (Oh et al. 2021). The surface of the nanoparticle can be coated with streptavidin molecules, which are subsequently treated with a biotin-conjugated affinity probe to detect the analyte (Lakshmipriya et al. 2016). Salinisation is another method for attaching biomolecules to an ahydroxyl surface, it uses silane molecules like APTES to bind biomolecules to an ahydroxyl surface. Using surface engineering, physical, electrical, chemical, electronic, mechanical, magnetic, wear-resistant, and corrosion-resistant capabilities may all be produced at the appropriate substrate surfaces (Hu et al. 2020). The ability to control interactions between nanoparticles and biosystems is critical for the optimal use of these materials in bioscience. For imaging, sensing, and delivery purposes, a wide range of nanoparticle surface structures have been produced. Surface functionalisation is a simple and efficient method of changing the surface properties of a material to achieve particular goals such as producing a desirable bioresponse or blocking a potentially dangerous reaction.

8.6 Characterisation of Nanomaterials and Probe

The advancement of biosensors as analytical devices has been profoundly impacted by the recent nanotechnology revolution (Banerjee et al. 2021). The development of advanced techniques, particularly X-ray, electron microscopy, and spectroscopy-based approaches, have resulted in extensive characterisation of the NPs immobilisation procedure (Zhang et al. 2016). Researchers face challenges when characterising nanomaterials due to differences in sample preparation, a lack of universal code for data processing, a lack of adequate reference material for the calibration of analytical tools, and the exploration of nanoparticles in complex matrices (Socas-Rodríguez et al. 2020). As a result, it is critical to describe nanoparticles in as many ways as possible in order to obtain reliable information on their shape, size, surface ligand, growth kinetics, and interactions with the surrounding materials (Mourdikoudis et al. 2018). All of these variables have a significant impact on its behaviour of the electrode and subsequent biosensing activity (Eissa et al. 2020). Ultraviolet-Visible Spectroscopy, similar to FTIR, is a method that is useful in the identification of pure drug compounds. It is typically the first characterisation technique used to monitor nanoparticle development by detecting the characteristic absorption bands. Many molecules contain chromophores, which absorb ultraviolet or visible light at particular wavelengths (Ang et al. 2015). A well-dispersed nanomaterial in a solution forms a distinct absorption band that may be used to evaluate the stability of nanoparticles in colloidal solutions based on concentration, agglomeration state, particle size, and refractive index (Eissa et al. 2020). Electron microscopes provide atomic-level resolution and are used to analyse the size and shape of nanomaterials in higher detail. An electron beam is passed over the surface of nanoparticles in a scanning electron microscope, and the scattered electrons are then used to generate a three-dimensional image of the surface properties (Mahmood et al. 2017). Despite its inability to reach an atomic-scale resolution, scanning electron microscopes are effective for analysing larger surface regions and bulk materials such as thin films. Because it employs transmitted electron beams to pass through thin materials and analyse the difference between the sample and the surroundings to acquire material information, transmission electron microscopy has a greater resolution (less than 101 nm) than scanning electron microscopy (McCarron and Chambers 2021). To evaluate the nanoparticle crystal structure, interplanar spacing, and other lattice parameters, selected area electron diffraction is employed in combination with transmission electron microscopy. X-ray techniques are frequently employed in conjunction with this diffraction-based technique (Mourdikoudis et al. 2018). X-ray powder diffraction is often used to offer information on the crystalline structure, phase nature, lattice parameters, and crystalline grain size to explain the optical and electrical behaviour of nanoparticles (Giannini et al. 2016). A scanning technology known as atomic force microscopy is utilised to generate a high magnification real topographical image of the surface of the nanoparticles (Mahmood et al. 2017). Atomic force microscopy is used to analyse surface properties such as nanoscale frictional force, surface hardness, surface charge distribution, and surface

magnetisation of nanoparticles (Wang and Wang 2018). It has 0.2 nm horizontal resolution and 0.1 nm vertical resolution. AFM imaging does not damage the materials and does not require any coating. However, a covering closely packed nanoparticles and a broken cantilever may create issues during the atomic force microscopy analysis. To monitor the surface functionalisation of nanomaterials as well as probe fabrication, a variety of characterisation techniques such as Fourier transform infrared spectroscopy, Raman spectroscopy, energy-dispersive X-ray spectroscopy, elemental mapping, and X-Ray photoemission spectroscopy are used (Mourdikoudis et al. 2018). Fourier transform infrared spectroscopy on a biochemically modified probe is used to analyse the type and strength of various bonds and functional groups, with information provided by a spectrum in the mid-infrared range of 4000–400 cm^{-1}. Therefore, one of the most important characterisation methods for structural study is Fourier transform infrared spectroscopy (Kowalczuk and Pitucha 2019).

8.7 Analytical Studies of Nanobiosensor

Biosensors are assessed using a common set of analytical performance criteria after probe fabrication (Artigues et al. 2017). A preliminary test is performed on the sensor surface to determine its sensitivity and selectivity for the target analyte. A regression plot is created based on the signal output at different analyte molecule concentrations and used as a reference for additional sensing applications (Merkoçi and Álvarez 2020). The sensitivity of a biosensor is shown by the slope of the regression line, which denotes a change in the output signal in response to a change in analyte concentration (Majer and Finšgar 2021). More sensitive biosensors can detect even little changes in analyte concentration in a solution (Bhalla et al. 2016). The limit of detection (LOD) of a biosensing system is the smallest quantity of analyte required to produce a detectable signal (Purohit et al. 2020). The smallest amount of a target analyte that a biosensor can detect is known as the limit of quantitation (LOQ). Specificity and selectivity are frequently used interchangeably. Biosensors recognise only one target analyte, which is ideal in any complex solution (Mehrotra 2016). The standard addition technique is used to create a calibration plot by adding a known quantity of analyte to the sensing solution. The concentration of the analyte may be determined after treating the unknown sample with the sensor by tracing the points on the standard plot to zero values. The technique of standard additions is established using an analytical instrument. The method is a technique for analysing a species quantitatively without the need of a calibration curve. In standard addition analysis, the spectroscopic intensity before and after adding precise aliquots of a known analyte standard solution is compared.

8.8 Probe Development for Biosensing

In order to provide the best analytical results for a variety of physical parameters, such as pH, ionic strength, and temperature, a biosensing probe is formed (Nguyen et al. 2019). The stages involved in developing a sensing surface were developed (Andersson 2017). The type of transducer, such as glassy carbon electrode, indium tin oxide, or paper-like matrix, is chosen for the sensing matrix. Based on the type of analyte, an antibody, enzyme, or aptamer is chosen and immobilised on the sensing surfaces in order to maintain peak performance. The sensing signal must be increased using a variety of techniques in order to detect very small amounts of analyte (Pirzada and Altintas 2020). Nanoparticles are employed in voltammetry-based biosensors to boost sensor conductivity and catalytic activity, which increases image intensity (Naresh and Lee 2021). Another technique is to use enzyme-tagged secondary antibodies to catalyse a second reaction, which will boost the signal and make analysis easier (Homaei et al. 2013). It should be emphasised that although this enzyme enhances the signal, it has no direct effect on the analyte detection process (Nguyen et al. 2019). The signal is enhanced by the use of a multi-enzyme cascade strategy. Several researchers have used a stimulant or irritant to directly extract a sizable quantity of analyte from human tissue, which was thereafter constantly monitored by the sensor (Loos et al. 2016). The result is a concentration-dependent plot that may be used as a guide for further research. The next step included determining how concurrent substances affected signal production. If the sensor matrix has a negative impact on signal production, anti-fouling surfaces or technologies are employed to decrease signal reduction.

8.9 Performance of Nanobiosensor at Physiological pH

In order to comprehend the mechanism of electrolysis, measurements of various pHs of a sample were made by measuring the potential of the sample in relation to the standard hydrogen. This process gives a value of zero for a 1-M solution of 'H' ions, giving the pH scales' initial value. The Nernst equation can be used to determine the cell potential for any other values with 'H' ion concentration:

$$E = E\mathrm{o} - \frac{RT}{nF} \ln Q \tag{8.1}$$

where 'n' is the number of moles of electrons transferred in the cell reaction or half-reaction, 'E' is the cell potential, 'Eo' is the standard cell potential at the temperature of interest, 'R' is the universal gas constant, 'T' is the absolute temperature, 'F' is the Faraday constant, and 'Q' is the reaction quotient. The relative amounts of products and reactants present during a reaction at a specific time are measured by the reaction quotient (Q). Distinct pH solutions can be used to test nanosensors at physiological pH values. Since each solution has a different level of ions, the abundance of each electrolyte in the solution causes changes in current dependent on the mechanism of

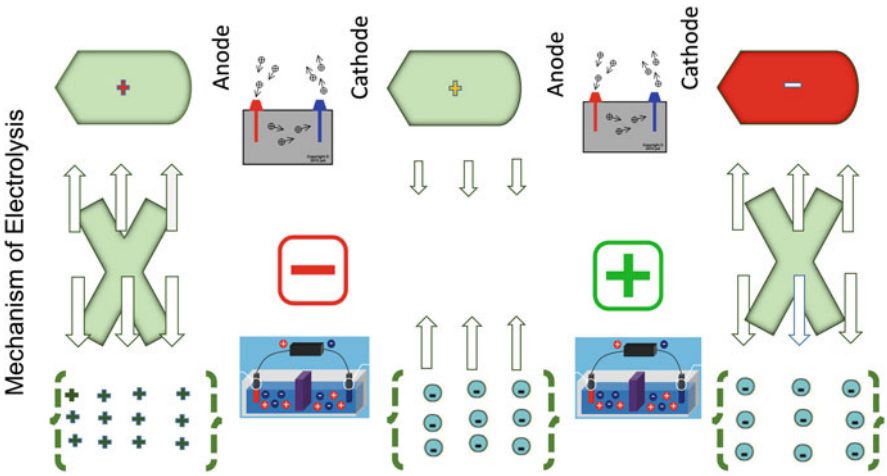

Fig. 8.5 Mechanism of electrolysis: In order to comprehend the mechanism of electrolysis, measurements on various pHs of a sample were made by measuring the potential of the sample in relation to the standard hydrogen. The pH is a measurement of the molar concentration of hydrogen ions

ion transfer (Fig. 8.5). The purpose of testing the nanosensor at pH is to determine the stability of the sensor at physiological pH (Saini et al. 2017).

8.10 Nanobiosensor for Biomarkers Detection

Nanobiosensors have been the subject of numerous investigations, and some scientists have shown that they may be used to measure material qualities at the nanoscale. Electrical detection systems provide a wide range of possibilities for the development of biomolecular detection sensors by utilising the unique electrical characteristics of nanoscale objects (Reinhardt 1981). For an accurate diagnosis and reliable findings, sensitive detection, and quantification of disease-associated biomolecules (protein and nucleic acid), in tissues and biological fluids is crucial (Kaminski et al. 2021). For example, early detection of neurodegenerative Parkinson's disease necessitates the discovery of biomarkers to prevent irreversible damages that may be caused by the disease (Kelley et al. 2014) (Fig. 8.6). Furthermore, an electrochemically generated highly exfoliated nitrogen-doped graphene was used to investigate Parkinson's disease biomarkers, this method improves the catalytic activity of poly nanocomposite electrodes for dopamine detection (Salahandish et al. 2019). Poly (anilineboronic acid) nanocomposites were electrodeposited on the surface of a glassy carbon electrode using cyclic voltammetry and in situ electrochemical polymerisation of anilineboronic acid monomers to create the sensing technique (Keteklahijani et al. 2019). They coated the electrode surface with nitrogen-doped graphene and carbon nanotubes with

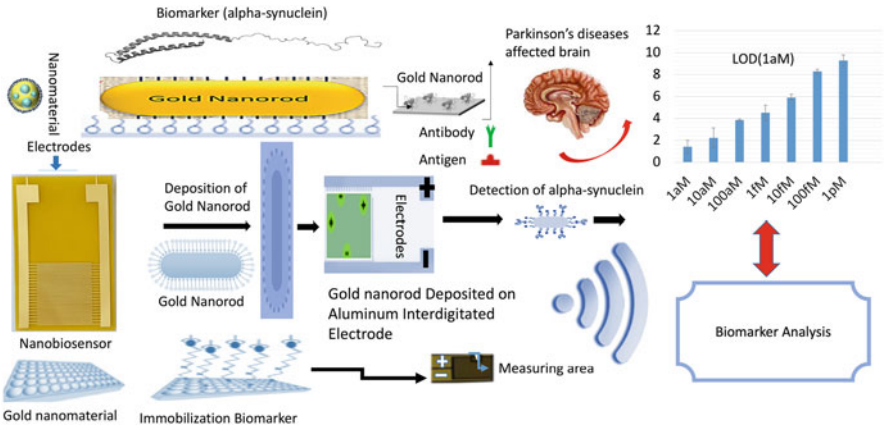

Fig. 8.6 Biomarker Identification: Nanobiosensors with surface engineering are utilised to detect a variety of biomarkers for disease diagnosis. Biomarker identification for early diagnosis of neurodegenerative Parkinson's disease, for example is critical before irreparable damage occurs. Nanobiosensors with surface engineering provide a stable sensing surface that could enhance the limit of detection (Adam et al. 2021)

ADNA functionalisation prior to electropolymerisation (Wang et al. 2020). This molecular anchoring significantly increases the density of boronic acid receptors for dopamine binding to detect Parkinson's disease, which promotes electrodeposition of the relevant nanocomposites on the electrode. They obtained a detection limit of 14 nM as well as a detection limit in a broad linear range of 0.02–1 M, which is a significant improvement for dopamine detection and opens the door to molecular diagnosis of neurological disorders such as Parkinson's disease. An ion channel mimetic self-assembled monolayer of macrocyclic polyamines was another modification used. It was connected to a gold electrode to be electrochemically identified as dopamine (Ribeiro et al. 2016). When dopamine was added, a corrole–dopamine complex formed on the monolayer, producing positive charges on the electrode and lowering the detection limit to the picomolar level. Differential pulse voltammetry was used to detect dopamine with urate and ascorbic acid in 3D zinc oxide nanowire arrays made of graphene (Wang et al. 2017b). The addition of nanowire to the system could offer a better electrical conductivity and a sensitivity to the electrochemical biosensors, which will make the detection limit to be decreased to one nanomolar for dopamine and uric acid in the serum of patients suffering from Parkinson's disease. The testing of this array leads to a discovery of a decreased level of uric acid in patients suffering from Parkinson's disease and this will eventually make uric acid a potential biomarker for the detection of Parkinson's disease. Zinc oxide nanowire arrays in 3D were also studied which comprised of graphene and their assembly might individually identify dopamine alongside urate and ascorbic acid by differential pulse voltammetry (Yue et al. 2014). The addition of nanowires to the system could offer a better electrical conductivity and a

sensitivity to the electrochemical biosensors, which will make the detection limit to be decreased to one nanomolar for dopamine and uric acid in the serum of patients suffering from Parkinson's disease. The testing of this array leads to a discovery of a decreased level of uric acid in patients suffering from Parkinson's disease and this will eventually make uric acid as a potential biomarker for the detection of Parkinson's disease. Another method of detecting Parkinson's disseise was by using gold nanoparticles. Due to their exceptional chemical stability, electrocatalytic capabilities, and sizable active surface areas, gold nanoparticles improve electrical signals (Cho et al. 2020b). The traditional ways to add gold nanoparticles to an electrode surface are as follows: (i) immobilisation of gold nanoparticles using chemical linkers, (ii) attachment of gold nanoparticles using electrical force, and (iii) direct gold nanoparticle formation via chemical/electrochemical reactions using a precursor solution containing gold chloride. These techniques have been used to control and modify the size, shape, aspect ratio, and density of gold nanoparticles, which are all significant properties. According to a recent study, a homogeneous cylindrical gold nanoelectrode (CAuNE)-modified platform is useful for measuring dopamine for the electrochemical diagnosis of Parkinson's disease (Kim et al. 2018). An aptamer that has been attached to gold nanoparticles was employed to increase the detection limit in order to identify the aberrant aggregation of alpha synuclein. The binding between alpha synuclein and aptamer was discovered to have a 10-pM limit of detection. On the basis of another investigation using methylene blue (MB)-tagged aptamers (Apt). The sensor makes use of an aptamer (Apt) that has been methylene blue (MB)-tagged and electrochemically reduced graphene oxide (ERGO) adsorbed on it (Jang et al. 2020). The detection limit was determined to be e 0.64 fM. The binding of alpha synuclein oligomer to the Apt triggers desorption of the Apt from the ERGO surface. Also, an immunosensor modified with ultrasensitive poly (D-glucosamine), gold nanoparticles, multi-walled carbon nanotubes, and reduced graphene oxide (PDG/AuNPs/MWCNTs/rGO) was proposed for the detection of alpha synuclein oligomer (−syno), with an estimated detection limit of 0.03 fM (Zoey et al. 2021). A DNA aptamer was used as the bioreceptor in a different study that also reported the label-free detection of alpha synuclein oligomer using the techniques of a colorimetric assay based on gold nanoparticles (AuNPs) (Sun et al. 2017). Additionally, using a fluorescent probe (MFC) examined the impact of citrate-capped gold nanoparticles on the aggregation kinetics of alpha synuclein. The detection limit was estimated to be 20 nM (D'Onofrio et al. 2020). Due to the existing difficulties in detecting early-stage Parkinson's disease, there is an urgent need to identify a specific biomarker using available biological materials that may accurately diagnose Parkinson's disease early and objectively monitor disease severity. Hence, biomarker analyses were enhanced by the formation of a powerful, highly affine non-covalent binding between alpha synuclein and anti-alpha synuclein antibody. A biosensor with a better limit of detection of 1 aM and a stable sensing surface was also developed to distinguish between aggregated and non-aggregated protein alpha synuclein in Parkinson's disease (Adam et al. 2021). The proposed method exhibited a satisfactory detection limit that may be utilised to mimic real biological and clinical fluids. In a recent research employing nanobiosensors, alpha synuclein was shown to be a viable biomarker for Parkinson's

disease since its detection limits are significantly lower than those of other biomarkers observed, such as uric acid.

8.11 Conclusion and Future Prospects

Nanobiosensors, which range in size from 1 to 100 nanometres, have piqued the interest of many researchers. The growing interest in nanobiosensors and their innovative applications for many applications has accelerated the development of nanodevices. Nanobiosensors are improving in terms of reliability and faster response. Nanobiosensors can detect even the smallest signal and are easy to make conventionally. Nanobiosensors are cost-effective and easy to miniaturise. Because of their advantages, they are useful in the development of present and future micro and nano-electronic components for devices and systems. Extensive research into nanobiosensors may lead to the creation of more complex nano-based systems or devices with higher sensitivity. Biosensors are bioanalytical tools that are used to quantitatively detect sensitive analytes. The research area has gone a long way from the early days of Clark electrodes to highly emerging biosensors. Optical, mechanical, and electrochemical nanobiosensors are the three main categories. Furthermore, new technologies are being widely implemented in order to improve the efficiency and affordability of nanobiosensors for everyday applications. Most of these biosensors are designed to detect incredibly tiny quantities of analyte in a complex biological matrix whilst requiring minimal sample and generating less fouling. The biosensor is kept stable and functioning for a long period using a variety of techniques. Nanomaterials and surface chemistry, for example are often used to successfully bind biorecognition components and build an anti-fouling surface, resulting in increased sensitivity and stability. Biosensors must thus be robust and effective at physiological pH, room temperature, or body temperature, as well as under external conditions. Nanomaterials improve effective surface area and signal intensity but not signal-to-noise ratio, which must be addressed for improved biosensor development. If the catalysis centre is incorporated in a 3D structure or if a co-factor is needed for the catalysis, new enzyme-based techniques should be used to create enzymatic biosensors. For the large-scale manufacturing of biosensors, anti-fouling, low-cost surfaces must be prioritised without compromising sensitivity.

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Peptide-Based Electrochemical Nanobiosensors for Clinical Diagnosis

9

Buddhadev Purohit and Winnie Edith Svendsen

Abstract

The selectivity of a biosensor toward its target analyte is highly dependent on the biorecognition element used in the sensing matrix. A carefully designed peptide can be an alternative to an antibody, with major advantages such as more tolerance toward environmental conditions, tunable sequence to detect a wide variety of targets, and cost-effective solid-phase synthesis suitable for large-scale sensor production. Especially the electrochemical peptide-based biosensors have generated a lot of interest due to their sensitivity, selectivity, and quick response time for clinical diagnosis. The possibility of miniaturization of electrochemical devices also adds to its popularity for on-site diagnosis. In this chapter, we discuss the role of peptides as a biorecognition element in recently developed sensors for clinical diagnosis. The use of nanomaterials in sensor matrix development, surface engineering strategies for peptide immobilization and antifouling effect, signal amplification strategies, and the long-term stability of the developed sensors is critically assessed.

Keywords

Point-of-care · Biomarker detection · Antibiofouling surface · Peptide design · Bioelectrochemistry · Nanomaterials · Peptide immobilization methods

B. Purohit (✉) · W. E. Svendsen
DTU Bioengineering, Technical University of Denmark, Kongens Lyngby, Denmark
e-mail: budpu@dtu.dk; wisv@dtu.dk

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Abbreviations

AgNP	Silver nanoparticle
AuNP	Gold nanoparticles
BREs	Biorecognition element
CA	Chronoamperometry
CA-125	Carcinoembryonic antigen 125
CV	Cyclic voltammetry
DPV	Differential pulse voltammetry
EIS	Electrochemical impedance spectroscopy
FBS	Fetal bovine serum
LDR	Linear dynamic range
LOD	Limit of detection
LSV	Linear sweep voltammetry
MMPs	Matrix metalloproteinases
PSA	Prostate-specific antigen
SPPS	Solid-phase peptide synthesis
SWV	Square wave voltammetry
β-CD	β-cyclodextrin

Amino Acid Code

A	Alanine
R	Arginine
N	Asparagine
D	Aspartic acid
C	Cysteine
Q	Glutamine
E	Glutamic acid
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
M	Methionine
F	Phenylalanine
P	Proline
S	Serine
T	Threonine
W	Tryptophan
Y	Tyrosine
V	Valine

9.1 Introduction

According to IUPAC, biosensors are defined as "A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" (Nagel et al. 1992). From the development of the first biosensor by Leland C. Clark, Jr., in 1956 to the present time, the design of biosensors has changed drastically, and new developments in different areas of science have been incorporated into biosensor making them even more sensitive and applicable for daily usage (Bhalla et al. 2016; Chandra and Prakash 2020; Hammond et al. 2016). The advancement in nanomaterial synthesis and characterization technologies in 2000–2010 fueled the meteoric rise of biosensors as an analytical device achieving it the status of a mainstream bioanalytical system for clinical diagnosis. No matter how advanced a biosensor is, it is an integration of three important components, i.e., biorecognition element (BRE) for selective recognition of target species, a transducer to convert this biorecognition event to a measurable signal, and a processor for analyzing and displaying the signal (Mahato et al. 2016; Purohit et al. 2020). One major class of biosensor is electrochemical biosensors, which offers robust analysis of biomarkers in various biological fluids for clinical applications. Due to the possibilities of miniaturization, low cost, ease of fabrication, and fast detection, electrochemical sensors have been utilized in the detection of many disease-related biomarkers. Micro-nanofabrication of the electrodes offers a very-small-sized sensor which can be functionalized easily to develop immunoassays for clinical diagnostics. The signal generation and amplification in the electrochemical biosensors are designed based on its modes of operation, such as amperometry, voltammetry, or potentiometry, and also depend on the use of signal label or label-free module (Akhtar et al. 2018; Kumar et al. 2019). By virtue of the advancement of synthesis and characterization of nanomaterials and surface engineering technologies, electrochemical biosensors can now be used for the detection of many important biomarkers. However, most of the electrochemical biosensors developed and reported are restricted to applications in laboratory environments, and very few have succeeded in field applications. The use of antibody or enzymes in the biosensor as BREs limits their application to standard laboratory condition only. The developed biosensor should be cost-effective, rapid, sensitive, and most importantly nonresponsive toward environmental parameters, leading to a lesser expenditure required for the storage and operation of the sensors. Using peptide as recognition molecule in the electrochemical sensor matrix can be a promising solution to these problems.

Peptides and antibodies share the same building block of amino acids, and a specific sequence of peptides can be used as an alternative to the selective binding of antibodies to a target. Peptides in their secondary structure can be used as a recognition molecule for a target due to their ability to form various noncovalent bonds (Chou et al. 2019). Also, peptides possess high stability against denaturation and environmental parameters making them more suitable for sensor applications than antibodies. The peptides can also be cost-effectively synthesized using standard

synthetic protocol, ease of chemical modifications, and can be evolved for better binding affinity to a target species. Also, peptides with similar amino acid sequence to a specific enzyme substrate can be used in the detection of enzymes and used in enzyme inhibition study (Karimzadeh et al. 2018). Parameters such as hydrophobic/hydrophilic nature, isoelectric point, storage condition, and solubility in different buffers are important aspects for the design of a peptide for biosensing (Wink et al. 1997). The biggest advantage of using the peptide-based screening is the use of solid-phase peptide synthesis (SPPS), from which a specific sequence or a library of peptides can be developed against the target, and can be mass manufactured, faster in comparison to developing a new antibody. The developed peptides by solid-phase synthesis have been used for the detection of metal ions, proteins, and DNA. The selection, design, and synthesis of specific peptide sequence is an important aspect for the efficient binding to the target molecule (Del Carlo et al. 2016; Gladich et al. 2015; Guida et al. 2018). The peptide sequence used in electrochemical biosensor usually contains a thiol-containing moiety to bind the Au surface of the electrode, a spacer sequence to maintain the flexibility or accessibility of the enzyme activity, and a molecular recognition ligand. The naturally occurring and synthetic amino acid building blocks can also be used for the mass production of the sequences using various chemical procedures with very high precision. These modified peptides can resemble the naturally occurring bioreceptors for the target analyte or can be artificially evolved with higher sensing parameters by screening large combinatorial libraries using in vitro display techniques. The detection principle of phage display was further incorporated to advance this area in different technologies such as mRNA display, ribosome display, bacteria display, and yeast display (Sfragano et al. 2021; Smith 1985). Once the peptide sequence is deciphered, various chemical synthesis procedures can be used to produce the peptide by solution-based or solid-phase SPPS compatible with batch and continuous-flow reactors (Palomo 2014). SPPS is a commonly used method for synthesis of peptide for both naturally occurring and semisynthetic nonnatural analogues. The SPPS follows a simple, rapid, and efficient protocol for higher yield of peptide and has been incorporated with various nanofabrication and sensing techniques (Beavers et al. 2014; Britland et al. 1992; Ngashangva et al. 2014). In silico methods were further used to study the peptide–peptide or peptide–small molecule interactions used in drug design or biosensor applications (D'Annessa et al. 2020). In silico methods are further used to study the kinetics and thermodynamics of peptide ligand–target interactions in its physical and chemical environment (Khayamian et al. 2021). This is followed by the decision of development of an electrochemically active sensor matrix development. The use of specific type of electrodes, nanomaterials, and type of electrochemical measurement is decided based on the nature of peptide–target interactions and optimized to detect the target analyte in a clinically relevant range. Novel signal amplification strategies are designed to attend a low limit of detection (LOD) and a wider linear dynamic range (LDR) for the developed sensor, and different blocking agents/antifouling layers are used to prevent the nonspecific binding of materials in a complex solution.

In this chapter, the recent trends in electrochemical biosensors using synthetic peptides have been summarized. The uses of peptides as BREs based on its affinity toward target molecules and as an enzymatic substrate sequences are described. The immobilization of the peptide sequences on electrode surface has also been highlighted with a reference to the nanomaterials used. Both label and label-free approaches for the detection of clinically important molecules are summarized here, and focus has been given to the integration of redox active molecules.

9.2 Design of Peptide-Based Electrochemical Biosensor

The design of a peptide-based electrochemical sensor depends on the peptide sequence used for biorecognition, the immobilization strategy of the peptide on the electrode surface, the type of electrochemical method used for analyte quantification, and the signal amplification strategy applied for ultrasensitive detection at lower concentrations. Figure 9.1 shows the different stages of a peptide-based biosensor development, where the first stage is the development of a specific peptide ligand. The design of the peptide can be optimized by using different in silico methods to achieve higher sensitivity toward its target analyte (Xiao et al. 2018) as shown in Fig. 9.1a. The peptides can be developed as an affinity-based BRE or as a substrate for the enzymatic action as shown in Fig. 9.1b. In the next step, the sensing matrix is developed using the peptide as BRE. Figure 9.1c shows different layers of developing a peptide-based electrochemical biosensor. A suitable electrode is first chosen based on the type of samples or operating electrochemical potential. Then, usually a layer of nanomaterials is deposited/transferred onto the electrode surface to increase the surface area or catalytic property of the sensor. The nanomaterials can be a metal nanoparticle or a polymer. Then the peptide is attached to the sensor surface through covalent interaction or ligand-receptor interaction (-SH linkage, EDC-NHS, or avidin–biotin interactions), and a blocking layer (MCH, BSA, or ethanolamine) is added to reduce the antifouling effect of the matrix. Then the sensor is interacted with the target analyte, and a signal amplification strategy is introduced to get the suitable sensor signal. Based on the nature of interaction and materials used, a suitable electrochemical technique is used for the detection of target analyte. Figure 9.1d shows the most commonly used electrochemical techniques, i.e., amperometry, potentiometry, voltammetry, and impediometric. The type of electrochemical technique used also depends on the peptide. The peptide itself is not redox active, so the peptide is modified with ferrocene (Fc) or methylene blue (MB) to generate the electrochemical signal (Gerasimov and Lai 2010; Santos et al. 2015), and the redox label modified peptides are usually used in voltammetric- or amperometric-based sensors. Label-free peptide-based biosensors can also be developed by using suitable electrochemical methods such as electrochemical impedance spectroscopy (EIS) (Cui et al. 2017; González-Fernández et al. 2018; Lim et al. 2018).

Peptide immobilization on electrode surface: Peptide can be immobilized on the electrode surface through either noncovalent or covalent interactions. These methods

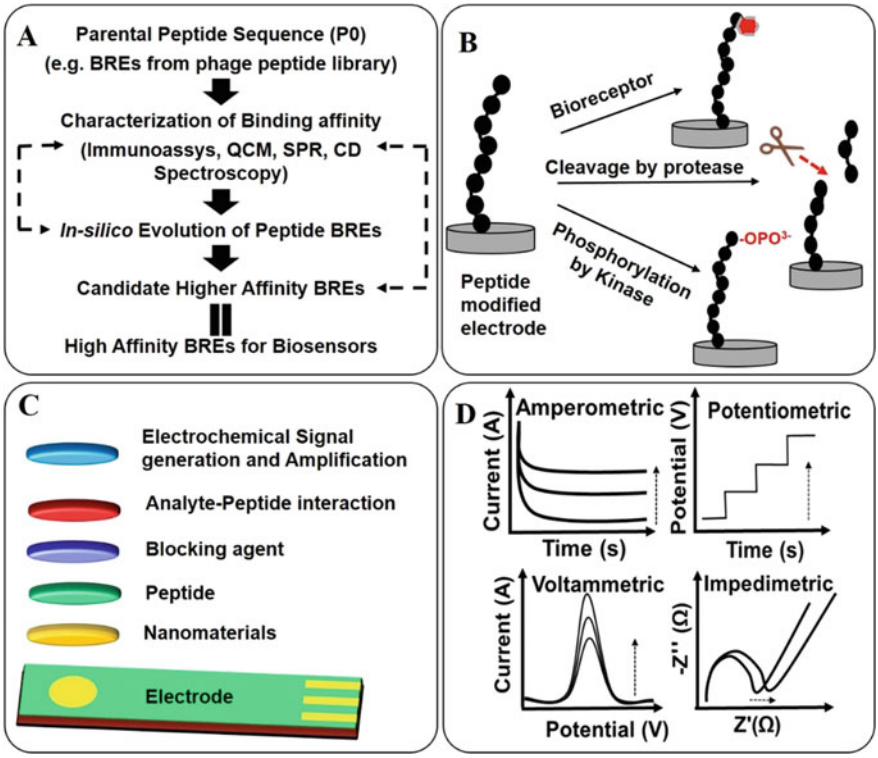

Fig. 9.1 The designing and working mechanism of a peptide-based electrochemical biosensor. (**a**) Schematic for evolving higher affinity peptide BREs using in silico methods (reproduced with permission from Xiao et al. 2018, ACS); (**b**) peptide can be used as a bioreceptor and enzyme substrate in both label-free and labeled electrochemical biosensor; (**c**) the general scheme of developing an electrochemical peptide-based biosensor; and (**d**) common electrochemical technique used for peptide-based biosensing

include electrostatic, direct (e.g., thiolated peptide Au electrode interaction), secondary interactions (e.g., biotin–avidin interactions), covalent attachment through direct ligand interactions (EDC–NHS interaction), or encapsulation method (Sapsford et al. 2013). The self-assembled monolayer (SAM) based on thiol linkage is commonly used for peptide-based sensors, whereas Staderini et al. reported a tripod anchor-based peptide (peptide with three anchoring thiol linkages on Au surface) performing better than a single thiol-linked peptide sensor (Staderini et al. 2018). Peptides can also be self-assembled to highly ordered structures driven by noncovalent intermolecular interactions such as electrostatic, hydrogen bonding, van der Waals, and hydrophobic interactions. Some of these structures can be synthesized under very mild conditions (such as aqueous media and room temperature) and can be functionalized with various nanomaterials for biosensing applications (Castillo-León et al. 2015). Peptide nanofibrils, nanospheres, and nanotubes can be utilized for the immobilization of a larger amount of biomarkers

for biosensing applications (Castillo et al. 2012, 2013; Gazit 2007). Dipeptide diphenylalanine, cyclic peptides, or octapeptides (such as NSGAITIG) can form a highly ordered structure under simple assembling conditions (Lakshmanan et al. 2012; Yan et al. 2010). The peptide nanotubes can be organized on a distinct location via molecular recognition or dielectrophoresis and can be easily modified with multiple recognition elements for multiplexing (de la Rica et al. 2011; Zhao et al. 2005; Zhao and Matsui 2007). The peptide structures are biocompatible and can also be used to stabilize antibodies and enzymes in the biosensors (Puiu and Bala 2018). This shows that peptides not only can act as a BRE molecule but can also be used for the development of sensing matrix.

9.3 Peptide as Affinity-Based Biorecognition Element

The affinity-based peptide biosensors can be developed either as a label-free biosensor or as a labeled biosensor. In the label-free biosensor, the peptide target interaction can be monitored by using a redox active molecule as a signal generator (potassium ferro-/ferricyanide) using electrochemical techniques such as CV, DPV, EIS, etc. The working principle behind affinity-based sensors is illustrated in Fig. 9.2a. The peptide can also be tagged with a signal molecule (ferrocene or methylene blue), and the peptide target interaction can be monitored by the change in signal in the different electrochemical methods as shown in Fig. 9.2b. In redox molecule-tagged sensor, (i) the interaction between peptide and target made the redox center closer to the surface of the electrode increasing the current signal, or (ii) the target analyte hindered the flow of electrons from redox center to the electrode by formation of a peptide–target bulky complex, and thus there is a decrease in signal.

Peptide as a biorecognition element can bind to a wide range of target analytes, from different metal ions, protein biomarkers, nucleic acid, and even cells/bacteria (Viguier et al. 2011). Liu et al. have compiled the recent developments in peptide-based metal ion detections using different biosensing methods (Liu et al. 2015). Different biosensors based on peptides (Domínguez-Renedo et al. 2013), mostly fluorescence and electrochemical biosensors, have been reported to detect Zn (Walkup and Imperiali 1997), Cu (GGDGGDGGDGGDGG) (White and Holcombe 2007), Cd (CPGCW) (Li et al. 2012b), Ag (Kim et al. 2012), Hg (White et al. 2008), etc. The metal-binding peptides are designed by mimicking the natural peptide sequences of metal-binding proteins, and additional amino acids are added to get a more stable structure (Kim et al. 2012; White et al. 2008). The same sequences can be further used to develop electrochemical biosensor as well as to measure the metal ion concentration in complex biological solutions. Thompson et al. reported the development of a 12-mer peptide sequence DKDGDGYITAAE-based sensing of uranyl ion (U(VI), where the sequence was originally from a calcium binding protein calmodulin (Thompson and Lai 2022). Phosphorylation of the ninth amino acid threonine in the peptide resulted in higher affinity for the target, even higher than the native target Ca (II), and the developed sensor was able to detect the ion with a LOD of 50 nM. Small molecules, like glucose, can also be detected by a cyclic peptide-

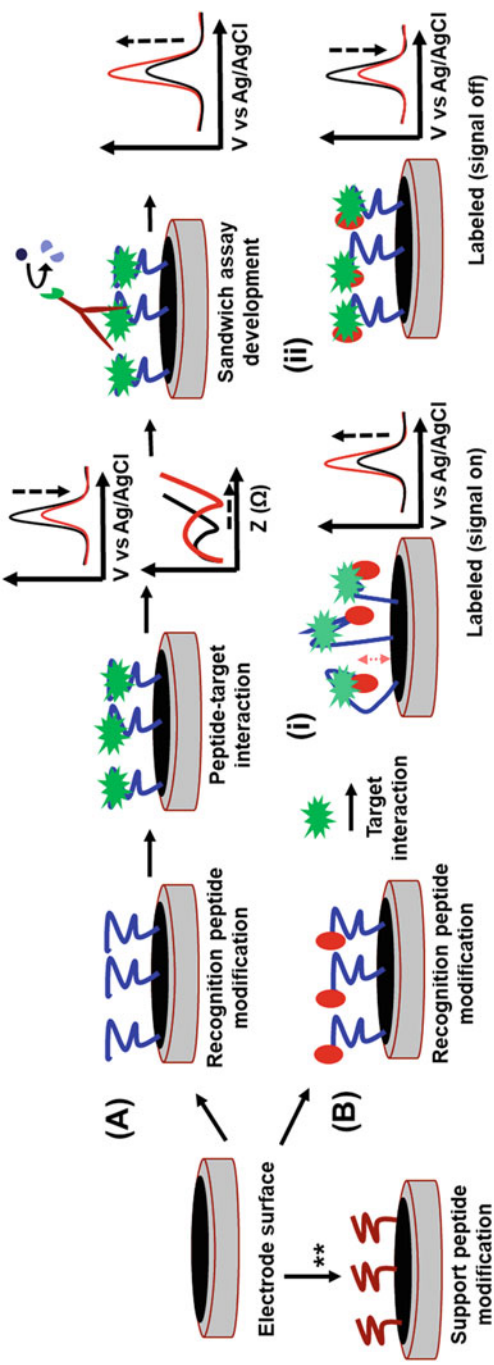

Fig. 9.2 The scheme of development of a peptide electrochemical biosensor where the peptide is used as an affinity-based biorecognition element, (a) without, and (b) with a labeling molecule attached to the peptide, respectively. Based on the nature of interaction of target molecule with labeled peptide, the target can be sensed from (b.i) increase or (b.ii) decrease in signal. The * mark shows that a peptide-modified surface can be used as a general platform for electrochemical biosensor development

based glucose receptor-modified electrochemical sensor (Li et al. 2017). The sequence used was cyclo[-CNDNHCRDNDC-] and can be used to detect glucose in the range of 10 nM to 5 mM using electrochemical impedance spectroscopy. Clinically important macromolecules can also be detected by peptide-based electrochemical sensors. Vancomycin, a last-line antibiotic used for the treatment of severe bacterial infection, can be detected by a dipeptide D-Ala-D-Ala (the dipeptide is found in the bacterial cell wall and binds to vancomycin). The sequence was earlier used for the development of nanomechanical system detection module (Ndieyira et al. 2008), and later on Tan et al. used the peptide for the capture of vancomycin and used it as a template for the development of molecular imprinted polymer development (Tan et al. 2021). Thus, developed impedance sensor can detect 10 pM of vancomycin.

For clinically important protein detection, enzyme-linked immunosorbent assay (ELISA) is most commonly used, and it has been established as the most commonly used method due to its sensitivity and selectivity. However, innovation in electrochemical biosensors is still required to overcome some disadvantages in ELISA (e.g., multiple washing and blocking steps, desorption of antibodies from the surface, unoriented adsorption of antigen in the surface). Here peptides can be used as a recognition element, as an immobilization platform for other antigens or antibodies, or as a specific binding to an antigen which in turn binds to the antibody for developing an alternative to ELISA. Li et al. developed a sensor for epidermal growth factor receptor (EGFR) detection using a ferrocene-modified YHWYGYTPQNVI ligand (Li et al. 2013). This is a "signal-on"-type biosensor, where upon the interaction with the protein the Fc unit comes in closer to the electrode surface resulting in an increase in the signal. Other important biomarker for disease progression is the antibodies produced in the body to fight the disease. Vanova et al. in their article have compiled a detailed account of peptide-based antibody detection as a predictive biomarker for disease progression (Vanova et al. 2021). Ding et al. used peptide nanowire as a platform to immobilize a larger amount of secondary antibodies and redox reporter for signal amplification to detect human I_gG with a LOD of 5 fg/mL (Ding et al. 2013; Taskin et al. 2013). The binding of a peptide to an antibody can be specific for that antibody, or a generalized sequence can also be used for binding to any antibody. A specific peptide sequence for binding a specific antibody should be preferred in that case. Rodovalho et al. used Ac-ACSSWLPRGCGGGS-NH_2 for the detection of juvenile idiopathic arthritis (JIA) IgG by monitoring the DPV and EIS responses (Rodovalho et al. 2018). Zaitouna et al. used a methylene blue modified HS–$(CH_2)_{11}$-EAAEWDRVHP-K-MB peptide sequence to detect the anti-p24 antibody (HIV detection) (Zaitouna et al. 2015). They modified the sequence by adding a hexapeptide sequence of hydrophilic amino acids (HS–$(CH_2)_{11}$-SGSGSGEAAEWDRVH P–K-MB or HS–$(CH_2)_{11}$EKEKEKEAAEWDRVHP-K-MB) and found that in both cases the sensitivity and the selectivity of the sensor increased for anti-p24 detection. Puiu et al. reported a novel method for the detection of antibody avoiding voltammetric baseline fluctuations and capacitance current drifts (Puiu et al. 2014). They first immobilized 9-mer support short peptide sequence (YAAAHAEAR) on the gold

electrode surface (through a lipoic acid and gold–thiol chemistry) and then modified with a MB for signal generation and a recognition peptide to detect anti-alpha-2 deamidated gliadin peptide IgG monoclonal antibody. Both the support and the recognition peptide interacted through the carboxyl groups of glutamate residues of the former peptide and the phenolic -OH of the tyrosine residues of the later peptide. The binding of the target antibody to the recognition peptide reduces the electron transfer from the MB to the gold surface leading to a decrease in the current response. This peptide platform can be modified with other recognition peptide for the detection of other analyte as well. In a similar approach, Arya et al. used a thiol-terminated coiled-coil peptide to modify the surface of a microelectrode array with a comb-like structure as a platform and used a peptide sequence from a viral protein as recognition element to detect the specific antibody (Arya et al. 2014). The recognition peptide was modified with Fc, and an interaction with the antibody would lead to decrease in signal. Hwang et al. have reported the detection of a norovirus capsid protein using QHIMHLPHINTL ligand and also achieved to detect human norovirus with a detection limit of 7.8 copies/mL (Hwang et al. 2017). Antimicrobial and cell-penetrating peptides are also used for the electrochemical detection of microbes and have been reported for detection of clinically important bacteria through impedance (Mannoor et al. 2010). Lim et al. developed a phage-displayed peptide ligand EHDRMHAYYLTR for the impedance and SWV-based detection of dengue fever biomarker NS1 with a LOD of 1.5–25 μg/mL and also the virus in the range of 10^5 to 10^9 copy/mL (Lim et al. 2018). Some of the recent reports in affinity based peptide based sensors are summarized in Table 9.1.

9.4 Peptide as Enzyme Substrate and for Enzymatic Activity

Custom-made peptide sequence can be used as a substrate for various proteases and therefore can be used for the development of a biosensor. Several proteases play a very important role in the growth and development, physiology, and metabolism, and also they are associated with a variety of different diseases. Matrix metallopeptidase (MMP), HIV protease, and trypsin are some of the most commonly detected enzymes by using peptide, and these enzymes are detected by cleaving a surface immobilized peptide substrate using an electrochemical sensor. These peptides are usually tagged with a redox molecules, and the action of the enzyme can be monitored by a "signal-off" or a "signal-on" mode (Yuan and Liu 2021). Figure 9.3a, b shows how peptide acts as an enzyme substrate for electrochemical biosensor development. In "signal-off" sensors (Fig. 9.3a), the protease cleaves the electroactive species from the immobilized peptide leading to a sharp decrease in the signal, and in the "signal-on" sensors (Fig. 9.3b), the action of the protease leads to considerable increase in current intensity. Also, some sensors are developed based on the phosphorylation of the peptide by a specific enzyme activity (Fig. 9.3c), and we will discuss those types of sensors with more details here.

Trypsin is an important pancreatic digestive enzyme, and an altered level of trypsin can be used as a marker for chronic pancreatitis and pancreatic cancers

Table 9.1 Affinity-based electrochemical peptide biosensors

Target molecule	Peptide sequence	Details	Ref.
Trypsin	Fc-[GRPS]-PEG disulfide	Electrode: Gold electrode Method: CV LOD: NA; LDR: 25 ng/mL to 100 μg/mL	Adjémian et al. (2010)
	RRF	Electrode: Polycrystalline Au electrode Method: SWV LOD: 88 pM; LDR: 90–800 pM	González-Fernández et al. (2018)
	CAGRAAADAD	Electrode: GCE/$NiCo_2O_4$-PAMAM Method: SWV LOD: 10 ng/mL; LDR: 10 ng/mL to 100 μg/mL	Lin et al. (2018)
PSA	CGGHSSKLQFWYFWY	Electrode: Gold electrode/Peptide/GO/AgNP Method: LSV LOD: 0.33 pg/mL LDR: 5-20,000 pg/mL	Meng et al. (2019)
Amyloid-β 1–42	RGTWEGKWK	Electrode: Gold electrode/MUA/peptide-Fc Method: SWV LOD: 240 pM; LDR: 480 pM to 12 nM	Li et al. (2012a)
	CPPPPTHSQW NKPSKPKTNMK	Electrode: Gold disk electrode/microporous gold nanostructure/peptide Method: DPV LOD: 0.2 pg/mL; LDR: 3–7000 pg/mL	Negahdary and Heli (2019)
JIA immunoglobulin	ACSSWLPRGCGGGS	Can detect the healthy blood sample from JIA positive sample	Rodovalho et al. (2018)
MCF-7 cell	WxEAAYQrFL	LOD: 3 cells/mm^2; LDR: 66–130 cells mm^2	Tian et al. (2021)
Apoptotic HeLa cell	FNFRLKAGAKIRFGRGC	LDR: 10^2 to 5×10^5 cells/mL; LOD: 30 cells/mL	Meng et al. (2016)
HepG2 cell	RGDS	Electrode: GCE/MWCNTs/RGDS ligand and antibody conjugated SiO2@Ru for signal amplification; LOD: 800 cells/mL	Wu et al. (2012)

detection. Peptide-based trypsin biosensor exploits its selectivity to cleave certain peptide sequences. Adjémian et al. reported a trypsin biosensor using a Fc-labeled and a flexible PEG linker-modified peptide (Fc-[GRPS]-PEG disulfide), where trypsin cleaves the peptide sequence leading to a significant drop in CV current

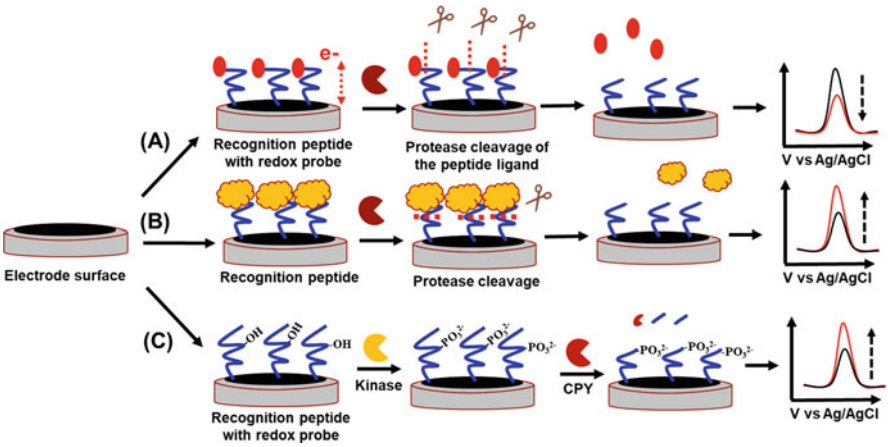

Fig. 9.3 Mode of action of peptide-based electrochemical biosensor, where the peptide acts as an enzyme substrate, (**a**) signal on, (**b**) signal on–off, and (**c**) phosphorylation. The image (**c**) is adopted from Vanova et al. (2021) and Yin et al. (2015)

response (Adjémian et al. 2010). In the same article, they also reported to detect thrombin by using RFSRPQL sequence as the peptide substrate using the same method. In this experiment, they concluded that the length of the peptide chain and surface coverage of the peptide on the electrode surface are very important to achieve a nanomolar range detection. Also, they have shown that MCH backfilling achieved higher signal compared to PEG in case of thrombin detection, even though in the case of trypsin the change was minimal. This article has shown that the blocking agent should be carefully selected. A different group tested the effect of different lengths of PEG spacers (PEG-4, PEG-6, PEG-8, and PEG-12)) in the peptide ligand for trypsin sensing and found that PEG-6 gives the most consistent and antifouling response (González-Fernández et al. 2018). These two articles show the important role of a spacer in the development of peptide-based enzyme sensor. Lin et al. used a nanomaterial enhanced detection strategy for trypsin detection, where they used $NiCo_2O_4$-PAMAM nanocomposite-modified GCE electrode and g-C_3N_4 nanocomposite-tagged peptide ligand CAGRAAADAD for signal amplification in ruthenium complex ($[Ru(NH_3)_6]^{3+}$). Trypsin cleaved the peptide leading to an increase in the current response, and the sensor can detect the enzyme with a limit of detection of 10^{-10} mg/mL. Prostate-specific antigen (PSA) is the most commonly used biomarker for prostate cancer detection (4.0 ng/mL or higher concentration). Meng et al. developed a PSA sensor based on the interaction of the peptide with graphene oxide (GO). In the absence of PSA, GO can interact with the peptide, and subsequent electrochemical signal generation by AgNP reduction on graphene surface can be monitored (Meng et al. 2019). In the presence of PSA, the peptide is cleaved, and the GO cannot interact with the peptide, and thus silver nanoparticles (AgNP) cannot form on the GO surface leading to a decrease in signal. However, affinity-based peptide sensors can also be developed for these enzymes, such as

CGGGGMERCPIKMFYNLGSPYMNI peptide ligand that was used for PSA detection in a sandwich electrochemical assay instead of a secondary antibody (Zheng et al. 2021). Matrix metalloproteinases (MMPs) are another important class of endopeptidases, associated with neurodegenerative and cardiovascular disorders, diabetes, sepsis, etc., usually detected using peptide ligand as enzyme substrate. MMPs can degrade many protein components in cell membrane and extracellular matrix and thus play a central role in cancer progression. Liu et al. have reported one of the earliest work on MMP-7 detection, where they used a Fc-modified RPLALWRSC peptide ligand (naming it as electrochemical proteolytic beacon) to achieve a LOD of 3.4 pM (Liu et al. 2006). Similarly, a Fc-modified CPLPLRSWGLK ligand was used for the detection of MMP-14 (Sun et al. 2020). These peptides can also be integrated with other BREs, such as nucleic acid or aptamer and nanomaterials for enhanced sensitivity (Jing et al. 2015). Similarly, other important biomarkers such as plasmin (Ohtsuka et al. 2009) and other proteins with enzymatic activity (Chan et al. 2015) are detected using peptide-based cleavage biosensors. Clinically important bacteria can also be detected using their specific proteolytic enzymes, such as *Listeria monocytogenes* and *Staphylococcus aureus* that have been reported to be detected by using specific sequence of peptide ligand (*S. aureus*, NH_2-Ahx-ETKVEENEAIQK Ahx-biotin, and *Listeria*, NH_2-Ahx-NMLSEVERE-Ahx-biotin) (Eissa and Zourob 2020). Specific enzymes from the bacteria acted on the substrate peptide sequence and resulted in the detection limit of 9 CFU mL^{-1} for *L. monocytogenes* and 3 CFU mL^{-1} for *S. aureus*. Some of the representative works on using peptides for enzymatic activity in electrochemical biosensing is summarized in Table 9.2.

Peptides can also be used as a phosphorylation substrate by certain enzymes, known as protein kinases, and this can be used for development of some innovative biosensors. Protein kinases are important for signal transduction and maintaining the body metabolism, and their activity measurement is important for normal body function. Wang et al. reported that the use of a protein kinase on a positively charged peptide SAM layer led to an increase in current response, when tested with $[Ru(NH_3)_5Cl]^{2+}$. The positively charged $[Ru(NH_3)_5Cl]^{2+}$ can interact better with a phosphorylated peptide SAM than a positively charged peptide SAM (due to electrostatic repulsion) (Wang et al. 2010). Li et al. used tyrosine kinase and an additional protein tyrosine phosphatase 1B (PTP1B), an enzyme that removes phosphorylation to study their interaction on an electrode, and they managed to profile enzyme activity using EIS as detection method (Li et al. 2012c). Yin et al. reported a protein kinase activity using a peptide-based sensor. They immobilized a peptide CDDDDDSDDDA-ATP on a AuNP-modified GCE, and the peptide was phosphorylated by casein kinase II (CK2) in the presence of ATP (Yin et al. 2015). A phosphorylated aspartic acid in the peptide led to a lower signal due to electrostatic repulsion with the negatively charged redox probe. The carboxypeptidase Y (CPY) degrades the peptide from C-terminus and leads to an increase in the signal. The group also reported that a phosphorylation at serine residue would lead to no further cleaving activity by CPY and a decrease in redox signal. This method was used to measure the CK2 activity in complex biological fluids. Some of the representative biosensor articles on peptide as an enzyme substrate are summarized in Table 9.2.

Table 9.2 Peptide sequence used as an enzymatic substrate in electrochemical sensing

Enzyme	Peptide sequence	Details	Ref.
Trypsin	MB-FRR-PEG-C-NH_2	Electrode: Gold electrode/ PEG_4-peptide Method: SWV LOD: 88 pM; LDR: 0.1–100 nM	González-Fernández et al. (2018)
	MB-FRRC-PEG_4-NH_2	Electrode: Gold electrode/tri-thiol peptide Method: SWV LOD: 1.2 nM; LDR: 1–100 nM	Staderini et al. (2018)
	MB-FRR-PEG_6 − Cys	Electrode: Gold electrode/peptide with a Pt microelectrode as reference electrode Method: SWV LOD: 1–100 nM; LDR: 1–100 nM	Ucar et al. (2020)
	FITC-[AEEA]FRR[AEEA]-Btn	Probe 1: biotin/peptide/FITC; probe 2: anti-FITC antibody/HRP complex Method: chronoamperometry LOD: 7 nM; LDR: 23–250 nM	Martín et al. (2020)
PSA	4-pentynoyl-GGGGHSSKLQL-biotin-OH	Electrode: GCE/juglone/4-azidoaniline/biotin-probe/streptavidin Method: SWV LOD: not provided; LDR: 1 pM to 1 μM	Strzemińska et al. (2016)
	Fc-CHSSLKQK-NH_2	Electrode: gold electrode/peptide-Fc Method: SWV LOD: 0.2 ng/mL; LDR: 0.5–40 ng/mL	Zhao et al. (2010)
	Fc-CGGHSSKLQFWYFWY-NH_2	Electrode: gold electrode/peptide/GO/AgNP Method: LSV LOD: 0.33 pg/mL; LDR: 5 to 20,000 pg/mL	Meng et al. (2019)
	biotin-K*QLKSSH*KKKKKD-rhodamine B	Electrode: NH_2-CuO/BSA/P-pep-RhB/β-CD/$AgInS_2$ NPs/ITO Method: EIS and photocurrent LOD: 0.06 ng/mL; LDR: 0.0001–100 ng/mL	Fu et al. (2021)

(continued)

Table 9.2 (continued)

Enzyme	Peptide sequence	Details	Ref.
MMP-7	RPLALWRSC	Electrode: gold electrode/peptide-Fc Method: SWV LOD: 3.4 pM; LDR: 0.1–10 ng/mL	Liu et al. (2006)
	NH_2-KKKRPLALWRSCCC-SH	Electrode: GCE/depAu/peptide1-PtNPs-S1/DNA nano-ladder Method: DPV LOD: 0.05 pg/mL; LDR: 0.2–20,000 pg/mL	Kou et al. (2016)
	NH_2-KKKEKEKEKRPLALWRSCEEE-COOH	Electrode: GCE/peptide/sodium alginate-graphene oxide-Pb^{2+}/Urease@ZIFs Method: SWV LOD: 24.34 fg/mL; LDR: 0.1–100,000 pg/mL	Zhang et al. (2022)
MMP-9	MB-GPLGMWSRC-NH2	Electrode: gold electrode/peptide-MB Method: CV LOD: 7 pM; LDR: 1 pM to 1 nM	Lee et al. (2017)
Caspase-3	Ac-GDEVDSK (FFFF)H–NH_2	Electrode: GCE/GO/peptide/Cu(II) and Ni(II)-binding ATCUN motif Method: CV LOD: 0.02 pg/mL; LDR: 0.5–2000 pg/mL	Deng et al. (2019)
Botulinum neurotoxin	Cys-Ahx-KTRIDEANQ-RATK (MB)M	Electrode: paper electrode Method: SWV LOD: 10 pM; LDR: 0.01–10 nM	Caratelli et al. (2021)

9.5 Peptide-Based Anti-Biofouling Materials

Custom-made peptide sequences with different degrees of surface hydration and charge can be used as an anti-biofouling layer. Peptides with high surface hydration/hydrophilicity are able to prevent the nonspecific binding of proteins by inhibiting the hydrophobic interaction, and the neutral charge-based peptide sequences do it by inhibiting the charge attraction. The mixed-charged peptides and hydrophilic peptides achieve their surface hydration via ionic solvation and hydrogen bonding, respectively (Chen et al. 2009). Keefe et al. screened multiple peptide sequences for antifouling property and found that the peptides with lowest fouling effects usually had an overall charge close to neutral (Keefe et al. 2013). Ye et al. performed an interesting experiment to observe the comparative antifouling activity of a

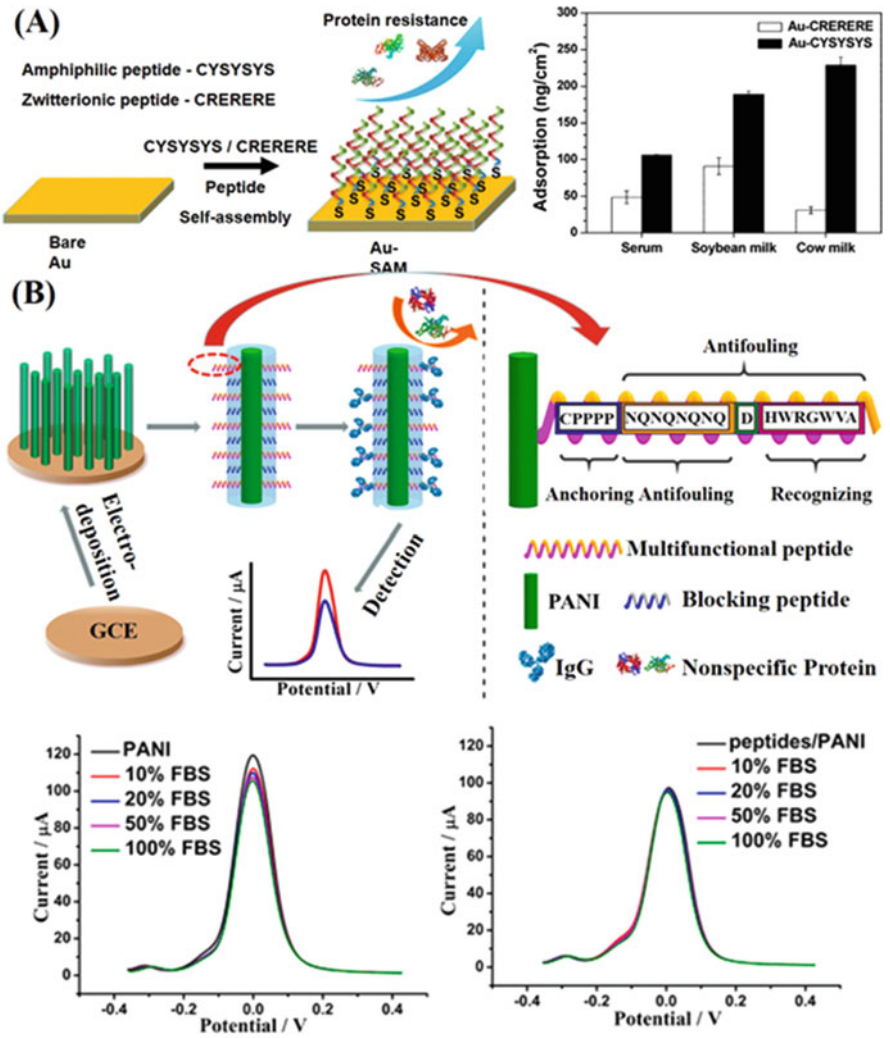

Fig. 9.4 Use of peptide-based material as anti-biofouling layer. (**a**) Modification of gold surface with an amphiphilic or zwitterionic peptide sequence resulted in differential adsorption in serum, soybean milk, and cow milk. Reused with permission from Ye et al. (2015). (**b**) The use of a single peptide sequence with an anchoring, an antifouling, and a recognition sequence for efficient binding as well as avoiding antifouling simultaneously. Reused with permission from Liu et al. (2018)

zwitterionic peptide sequence (CRERERE) and an amphiphilic sequence (CYSYSYS) (Ye et al. 2015). Both the peptides were attached to gold surface via thiol linkage, and their surface fouling in response to some common biological solutions was tested. Both the peptide sequences have shown a very low protein adsorption; however, the zwitterionic sequence proved to be a superior antifouling layer as shown in Fig. 9.4a. Higher surface hydration in zwitterionic peptide

sequences resulted in lower fouling effect in the protein solutions. Nowiniski et al. compared the antifouling property of a nonfouling peptide (EKEKEKE) in two different combinations, i.e., (i) attached to a gold surface through cysteine and (ii) attached to a gold surface through an additional linker with a length of four residues –PPPPC. They found that the peptide with –PPPPC forms a secondary structure instead of a random structure (found in case of only –C) and exhibits more nonfouling activity (Nowinski et al. 2012). They also found that –PPPPC showed better nonfouling activity than –PC, –PPC, and –PPPC. So, the peptide sequence RGD-EKEKEKE-PPPPC was used for specific cell sensing. This work showed that instead of cysteine, a linker-modified cysteine can act as a better peptide-modified sensor surface. In another interesting approach, Liu et al. designed a multifunctional peptide sequence for the electrochemical detection of human immunoglobulin G (IgG) as shown in Figure 9.4b. The peptide contains an anchoring domain (–CPPPP), an antifouling domain (–NQNQNQNQDHWRGWVA), and a recognition domain (Liu et al. 2018). The peptide was used on a polyaniline (PANI) nanowire-modified GCE electrode surface and was tested to detect IgG in serum samples. The peptide-modified sensor surface was incubated with different concentrations of fetal bovine serum (FBS) and found to be less sensitive to FBS as compared to PANI-modified electrode surface as shown in Fig 9.4b. The same group later on developed another sensor for PSA detection using two different peptides for a more reliable signal generation (Ding et al. 2019). The electrode surface was functionalized with two different antifouling peptides, where the longer peptide (Pep1) contains sequence HSSKLQK which can be recognized and cut by PSA. Pep 1 was functionalized with a peroxidase activity mimicking graphene oxide−Fe_3O_4 − thionine (GO–Fe_3O_4–Thi) probe. The GO–Fe_3O_4–Thi acts as an electrochemical probe due to the presence of Thi, and in the presence of PSA, this probe was cut resulting in a decrease in signal. The shorter peptide (Pep 2) was functionalized with ferrocene (Fc), which was used for a steady electrochemical signal generation. The signal of Fc of Pep 2 was constant and used as a reference for reliable signal generation. The sensor achieved a linear dynamic range of 5 pg/mL to 10 ng/mL, with low detection limits of 0.76 pg/mL (through DPV) and 0.42 pg/mL (through CA). Song et al. reported another multifunctional nonfouling peptide YVEYHLCREKEKEKEKAKAKAKAK that can be attached to a PEDOT-modified glassy carbon electrode surface (Song et al. 2020). One end of the peptide with amine-rich sequence binds to electrodeposited PEDOT–citrate film, while the other end recognizes aminopeptidase N (APN) and human hepatocellular carcinoma cells (HepG2 cells). The middle part of the peptide contains a nonfouling surface, and the developed sensor detects APN and HepG2 cells in its clinical range. Based on the concept of neutral-charged peptide sequence, specific sequence of additional linker peptide, and covering the lower end of peptide with monomers/polymers to avoid the nonspecific binding, many other peptide-based antifouling surfaces have been reported. Some of these representative works are mentioned in Table 9.3.

Table 9.3 Peptide sequence used as antifouling surface

Antifouling peptide (with recognition sequence)	Solutions tested	Remarks	Reference
CAEAEPPPP*QEQKQEQK*	Human serum samples	Retained antifouling properties for 30 days	Han et al. (2020)
CDSDSPPPPAEAKAEAK	Human serum, different protein solutions, and human lung cancer cell culture medium	Retained antifouling properties for 20 days	Wang et al. (2020)
$(CKEKEKEKE)_2$ -KEPPPPKEKEKEKEK-biotin	Human serum samples	Incubated for 2 h and showed the least fouling by complex solutions	Song et al. (2021)
NH_2-KKK*EKEKEK* RPLALWRSCEEE-COOH	Human serum samples and other small biomolecules	–	Zhang et al. (2022)
EKEKEKE-PPPPC	Human serum samples and whole blood	Combined with aptamer for sensitive detection of ATP	Wang et al. (2018)

9.6 Future Prospect and Conclusion

In this chapter, the recent developments in peptide-based electrochemical biosensors are discussed in details. The last two decades have seen a tremendous increase in the potential for in silico-based peptide sequence development for selective binding to a particular analyte. This feature combined with advanced nanomaterials and BRE immobilization strategies has led to the development of many new peptide-based biosensors. Several antifouling peptides have been developed based on the hydrophilic amino acids to reduce the amount of nonspecific protein adsorption, and also PANI-like materials are also used to get a higher signal with higher antifouling effect. However, specificity, sensitivity, and actual device development is still a problem for these biosensors. Even though there are a lot of articles reported to develop peptide-based biosensors, a fraction of those has translated into a product for use by a larger population. Miniaturization of the sensing device, smartphone integration, and development of antifouling layers for sensing in whole blood can further improve the peptide-based electrochemical sensors.

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Recent Trends in Enzyme-Based Electrosensing Devices Modified with Nanomaterials

10

Olga Domínguez-Renedo, A. Marta Navarro-Cuñado, and M. Asunción Alonso-Lomillo

Abstract

Enzyme-based electrochemical biosensors are analytical devices with great potential in various fields, thanks to their specificity, high sensitivity, and the possibility of automation and miniaturization. The analytical performance of these electrochemical devices can be remarkably improved by the employing of advanced nanomaterials due to the important features of these materials, including great effectiveness in electron transfer related to its high surface area and conductivity. This chapter reports the recent applications of different enzymatic biosensors based on the modification of the working electrode with nanomaterials, including fullerenes, graphene, carbon nanotubes (CNTs), carbon and graphene quantum dots (QDs), metallic nanoparticles (NPs), and inorganic QDs. The reported devices are categorized according to the target biomolecule, and their description has considered not only the nanomaterial used but also the type of electronic transfer that takes place (direct or mediated) as well as the enzymatic mechanism involved.

Keywords

Electrochemical biosensor · Enzymatic biosensor · Glucose oxidase · Biogenic amines detection · Bioanalysis

O. Domínguez-Renedo · M. A. Alonso-Lomillo (✉)
Analytical Chemistry Department, Faculty of Sciences, University of Burgos, Burgos, Spain
e-mail: malomillo@ubu.es

A. M. Navarro-Cuñado
Physical Chemistry Department, Faculty of Sciences, University of Burgos, Burgos, Spain

B. Purohit, P. Chandra (eds.), *Surface Engineering and Functional Nanomaterials for Point-of-Care Analytical Devices*, https://doi.org/10.1007/978-981-99-3025-8_10

10.1 Introduction

Electrochemical biosensors with enzymes as the biorecognition elements are one of the largest and most commercially successful groups of biosensors. The sensor usually consists of an enzyme-modified working electrode, a reference electrode, and an auxiliary electrode, and the sensor performance is measured by correlating the variations in the current response with different concentrations of target substrate. Over the last half decade, nanomaterials have become the key component of many enzyme-based electrochemical sensors, as evidenced by hundreds of related research papers published in specialized journals (Kucherenko et al. 2019; Kumar et al. 2020; Mahato et al. 2020). The present chapter covers the recent applications of various types of nanomaterials, namely, fullerenes, graphene, carbon nanotubes (CNTs), carbon and graphene quantum dots (QDs), metallic nanoparticles (NPs), and inorganic QDs, to improve the analytical performance of electrochemical enzyme-based sensors. The chapter is structured according to target biomolecule and highlights the evolution in the construction of these types of biosensors, based on mediated and direct electron transfer mechanisms, for different analytical purposes, that is, detection of biomolecules, environmental pollutants, food contaminants, and clinical biomarkers.

10.2 Enzymatic Electrochemical Biosensors

The most important step in designing an enzyme-based electrochemical biosensor is the proper immobilization of the enzyme onto the electrode surface. This helps the enzyme in optimum enzymatic reaction catalysis and protects the enzyme from external environmental factors. In addition, different properties of the enzymes, such as selectivity and specificity of an enzyme, can be optimized or altered after its immobilization by changing its conformation (Alonso-Lomillo et al. 2009; Lipińska et al. 2021). The most important and common methods of enzyme immobilization are as follows (Alonso-Lomillo et al. 2009):

- Adsorption: The physical adsorption of the enzymes on the electrode surface occurs mostly due to van der Waals interaction. These types of interactions are generally weak, and covalent bonding is preferred for a stronger interaction.
- Covalent bonding: Covalent bonding is more stable and stronger than the physical interactions (more detail of this kind of interaction is illustrated in Chaps. 1 and 2).
- Entrapment of the enzyme: The physical retention in the inner cavities of the porous matrix, such as Nafion or polypyrrole.
- Microencapsulation of the enzyme: Enzymes are trapped in semipermeable membranes which allow the substrate molecules and products to pass through but block the movement of enzymes.
- Cross-linking of the enzyme involves the use of reagents (such as glutaraldehyde), which forms intermolecular bonds between enzyme molecules.

- Enzyme-containing ink: The rise of screen-printed electrodes and wearable sensors on flexible substrate gives rise to the use of enzymes in a conductive ink solution. The solution mostly contains a binder (Wang et al. 1996).

The enzymes used in the designed biosensors are very selective toward the target substrate, and either the substrate or the product of the enzymatic catalysis is an electroactive species which can be monitored through the electrochemical sensing. The generation of electrons, charged species, or molecules like hydrogen peroxide led to a measurable change in potential or current response, and this is monitored as a direct measurement of the target molecule concentration (Rathee et al. 2016). Glucose biosensors are the perfect example of how an enzyme-based sensor is designed, where the three generations of the biosensors based on the sensing mechanism are explained (Purohit et al. 2022). In the first generation of enzymatic sensors, the byproduct of the reaction hydrogen peroxide is measured as a function of glucose concentration. In the second generation of enzyme electrodes, electron- or charge-carrying mediator molecules were incorporated to facilitate the electronic transfer between the active center of the enzyme and the surface of the electrode. This type of sensing mechanism changed the landscape of glucose sensing and led to the development of miniaturized sensors. The third generation of biosensors, the most advanced form of enzyme-based biosensors, in which the electronic transfer between the active center of the enzyme and the electrode surface takes place directly without the use of mediators, works at potentials that are very close to the intrinsic potentials of the enzyme itself, reducing their exposure to possible interfering molecules (Alonso-Lomillo et al. 2009; Teymourian et al. 2020). Later on, nanomaterials, both organic and inorganic, have been combined with oxidoreductase enzymes as tools for immobilization, leading to high catalytic efficiencies and promising applications in sensing, bypassing sometimes the need for an additional electron mediator (Dimcheva 2020; Kurbanoglu et al. 2017a; Ratautas and Dagys 2019).

10.3 Nanomaterial-Based Enzymatic Electrochemical Biosensors

The past two decades have seen a tremendous rise in the synthesis and characterization of nanomaterials and their use to advance the sensitivity and selectivity of different types of biosensors. The nanomaterials were used in the design of electrochemical enzymatic biosensors to achieve higher signal-to-noise ratio, antibiofouling property, and enhanced enzymatic catalysis. The most important properties of the nanomaterials being used in the biosensors are their higher surface-to-volume ratio, catalysis, electrical conductivity, and excellent magnetic properties (Akhtar et al. 2018; Chandra et al. 2010; Chandra and Prakash 2020; Mahato et al. 2016). Nanomaterials can be tuned to get a particular chemical or physical property according to the need of the biosensors, and also nanomaterials with different active

sites and functional groups on their surface can be synthesized for high adsorption and catalysis activity (Kurbanoglu et al. 2017a).

Among the different nanostructure materials, carbon nanomaterials have received great attention in the development of electrochemical enzymatic biosensors owing to their exceptional electrical properties. Moreover, the possibility to customize their synthesis with attached functional groups or to assemble them into three-dimensional arrays has allowed the design of new interesting materials (Pilehvar and De Wael 2015). Examples of such materials include fullerenes such as the closed cage, nearly spherical C_{60}, and related analogues (Pilehvar and De Wael 2015). Carbon nanotubes (CNTs) have also been used for low-level detection of different analytes using enzymatic biosensors (Ghanei et al. 2020). These hollow graphitic cylinders improve the electrode surface coverage and can be used as the interface between electrodes and the oxidation–reduction center in biomolecules. Two-dimensional graphene, graphene oxide (GO), and reduced GO (rGO) nanomaterials create an ideal immobilization support for various biomolecules including enzymes. This immobilization capacity can be improved by grafting desirable functional groups on their surface and providing functionalized nanomaterials with tailored properties that allow protection from enzymatic cleavage, improve transport capacity in living cells, or enable the incorporation of enzymes in microdevices and microchip bioreactors (Pavlidis et al. 2014). CNTs and graphene-based nanomaterial-modified electrodes exhibit excellent electrocatalytic activity for the reaction of hydrogen peroxide and nicotinamide adenine dinucleotide (NAD) frequently used as the base of enzymatic electrochemical biosensors (Kuila et al. 2011; Shao et al. 2010; Xiong et al. 2018; Yang et al. 2015).

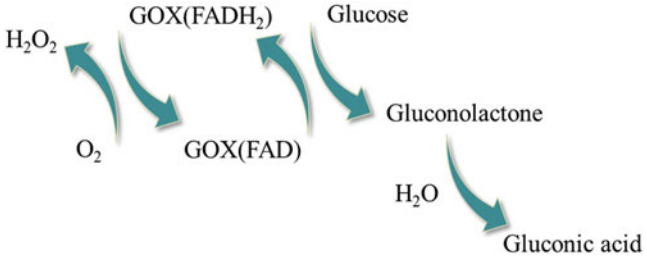
Noble metal nanostructures offer some additional advantages of great importance for electrochemical research, in general, and for biosensor technologies, in particular (Dimcheva 2020). Metallic NPs, including gold (AuNPs), silver (AgNPs), platinum (PtNPs), and palladium (PdNPs), can effectively catalyze hydrogen peroxide reduction, as well as facilitate more efficient electron transfer between immobilized biomolecules and electrode substrates, resistance to corrosion, biocompatibility, and preservation of biological activity keeping the immobilized enzyme in a suitable microenvironment and easy functionalization of its surface with organic molecules (Dimcheva 2020; Wang et al. 2020a; Xiong et al. 2018).

QDs are well-known nanostructured semiconductor materials ranging in a very small size range of only several nanometers, which provide a larger surface area for binding of enzymes on the surface of the electrodes. The sensitivity of the electrochemical signal can be further enhanced by using QDs as carriers to load many electroactive species or enzymes due to their unique electronic properties that differ from those of larger particles of the same material due to quantum-level effects (Ali et al. 2018; Dhanjai et al. 2018; Ndangili et al. 2011).

10.4 Applications of Enzyme-Based Electrosensing Devices Modified with Nanomaterials

10.4.1 Glucose

The use of electrochemical enzyme sensors for the fast and reliable determination of glucose in food industry and medical applications is probably the most widespread since the 1950s. Biosensors based on the immobilization of the enzyme glucose oxidase (GOx) have been improved through the use of new types of materials and immobilization procedures that allow a miniaturization of the devices, with reduction in manufacturing costs and used materials, while increasing their sensitivity and selectivity (Juska and Pemble 2020; Pohanka 2021; Teymourian et al. 2020). Glucose dehydrogenases (GDHs) constitute another major class of enzymes for glucose analysis but provide lower specificity than GOx (Teymourian et al. 2020). Basically, GOx oxidizes glucose to gluconolactone, via reduction of the cofactor flavin adenine dinucleotide (FAD) to $FADH_2$, resulting in the formation of hydrogen peroxide and the depletion of O_2:



The last two decades have seen a tremendous progress in the use of nanomaterials for increasing the activity and stability of glucose biosensors (Juska and Pemble 2020; Pohanka 2021; Teymourian et al. 2020). Carbon and inorganic nanomaterials have been used as ideal enzyme supports, acting primarily to catalyze electrooxidation of the H_2O_2 product (Pohanka 2021; Teymourian et al. 2020). AuNPs, AgNPs, PtNPs, PdNPs, and oxide NPs (CuO, Cu_2O, NiO, Fe_2O_3) and bimetallic systems (Au-Pt, Au-Pd, and Cu-Ag) have been used for glucose detection. Nevertheless, the use of AuNPs has led to the major benefit, that is, a higher glucose oxidation current. Thus, extensive research in GOx immobilization on AuNPs for glucose detection has been carried out over the last half decade using potential, current, and impedance-based methods, as well as the most commonly applied methods: covalent bonding, adsorption, cross-linking, entrapment, and self-assembled monolayers (Lipińska et al. 2021). Titanium provides a new way to prepare implantable GOx biosensors, considering its good compatibility to human tissue and body fluids, its corrosion resistance, and chemical stability. One proposal has been described by Ma et al. (Ma et al. 2020), loading GOx on hydrogen titanate nanotubes by cross-linking to form an amperometric biosensor with good sensitivity

performance for glucose. CNTs and fullerene-based composites with metal nanocatalysts have also been applied as biocompatible matrices for glucose sensing using glassy carbon (CGE), graphite, and gold electrodes. In this way, GOx/PtNPs/CNTs/GCE (Wen et al. 2009), GOx/ferritin/ZnONPs/polyindole/CNTs/GCE (Inamuddin et al. 2020), GOx/anthracene- and isoindigo-based polymer/CNTs/graphite electrode (Soylemez et al. 2019), GOx/rGO/-Pt NPs@Zn-MOF-74/GCE (Uzak et al. 2020), and GOx/Prussian blue/PtNPs/graphene/Au (Pu et al. 2021) have successfully been used as sensitively amperometric sensors for low-potential determination of glucose, as well as GDH-AuNPs-C_{70} fullerene-GCE (Piotrowski et al. 2017).

Third-generation glucose biosensors, which rely on direct electron transfer from glucose to electrode surfaces via the enzyme's redox center (FAD), have long been a sought-after research target (Teymourian et al. 2020). FAD is deeply buried inside the protein structure, so the reoxidation of $FADH_2$ at the electrode is not easily achieved (Juska and Pemble 2020; Pohanka 2021; Teymourian et al. 2020). The attachment of conductive NPs to the enzyme cofactor has proven to be a successful strategy (Juska and Pemble 2020; Lipińska et al. 2021; Muthurasu and Ganesh 2016). For instance, AuNPs and fluorescent AuNPs chemically attached to GOx and immobilized by drop casting onto a GCE have been used for voltammetric glucose detection in human blood serum samples at −0.5 V (Muthurasu and Ganesh 2016). GOx has also been entrapped in a TiO_2 functionalized SnS_2 matrix onto a GCE using Nafion to develop an amperometric glucose biosensor, working at −0.45 V (Yao et al. 2019). Recently, graphene has attracted considerable attention to be used as a promising electrode material for real-time glucose detection. A GOx biosensor has been constructed by depositing a porous film of $Ti_3C_2T_x$ MXene nanosheets and graphene sheets onto a GCE, followed by the entrapment of GOx with Nafion (Gu et al. 2019). Moreover, a graphene-laminated structure by combining GO and edge-functionalized graphene layers together onto GCE surfaces for high GOx loading has been developed for the highly sensitive amperometric glucose detection at −0.5 V (Hao et al. 2021). The electron transfer from FAD-dependent glucose dehydrogenase to single-sheet graphene electrodes has also been reported (Filipiak et al. 2020).

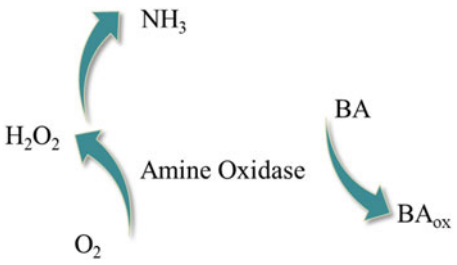
10.4.2 Fructose

D-fructose, widely used in food industry as sweetener, is generally monitored during food and beverage production to evaluate freshness and quality of products, along with other criteria. Its careful monitoring in human diet is also vital. Fructose dehydrogenase (FDH) has been noted as the most promising enzyme for the development of D-fructose electrochemical biosensors, since it is not oxygen-dependent and able to perform direct electron transfer to certain electrodes (Okawa et al. 2018; Voitechovič et al. 2020): D fructose + oxidant → 5-keto-D-fructose + reduced oxidant.

The influence of earlier trends in electrochemical transducers is also reflected in occasional studies of bioelectrochemical systems with FDH (e.g., application of numerous nanostructured carbon materials as CNTs and GO) (Voitechovič et al. 2020). Direct electron transfer FDH capability plays an significant role in the construction of mediator-free and simple bioelectrochemical devices for biosensing (Bollella et al. 2018; Hibino et al. 2017; Kaida et al. 2020; Kawai et al. 2016). Nanostructuration of electrodes seems to play a vital role: the shape of the NPs has a critical effect on the catalytic current related to the oxidation of D-fructose at a drop-casting FDH-modified electrode surface. In particular, it has been found that graphite electrode-modified triangular AuNPs have a higher effect compared with the spherical ones (Bollella et al. 2019). FDH electrodes built using a hollow nanostructure composed of gold and silver, casted onto an indium tin oxide (ITO)-coated glass plate or a carbon paper as base electrode, have shown good performance on electrocatalyst for the direct electron transfer (Okawa et al. 2018). FDH solution drop casted to CNT-modified GCE electrodes was used, where the anthracenyl groups in the electrode interacted with hydrophobic binding site within the enzyme. The analytical properties of these devices were thoroughly investigated to use this electrode platform as a third generation of amperometric biosensor, working at +0.25 V (Bollella et al. 2018). Kizling et al. have presented electrochemical evidence that magnetic interactions between the paramagnetic heme centers of FDH subunit II, electrostatically adsorbed, and superparamagnetic iron oxide NPs, adsorbed on gold electrodes, enable a suitable orientation of the enzyme molecule and enhance the rate of direct electron transfer (Kizling et al. 2018).

10.4.3 Biogenic Amines

Biogenic amines from the spoiled food products (fish, meat, fermented foods, etc.) upon ingestion cause different medical conditions (hypotension, nausea, vomiting, headaches, and diarrhea), and therefore their detection is important (Apetrei and Apetrei 2015, 2016; Dalkıran et al. 2019; Medyantseva et al. 2015b). The accurate detection (especially the structurally similar amines) of these molecules in food products needs selective enzymes in the electrochemical biosensors. Amine oxidase-based electrodes catalyze the reactions of the bioamines to measurable byproducts (ammonia and hydrogen peroxide).



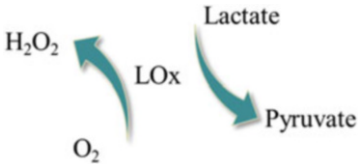
Measurements of the hydrogen peroxide production are commonly used for the quantification of these compounds in different samples. The application of nanostructured materials such as carbon nanomaterials, which can make the electrode surface more developed and more electrically conducting, and NPs, which can provide the necessary charge density to directly affect the sensitivity of a sensor and maintain its high electrical activity, as surface modifiers of various electrodes has led to biosensors with improved analytical characteristics (Medyantseva et al. 2015b). Increasing selectivity is also expected, since the operation potential during amperometric measurements may be lowered and interfering substances excluded (Guarda et al. 2017). Like this, monoamine oxidase has been immobilized on graphite electrodes with CNTs and AgNPs by bovine serum albumin or chitosan and glutaraldehyde (Medyantseva et al. 2015b), as well as on CNTs, AuNPs, and PtNPs containing pastes using a dialysis membrane for phenylethylamine amperometric biosensing, at +0.4 V, as diagnostic tool in monoamine-related diseases and research tool in brain and neuroscience (Aigner et al. 2017). Diamine oxidase has also been immobilized onto nanostructurated surfaces, rGO and PtNPs, of screen-printed carbon electrodes (SPCEs) using chitosan for histamine amperometric biosensing, at +0.4 V, in fish samples (Apetrei and Apetrei 2016). Lately, SPCEs modified with Prussian blue/ITONPs (Kaçar et al. 2020), PtNPs (Dalkıran et al. 2020), or TiO_2NPs/CNTs/hexaammineruthenium chloride/chitosan (Koçoğlu et al. 2020) have been further modified with monoamine oxidase or diamine oxidase enzymes in order to investigate their effect on the performance of the biosensors for biogenic amine sensing in cheese and fish samples. For these purposes, the enzymes have been both Entrapped within Nafion membrane (Dalkıran et al. 2020; Kaçar et al. 2020) or by covalent bonding using EDC–NHS-based amine coupling chemistry (Koçoğlu et al. 2020).

In addition, tyrosinase-based biosensors have also been built for biogenic amine determination. Tyrosinase catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones. In the case of tyramine, tyrosinase catalyzes its oxidation to *o*-dopaquinone via dopamine, which can be electrochemically reduced at low potentials (Apetrei and Apetrei 2015; Dalkıran et al. 2019). Tyrosinase/carboxyl-functionalized CNTs/SPCEs, using glutaraldehyde as cross-linker (Apetrei and Apetrei 2015), and tyrosinase/Fe_3O_4NPs-chistosan/poly-L-lysine/SPCEs, using a Nafion membrane (Dalkıran et al. 2019), have been reported for the amperometric detection of tyramine in pickled and smoked fish samples and cheese samples, respectively.

10.4.4 Lactate

L-lactic acid is an important marker for the freshness and storage quality of different food products and also for the fermentative processes. Lactate is also a byproduct of glucose metabolism in the muscle cells and an important biomarker in fitness and sports science. Lactate is also a biomarker for ischemic conditions and diabetes (Loaiza et al. 2015; Rathee et al. 2016; Rattu et al. 2021; Sun et al. 2015a). Lactate

oxidase (LOx) catalyzes the oxidation of L-lactate to pyruvate in the presence of dissolved oxygen and forms hydrogen peroxide, which can be either oxidized or reduced on the surface of a working electrode, resulting in a current directly proportional to the lactate concentration:

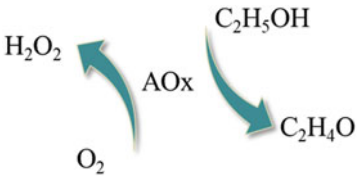


Ali et al. follow this scheme to develop a lactate biosensor using a screen-printed modified electrode with poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonate) and CNTs for the covalent immobilization of LOx (Ali et al. 2021). Diamond NPs have gained attention considering their additional advantages compared to other carbon nanomaterials, such as an excellent biocompatibility, a noncytotoxic nature. Diamond NPs can be deposited on a gold electrode or a 3-mercaptopropyl trimethoxysilane-modified gold electrode by adsorption, as well as LOx, leading to a lactate biosensor, based on the enzymatic oxidation of this analyte to pyruvate in the presence of hydroxymethyl ferrocene as redox mediator (Briones et al. 2015, 2016a, 2016b). Carbon nanodots have also been used as platform to immobilize LOx onto screen-printed gold electrodes to be employed in the quantification of L-lactate in human serum (Bravo et al. 2019). Recently, a dual-channel biosensor has been reported for real-time analysis of glucose and lactate in sweat. The biosensor has been built using highly integrated sensing paper combining hydrophobic protecting wax, conducting electrodes, and $Ti_3C_2T_x$ MXene/methylene blue active materials for immobilizing GOx and LOx (Li et al. 2021).

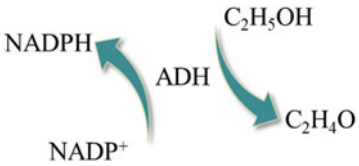
The development of hybrid nanomaterials for the highly efficient electrical detection of L-lactic acid using voltammetric and amperometric biosensors has also attracted great attention. Sun et al. (2015a) have synthesized polypyrrole-Pluronic F127 NPs with conducting and biocompatibility properties to construct a L-lactic acid biosensor that could be applied in biochemical assays. LOx has been electrostatically adsorbed on polypyrrole-Pluronic F127 NPs dropped on a GCE and protected by a Nafion film. In this case, a nice reduction peak recorded at +0.2 V by differential pulse voltammetry (DPV) has been related to L-lactic acid concentration in pig muscle samples (Sun et al. 2015a). LOx has also been immobilized using polyethyleneimine and glutaraldehyde (Loaiza et al. 2015) or chitosan and glutaraldehyde (Yang et al. 2018) onto PtNPs or graphitized carbon nanofibers modified SPCEs and onto graphene supported PtNPs modified GCEs for the analysis of lactic acid in alcoholic beverages and in human serum samples by amperometry at +0.3 V and +0.4 V, respectively. Moreover, a L-lactate biosensor based on covalent immobilization of LOx onto carboxylated CNTs/CuNPs/polyaniline-modified pencil graphite electrode has been described as well for the analysis of plasma samples (Dagar and Pundir 2017, 2018).

10.4.5 Ethanol

Ethanol sensing and measurement is important for both healthcare and different beverage industries. The recent focus on development of ethanol as more sustainable alternative of petroleum has also contributed toward the need of sensitive ethanol measurement methods (Gómez-Anquela et al. 2015). Alcohol oxidase (AOx)- and alcohol dehydrogenase (ADH)-based electrochemical devices modified with nanomaterials have commonly been used for the sensitive amperometric detection of ethanol. AOx catalyzes the oxidation of ethanol to its corresponding aldehyde with hydrogen peroxide production (Hooda et al. 2018a):



Ethanol was amperometrically detected using H_2O_2 as redox indicator at +0.6 V using a polyaniline-encapsulated AuNPs-AOx assembly stabilized on a GCE by chitosan–Nafion mixture (Chinnadayyala et al. 2015). The quantification of ethanol has also been reported through macromolecular designs involving polyfluorene-*g*-poly(ethylene glycol)/CNTs/AOx/graphite electrodes (Bekmezci et al. 2019), poly (brilliant green)/CNTs/AOx/carbon film electrodes, and poly (3,4-ethylenedioxythiophene)/CNTs/AOx/carbon film electrodes (Barsan et al. 2016). To avoid the presence of interfering compounds in the samples, horseradish peroxide (HRP) has been used as an attractive alternative for the measurement of hydrogen peroxide. Thus, the released hydrogen peroxide in the AOX reaction can be bioelectrocatalytically reduced by HRP through direct electron transfer mechanism to generate the current response (Hooda et al. 2018a, 2018b). Based on this mechanism, both enzymes have been immobilized onto AgNPs/polyaniline/GCEs (Santos et al. 2017) and onto hybrid nanomaterial-based biosensors (Hooda et al. 2018b, 2020). Hooda et al. have described biosensor assemblies by covalently immobilizing AOx with polyvinylchloride and glutaraldehyde followed by HRP onto AgNP-, chitosan-, and CNT-modified gold electrode (Hooda et al. 2018b) or HRP, AuNPs, chitosan, CNTs, and Nafion nanocomposite onto the surface of a gold electrode (Hooda et al. 2020). ADH is more stable than AOx-based biosensors, where the catalysis of oxidation of alcohols utilizes NAD^+ or $NADP^+$ as electron acceptor.



This prerequisite of external addition of a cofactor during the chemical reaction involved in ADH biosensors poses a limitation to them (Hooda et al. 2018a). Many efforts have been made to improve their sensitivity by using mediators and signal promoters such as nanomaterials or redox polymers. For instance, Eguílaz et al. immobilized ADH by entrapment with Nafion on GCEs modified by CNTs covalently functionalized with polytyrosine (Eguílaz et al. 2016). ADH has also been immobilized by adsorption onto GCEs modified with aluminum hydroxide/iron hydroxide/CNT composites (Wang et al. 2020b), magnetic-AuNPs/carbon paste electrodes (Samphao et al. 2015), magnetic-AuNPs/MnO_2/carbon paste electrodes (Samphao et al. 2015), poly(brilliant green)/CNTs/carbon film electrodes (Barsan et al. 2016), and poly(3,4-ethylenedioxythiophene)/CNTs/carbon film electrodes (Barsan et al. 2016). Ethanol biosensors based on the combination of ADH with several nanomaterials have been reported as well: ADH has been cross-linked with glutaraldehyde to SPCEs modified by CNTs, AuNPs, and polyneutral red (Bilgi and Ayranci 2016, 2018), entrapped within Nafion onto RuO_2/graphene nanoribbon composite supported on SPCEs (Vukojević et al. 2018), and dropped onto SPCEs modified by CNTs or CNTs blended with TiO_2 NPs combined with room-temperature ionic liquids, which facilitate the enzyme immobilization and the kinetics of electron transfer due to their intrinsic electrical conductivity (Zappi et al. 2019).

10.4.6 Phenolic Compounds

Many different industries, such as textile, pesticides, petrochemical or pharmaceutical, release multiple phenol and their derivatives into the environment. These molecules exhibit severe toxicity, and their sensitive detection is important to restrict their concentration to a minimum level. Several enzyme-based electrochemical sensors are reported for this cause (Bensana and Achi 2020). Laccase is one of the most commonly used enzymes for the phenol detection owing to its high stability and its wide spectrum of substrates (Othman and Wollenberger 2020). Laccase is a multi-copper enzyme, which catalyzes the oxidation of phenols to quinone by a four-electron staggered reaction, and quinone is measured to determine the concentrations of phenol (Sarika et al. 2017). In this way, laccase-based nanostructured biosensors built by the modification of different electrodes with nanomaterials have often been used. These biosensors are based on the modification of the working electrode with organic nanomaterials including CNTs (Coelho et al. 2019; Othman and Wollenberger 2020; Uc-Cayetano et al. 2020) and GO, using different

immobilization strategies, for the analysis of different phenolic compounds (Boujakhrout et al. 2016; Nazari et al. 2019). Modified electrodes with different kinds of NPs have also been constructed for the immobilization of laccase. Thus, AuNPs/GCEs (Maleki et al. 2019) and AuNPs/SPCEs (Mazlan et al. 2017) have been used in the determination of catechol and azo-dye tartrazine, respectively. ZnO NPs and α-Fe_2O_3 NPs have also been incorporated to carbon paste electrodes (Mendes et al. 2017; Sarika et al. 2017).

Laccase-based biosensors, in which the electrode has been modified by hybrid nanomaterials, have been developed for the analysis of catechol and hydroquinone. Thus, AuNPs mixed with CNTs (Albayati et al. 2019) and graphene nanoplatelets (Zrinski et al. 2020) have been used in the modification of GCEs and SPCEs, respectively. A new nanomaterial, composed of Cu and carbon nanofibers decorated with Ag-doped TiO_2 NPs, has been prepared to obtain composites that have been mixed with laccase and Nafion to construct a novel hydroquinone biosensor (Yang et al. 2016).

Tyrosinase has also been selected as the biosensing element for the development of nanostructured biosensors for the determination of phenolic compounds. This enzyme catalyzes the oxidation of these compounds to quinone species, which can be further reduced at the enzyme electrode to generate electrochemical signals (Wee et al. 2019). Thus, highly sensitive phenolic biosensors have been developed by means of the modification of SPCEs with carbon nanomaterials, including CNTs (Wee et al. 2019) and functionalized rGO (Hua et al. 2016). Different NPs, namely, AuNPs (Cerrato-Alvarez et al. 2019), gold nanorods (Ribovski et al. 2019), IrOx NPs (Kurbanoglu et al. 2015), and ZrO_2 (Ahmad et al. 2016), have also been used in the modification of disposable electrodes. GCEs have also been selected for the immobilization of tyrosinase after the modification with AuNPs (Dong et al. 2017; Vicentini et al. 2016) and poly(3,4-ethylenedioxythiophene) NPs decorated graphene QDs (Erkmen et al. 2021) and CdS QDs (Han et al. 2015). Hybrid nanomaterials have been used as well for the modification of both types of working electrodes. In this way, carbon nanofibers/AuNPs and graphene-decorated AuNP materials have been used in the modification of SPCEs (Bounegru and Apetrei 2020; Fartas et al. 2017). Hybrid graphene nanomaterials have also been used in the modification of GCEs by means of their combination with Fe_2O_3 NPs (Sethuraman et al. 2016) or choline-functionalized AuNPs (He et al. 2017).

HRP reacts faster with a wider range of phenolic compounds and shows higher sensitivity compared to phenol oxidases. Thus, this enzyme has been frequently used to construct nanostructured biosensors for phenolic compounds. The reduction current registered in this kind of biosensors is proportional to the phenolic compound concentration in the sample (Hernández-Cancel et al. 2015). A comparative study of CNTs, fullerene, and hydroxylated fullerene-modified carbon paste electrode has been performed by *Santos* et al. (Santos et al. 2015), founding that all the three nanostructured matrices show a sensitivity improvement. CNTs have also been used for the modification of SPCEs by cross-linking (Duarte et al. 2021) or adsorption (Chekin et al. 2015) of the enzyme. Covalent immobilization has been performed

using GCEs modified with AuNPs (Hernández-Cancel et al. 2015) and poly(dopamine)-modified magnetic NPs (Martín et al. 2015).

10.4.7 Ascorbic Acid

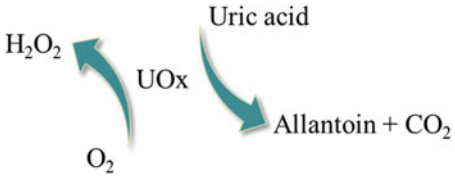
One of the most interesting areas of research is still the accurate determination of vitamin C due to its high importance for both human life and industrial applications. In recent years, research results related to nanomaterials have been increased in the ascorbic acid field due to the development in the preparation and application of carbon nanomaterials and metal/noble metal NPs (Lawal 2018; Siddeeg et al. 2020; Yang et al. 2015). The enzyme ascorbate oxidase (AAO) has been used to increase the specificity of the sensor. This multi-copper protein and oxidoreductase can specifically catalyze ascorbic acid to generate dehydroascorbic acid and water in the presence of molecular oxygen (Ma et al. 2018).

AAO has been immobilized onto fullerenes/carbon rods and CNTs/carbon rods by using bovine serum albumin and polyurethane (Barberis et al. 2015), onto GO/ZnO membrane-based arrayed screen-printed electrodes with 3-glycidoxypropyl-trimethoxysilane as cross-linker (Chou et al. 2019), and onto gelatin B/ZnONP-based nanocomposite film deposited on indium tin oxide glass plates with glutaraldehyde (Rawat et al. 2015).

Different enzyme-based nanohybrid materials have been fabricated as well via immobilizing AAO on the surface of electrodeposited AuNPs and rGO-modified GCEs (Ma et al. 2018), onto poly(l-aspartic acid) film fabricated on carbon nanofiber and nanodiamond particle-modified GCEs (Kaçar and Erden 2020), and on hydroxyapatite nanowire/rGO/AuNPs/GCEs (Zhao et al. 2020).

10.4.8 Uric Acid

The antioxidant uric acid is the main end product of purine metabolism in humans (Jain et al. 2019; Yu et al. 2018). It is found in urine or blood serum and excreted by the kidneys, and it is responsible for many biological changes in the human body (Jain et al. 2019; Lawal 2018; Tiwari et al. 2016). Monitoring uric acid levels is of great importance (Lawal 2018; Tiwari et al. 2016; Yu et al. 2018). Recent studies in the field of nanostructures and biocompatible nanomaterials together with biotechnology have led to improved biosensors for this target (Ahmad et al. 2015; Jain et al. 2019; Tiwari et al. 2016). Uricase (urate oxidase, UOx) catalyzes the oxidation reaction of uric acid to produce allantoin and hydrogen peroxide. This selective detection has been proved to be hugely fruitful (Verma et al. 2019). The most important challenge here is the monitoring of hydrogen peroxide in the presence of many different interfering molecules with similar redox potential and overlapping current response. To avoid this problem, different redox mediators and/or a suitable transducer matrix is used for optimum enzymatic activity (Jirakunakorn et al. 2020; Verma et al. 2019).



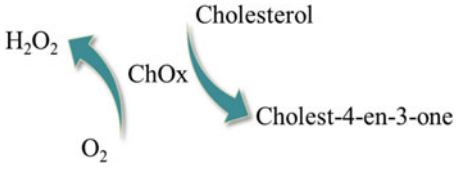
Enzymatic biosensors whose electrode has been modified by metal NPs and CNTs are particularly noteworthy. Uric acid detection has been performed by immobilizing UOx on Fe_3O_4 NP-modified carbon paste electrodes (Erdogan and Kucukkolbası 2021), on Cu_2ZnSnS_4 NPs/ITO glass electrodes (Jain et al. 2019), or on cysteamine self-assembled monolayers created on the surface of a screen-printed gold electrodes modified with AuNPs (Hernandez et al. 2021). UOx biosensors have also been constructed based on poly(vinylferrocene), CNTs, and gelatin-modified GCEs by using *N*-ethyl-*N*′-(3-dimethyaminopropyl) carbodiimide and N-hydroxysuccinimide chemistry (Erden et al. 2015), on CNTs/CuO/carbon paste electrodes (Buenaventura et al. 2020), and on CNTs/gold thin film with Nafion (Fukuda et al. 2020). The attractive features of graphene, GO, and graphene QDs also play an important role in providing a favorable microenvironment for enzyme immobilization. UOx has been successfully immobilized on porous cryogel platform of graphene-incorporated chitosan on top of a Prussian blue layer electrodeposited on SPCEs (Jirakunakorn et al. 2020) or GCEs (Zheng et al. 2019), on GO/GCEs (Omar et al. 2016), on GO/Pt (de Fátima Giarola et al. 2018), and on graphene QDs/GCEs (Yu et al. 2018).

UOx has been immobilized to ZnS nanomaterials/ITO glass electrodes via entrapment method (Zhao et al. 2017), to ZnO nanosheets deposited onto Ag/Si electrodes (Ahmad et al. 2015), to ZnO QDs/SPCEs (Ali et al. 2018), and to hybrid nanostructures. Likewise, AuNPs decorated rGO nanocomposite thin films with enhanced electroactive characteristics have been prepared and covalently immobilized with UOx onto ITO-coated glasses (Verma et al. 2019). Chitosan/UOx–poly(furan-3-boronic acid)–PdNPs/plated Pd/CNTs/Au electrodes (Sun et al. 2015b), UOx/CeO_2 with extensive oxygen vacancies–C–rGO nanocomposites/GCE (Peng et al. 2018), UOx/bull serum albumin/ß-lactoglobulin-CNTs-PtNPs/GCEs (Han et al. 2019), and UOx/AuNPs-hydroxyapatite nanowires-rGO/GCEs (Chen et al. 2020) have been described as well. A bienzymatic biosensor (UOx for uric acid oxidation and HRP for higher electron transfer) has been reported for uric acid measurement in different biological fluids (Bhushan et al. 2019).

10.4.9 Cholesterol

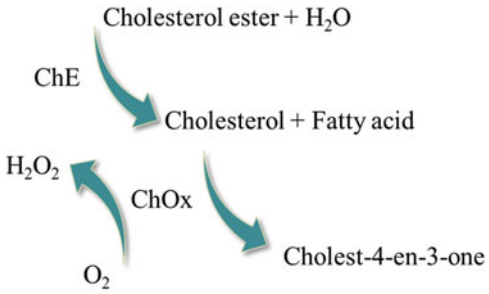
Cholesterol is a very important molecule required for the structural stability of the cells and also acts as a precursor molecule for different vitamins and important

hormones, e.g., bile acids and vitamin D (Gholivand and Khodadadian 2014; Huang et al. 2018; Nandini et al. 2016). The presence of high levels of cholesterol in the blood has been related to serious diseases, including arteriosclerosis, cerebral thrombosis, and coronary diseases (Gholivand and Khodadadian 2014), while the presence of low levels of cholesterol is associated with diseases such as hypolipoproteinemia, hyperthyrea, and septicemia (Huang et al. 2018). Therefore, the development of a sensitive cholesterol biosensor is very important, where cholesterol oxidase (ChOx) is the most commonly used bio-sensing element. ChOx is a FAD-dependent oxidoreductase enzyme that catalyzes the oxidation of cholesterol. The enzymatic activity can be illustrated as:

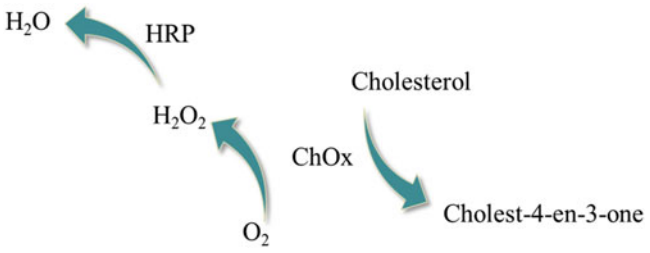


The electrochemical measurement of H_2O_2 generated by oxidation or reduction allows the quantification of cholesterol levels. Different nanomaterials have been used for the development of ChOx-based electrodes including ZnS QDs (Suganthi et al. 2016) deposited over a conductive carbon paper support and SnO_2 QDs mixed with the enzyme on a carbon paste electrode (Tığ et al. 2016). However, hybrid nanomaterials have more frequently been used in the construction of this type of biosensors. In this way, CNTs have been combined with different nanomaterials including NPs such as nickel ferrite NPs (Moonla et al. 2017) and PtNPs which have a high catalytic activity for H_2O_2 electrooxidation (Xu et al. 2016). Other hybrid nanomaterials include mixtures of graphene with TiO_2 deposited on an ITO electrode (Komathi et al. 2016) and rGO combined with PtNPs using SPCEs (Phetsang et al. 2019). MoS_2 QDs joint with AuNPs in a GCE have also led to good results in the analysis of cholesterol in real samples (Lin et al. 2016). Amperometric ChOx biosensors based on the electrochemical response of a redox mediator have also been described. In this way, Prussian blue has been used as a mediator for the construction of CNTs/SPCEs (Salazar et al. 2019) and AuNPs/GCE (Wang et al. 2019) biosensors. Ferricyanide has also been used as an effective electrochemical mediator in ChOx-based biosensors based on the modification of different electrodes with AuNPs (Sharma et al. 2017), AgNPs (Nantaphol et al. 2015), and AuNPs/CNTs (Alagappan et al. 2020). Voltammetric biosensors for the analysis of cholesterol have been developed using carbon nanomaterials such as graphene, GO, and rGO (Li et al. 2015; Pramanik et al. 2018) and CdTe/CdSe/ZnSe QDs (Mokwebo et al. 2018). These biosensors have frequently been based on the consumption of dissolved oxygen during cholesterol oxidation resulting in a decrease in current response, obtained by DPV, with increase in cholesterol concentration. There are also electrochemical ChOx-based electrodes based on the measurement of the analytical response related to the electrochemical oxidation of the enzyme after the

enzymatic reaction has taken place ($ChOx_{red} \rightarrow ChOx_{Ox} + e^-$). For example, a GCE modified with different ZnO@ZnS nano-heterostructures and ZnS nanotubes has been used for the sensitive analysis of cholesterol (Giri et al. 2016). The modification of CNTs with metal oxide NPs can also improve the performance of an electrochemical biosensor. Thus, *Jayanthi* et al. (Jayanthi and Suja 2020) describe an electrochemical biosensor based on a carbon paste electrode, CNTs, and TiO_2 NPs using DPV as the analytical technique. The biosensor takes advantage of the interesting features exhibited by the TiO_2 NPs like low cost, stability, and biocompatibility. DPV has also been used for the analysis of cholesterol in human blood serum using CNTs modified with ZnO NPs deposited on a fluorine-doped tin oxide glass substrate by dip coating (Ghanei et al. 2020). Cholesterol can be present in different forms: the free form and the esterified form. In order to determine total cholesterol, several electrochemical biosensors have been developed based on the immobilization of ChOx together with cholesterol esterase (ChE). These biosensors are frequently based on the electrochemical measurement of the H_2O_2 generated according to the following scheme:



Both enzymes have been used in the generation of amperometric biosensors based on the modification of a Pt electrode (Rahim et al. 2018) and a SPCE (Feng and Liu 2015) with AuNPs and a Pt-Ir wire electrode modified with CNTs (Taogesi et al. 2015). There are also several works describing the determination of cholesterol by means of linear sweep voltammetry using different electrodes modified with both enzymes. These works are based on the reduction of Ag^+ by the enzymatic reaction products. The deposited Ag produced a detectable anodic stripping signal. The nanomaterials used in the modification of these kinds of biosensors have been hybrid combinations of AuNPs/GO (Huang et al. 2018) and AuNPs/rGO (Zhu et al. 2019). Cyclic voltammetry (CV) has also been used as an electrochemical technique for the determination of cholesterol with ChOx/ChE-based biosensors. In this case, ITO electrodes have been modified by entrapment using CdS QDs (Dhyani et al. 2015) or by cross-linking using AuNPs (Sharma et al. 2015). Bienzymatic biosensors based on the immobilization of ChOx and HRP enzymes have been developed for the sensitive and selective determination of cholesterol based on the following mechanism:



As it has been above-mentioned, the subsequent reduction of the enzyme can be followed using different modified electrodes. Amperometric (Xu et al. 2015) and CV (Satvekar et al. 2015) measurements have been performed using ITO electrodes modified with AuNPs (Xu et al. 2015) and DNA-assembled Fe_3O_4@Ag nanorods (Satvekar et al. 2015). Multienzyme biosensors based on the incorporation of nanomaterials and ChOx, ChE, and HRP enzymes in different electrodes have also been developed. Amperometric determination of cholesterol has been performed using hybrid nanomaterials based on AuNPs and CNTs. The mixture of both materials with paraffin formed the electrode surface for the covalent immobilization of the enzymes (Lata et al. 2016). A hybrid material has also been used by *Rashidi* et al. (Rashidi et al. 2018) for the generation of a cross-linking multienzymatic biosensor using a GCE modified with AuNPs/chitin–ionic liquid/poly (3,4-ethylenedioxypyrrole)/graphene-CNTs-1,1′-ferrocenedicarboxylic acid–ionic liquid. Core–shell NPs are promising nanomaterial for the development of electrochemical biosensors, which can be customized by changing the core-to-shell ratio of constituting materials. For example, Fe_3O_4@C@Ag NPs have been used for the modification of an ITO electrode based on the solgel entrapment of the mixture of enzymes (Satvekar and Pawar 2018).

10.4.10 Drugs

The accurate measurement of different active compounds in drug molecules is very important to study the effect of these molecules. Sensitive assays for the drug molecules are required for both medical science and pharmaceutical development, where the effect of small changes in drug concentration leads to huge change in response to different diseases or leads to unnecessary side effects. Thus, the successful analysis requires high sensitivity and selectivity that can be obtained using enzyme electrochemical biosensors (Aydin et al. 2018). Cytochrome P450 (CYP450) is one of the most commonly used enzymes in electrochemical biosensor for drug analysis. These enzymes are responsible of metabolic reactions of exogenous and endogenous compounds, including drugs (Tian et al. 2017). In this way, the direct determination of several drugs can be performed by means of CV (Ajay et al. 2020; Feleni et al. 2019, 2020) using a GCE modified with a hybrid nanomaterial consisting of GO and AgNPs in which the enzyme was immobilized by adsorption

(Ajay et al. 2020) and gold electrodes modified with PdTe QDs using a covalent immobilization of the enzyme (Feleni et al. 2019, 2020). Amperometry has also been used in the analysis of drugs such as omeprazole using a GCE modified with CeNPs and rGO. In this case, the enzyme was immobilized by cross-linking (Tian et al. 2017). The inhibition effect of drugs, such as abiraterone, in CP450 activity has also been used by the adsorption of the enzyme on a SPCE modified with CNTs (Aliakbarinodehi et al. 2016). This inhibiting effect of some drugs also forms the basis for different biosensors based on tyrosinase (Beilinson et al. 2021; Kurbanoglu et al. 2015, 2017b), monoamine oxidase (Medyantseva et al. 2015a, 2017), and α-glucosidase (Mohiuddin et al. 2014, 2016). Tyrosinase amperometric biosensors using different disposable electrodes, such as SPCE modified with IrOxNPs (Kurbanoglu et al. 2015) or IrOxNPs/rGO (Kurbanoglu et al. 2017b) and SPPtE modified with several nanomaterials (Beilinson et al. 2021), have been developed. SPCE disposable electrodes have also been used for the immobilization of monoamine oxidase using CNTs (Medyantseva et al. 2015a) and CNTs/AuNPs or CNTs/AgNPs (Medyantseva et al. 2017). In the α-glucosidase-based biosensors, the decrease in the analytical signal due to the inhibitory effect was followed by CV using SPCE modified with CNTs using a covalent immobilization of the enzyme (Mohiuddin et al. 2014, 2016). HRP has also been selected as the bioelement in electrochemical biosensors for the analysis of drugs following the analyte oxidation by DPV. Thus, a GCE modified with CNTs and TiO_2NPs (Chokkareddy et al. 2017) and a GCE modified with rGO and ZnO QDs (Chokkareddy et al. 2018) have been used for the analysis of isoniazid and ethambutol, respectively. In both cases, the enzyme was immobilized by adsorption. Other enzymes have been used in the development of nanostructured biosensors for the direct analysis of different drugs: acetylcholinesterase (AchE) using a graphene/AuNPs/SPCE (Long et al. 2015), amidase using inkjet-printed CeO_2/graphene/CNT electrode (Stanković et al. 2019), and human flavin-containing monooxygenase 3 (hFMO3) using a GCE/GO (Castrignanò et al. 2015).

10.4.11 Hormones

Hormones are signaling molecules important for the proper growth and development and also to maintain the physiology/metabolism of the organism. These hormones are secreted by different glands, and travel, either by simple diffusion or by circulation in the bloodstream, to specific target cells for its action. Their detection is interesting for clinical and pharmaceutical studies and also for disease diagnosis. The physiological concentration of the hormones is in nM or even less. Thus, highly sensitive analytical methods are required for the accurate measurement of these molecules, where enzymatic electrochemical biosensors are used widely (Bahadir and Sezgintürk 2015). Different enzymes have been used in the construction of this type of biosensors, with laccase being one of the most used. The analysis of the target analyte (substrate) with this kind of biosensors can then take place following three different procedures: direct oxidation of the substrate, oxidation of the

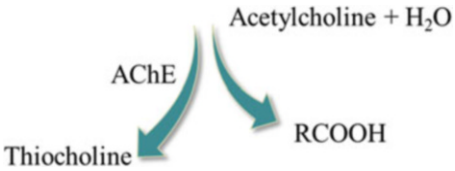
substrate with the participation of a mediator, or during the coupling reactions (Spychalska et al. 2020). Direct oxidation of hormone estriol has been analyzed using an amperometric method based on a GCE modified with rGO/Sb_2O_5 hybrid nanomaterial. Sb_2O_5 resulted in an excellent metal oxide due to its exceptional acidic properties and surface defects, which benefit the incorporation of other species on its surface and the electrocatalytic transformation of important compounds (Cincotto et al. 2015). The direct oxidation of 17α-ethinylestradiol has also been registered using and amperometric biosensor based on a novel electrospun nanofibers of polyvinylpyrrolidone/chitosan/rGO deposited onto a fluorine-doped tin oxide electrode (Pavinatto et al. 2018). CV has also been used in the direct oxidation analysis of β17-estradiol using a gold electrode modified with graphene QDs. Laccase was immobilized on the surface of the modified electrode by covalent cross-linking with glutaraldehyde (Spychalska et al. 2020). The analysis of β17-estradiol has also been performed using the participation of a thionine as mediator (Povedano et al. 2017). In this case, DPV measurements have been carried with a GCE modified with an rGO/RhNP nanohybrid material. The working electrode was coated with the nanocomposite and used to support the cross-linking of the enzyme–glutaraldehyde for the development of a voltammetric 17β-estradiol biosensor.

Other enzymes used in the development of electrochemical biosensors for the analysis of hormones have been tyrosinase (Erkmen et al. 2021) and CYP3A4 (Xu et al. 2018). The amperometric determination of epinephrine and norepinephrine has been carried out using poly(3,4-ethylenedioxythiophene) NPs decorated graphene QDs using a SPCE platform. Norepinephrine and epinephrine acted as substrates of the tyrosinase enzyme immobilized by cross-linking with glutaraldehyde in the described modified electrode (Erkmen et al. 2021). A facile electrochemical enzymatic CYP3A4 nanoreactor for the amperometric analysis of steroid hormones has been developed by using a polydopamine/nanoporous graphene foam-modified GCE electrode. The enzymatic activity of CYP3A4 can be effectively regulated by changing the pore diameter of PNGFs.

10.4.12 Pesticides

Pesticides are widely used to manage the pest and diseases in agricultural practices and thus to increase the productivity of the crops. However, the adverse effects of these compounds, especially on soil, water, and animals, are tremendous. The current high consumption of pesticides has made their effects on the environment a problem of global concern. Therefore, many nations and international organizations have restricted the use of several acute pesticides and slowed down the approval of new active pesticide compound. Developing analytical methods, including nanomaterial-based electrochemical biosensors, for pesticide detection is a great challenge for analysts and a crucial research and development topic for analytical chemistry (Dhull et al. 2021; Karadurmus et al. 2021; Kurbanoglu et al. 2017a; Wang et al. 2020a; Xiong et al. 2018). According to the reaction mechanism, the electrochemical enzyme-based biosensors for pesticide detection can be

classified as either inhibition biosensors or catalytic biosensors (Wang et al. 2020a). The inhibition biosensors used in the determination of pesticides have been based on the detection of the compound of interest by analyzing the residual activity of various enzymes such as AChE, butyrylcholinesterase (BChE), tyrosinase, and alkaline phosphatase, with AChE being the most used (Karadurmus et al. 2021; Pundir et al. 2019; Wang et al. 2020a). In catalytic mode, the enzyme organophosphorus hydrolase (OPH) catalyzes the hydrolysis of organophosphorus pesticides as direct substrates providing an amperometric signal (Karadurmus et al. 2021; Wang et al. 2020a). Most recent works for the determination of pesticides using nanomaterial-modified enzyme biosensors are based on the inhibition of the enzyme AChE. This enzyme catalyzes the hydrolysis of compounds such as acetylcholine (ACh) or acetylthiocholine (ATCh), generating electroactive products that can be detected using an electrochemical technique:



Recently, an amperometric biosensor based on PPy/AuNPs nanocomposite deposited on a Pt modified ITO electrode by means of Electropolymerizaton has been developed for the carbaryl pesticide detection (Loguercio et al. 2021). Soulis et al. developed an amperometric biosensor to study the effects of surface functionalization of SPCEs with carbon black (as an alternative to graphene and CNTs) on the detection of pesticides. The sensing of an organophosphorus (chlorpyrifos) and a carbamate pesticide (carbofuran) was performed based on the rate of enzyme inhibition using ATCh as the substrate (Soulis et al. 2020).

Voltammetric techniques including DPV and square wave voltammetry (SWV) have also been used in the recent development of biosensors based on the inhibition of AChE. In this way, the determination of pesticides such as malathion and methyl paraoxon in cabbage and apple samples has been performed using a GCE modified with nitrogen-doped Mo_2C nanoflowers, and the enzyme was immobilized by adsorption. The built biosensor was based on the special properties of transition metal carbides such as Mo_2C that make them a potential alternative to noble metals (Zhou et al. 2021). Wang et al. (2020c) have also taken into account these special properties for the construction of a biosensor sensitive to the concentration of dichlorvos pesticide. The biosensor was based on the immobilization of the AChE enzyme on a $Ti_3C_2T_x$–chitosan matrix deposited on a graphene-modified GCE. Chitosan nanocomposites with SnS2 (Liu et al. 2020) and TiO2 (Hu et al. 2020) NPs deposited on a GCE have also been described for the determination of chlorpyrifos and dichlorvos, respectively. Other nanocomposite used in the construction of an AChE inhibition biosensor has been a mixture of zinc oxide nanoflowers and

rGO (Singh et al. 2020). In this case, a gold electrode was selected as the transducer, and a biosensor based on AuNPs-MoS_2-rGO/polyimide flexible film was used for paraoxon detection obtaining a large current response by DPV, demonstrating successful immobilization of AChE based on the specific strong interaction between AuNPs and S (Jia et al. 2020).

Other recently developed biosensors in pesticide determination are based on the use of bienzymatic systems combining AChE and HRP enzymes (Jiaojiao et al. 2020) or of new enzymes such as urease (Thanh et al. 2021). In the first case, functionalized CNTs (Cl/CNTs) deposited on a GCE have been used for the adsorptive immobilization of both enzymes. The bi-enzyme system was finally used for the determination of monocrotophos pesticide by means of DPV. The urease biosensor was based on the inhibition of the hydrolysis reaction of urea, catalyzed by this enzyme. The enzyme was immobilized by cross-linking on a SPAuE modified with an rGO/CNTs/octahedral-Fe_3O_4/chitosan composite material, and the inhibition response was followed by SWV. The developed biosensor exhibited good sensing performance and was able to detect glyphosate herbicide in river water with high accuracy and good recovery (Thanh et al. 2021).

10.5 Conclusions

In recent years, it has been observed a great development in enzymatic biosensors for numerous analytical applications in fields of special interest such as environmental science, food industry, clinical trials, and pharmaceuticals, considering the large number of references described and their business potential (Kumar et al. (2022), Mahato et al. (2022) and Purohit et al. (2022)). The high specificity of the biological recognition processes in enzymatic reactions has allowed the development of numerous electrochemical devices, which have become very useful in the determination of numerous substances. On the other hand, recent advances in nanotechnology offer opportunities to design and construct new effective biosensors in different fields of analysis due to the unique features of nanomaterials. Thus, the modification of the working electrode with different nanomaterials, previously to the enzyme immobilization, has attracted attention because of their valuable properties, as they are biocompatible, selective, and sensitive and provide highly accurate results. In addition, it has been shown that the use of these nanomaterials produces an interesting catalytic effect, facilitating a more efficient transfer of electrons between immobilized biomolecules and electrode substrates. Moreover, they present resistance to corrosion, biocompatibility, and preservation of biological activity while keeping the enzyme immobilized in a suitable microenvironment. These properties together with the great versatility and low cost of this technology are responsible for its current continuous development.

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Fluorescent Aptasensors for Point-of-Care Detection of Environmental Pollutants

11

Sanjay Singh, Surbhi Goel, and Neeti Kalyani

Abstract

Early detection or accurate sensing of toxic contaminants is one of the many problems worldwide. The growth of industries and the controlled or uncontrolled discharge of toxic pollutants are the main causes of generating huge amount of environmental pollutants. To solve this problem and meet the growing demands of the modern world, studies have been conducted to design fast-track diagnostic. Due to the great need for clinical trials, occupational health, public health and social security, the quantification of pollutants is becoming increasingly important. Due to improvements in biosensing techniques, rapid and consistent methods have been realized for the precise and sensitive diagnosis of environmental pollutants. For the detection of pollutants, optical methods are ideally suited as no sample preparation is needed which results in rapid, easy and economic analysis. In this chapter, fluorescence-based aptasensors for the detection of environmental pollutants are discussed. This chapter highlights the various fluorescence-based methods that can be utilized for the detection of the pollutants at the point of sample collection.

S. Singh
Tulane University, New Orleans, LA, USA

S. Goel
Indian Institute of Technology Delhi, Delhi, India

N. Kalyani (✉)
Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark

B. Purohit, P. Chandra (eds.), *Surface Engineering and Functional Nanomaterials for Point-of-Care Analytical Devices*, https://doi.org/10.1007/978-981-99-3025-8_11

Keywords

Fluorescence sensor · Aptamer · Environment pollutants · Optical sensor · Aptasensor

11.1 Introduction

Industrial revolution and urbanization, on the one hand, improved the overall quality of life and fulfilled the increasing demands of ever-increasing population. However, on the other hand, rapid industrialization and climate changes lead to generation and release of excessive waste polluting our soil, water and air, and to meet food requirements, the use of chemical pesticides in agriculture has been increased (Khan and Ghouri 2011; Rykowska and Wasiak 2015). These environmental pollutants including pharmaceutical wastes and pathogens are not just life-threatening to humans but also to other flora and fauna. A certain amount of these pollutants is expectable and can be tolerated by living beings, but even at small concentration if exposed for longer duration, these pollutants can pose serious health hazards (Yan et al. 2018; He et al. 2020b; Kalyani et al. 2021b). In present fast-paced life, relying on conventional methods like chromatography and spectrophotometric techniques for the detection of these pollutants can be troublesome as it requires expensive chemicals, time for sample preparation and result generation and skilled professional for operation of instruments (Long et al. 2013; Justino et al. 2017). The need for rapid detection that too at a site of sample generation is the need of the hour along with lowest possible limit of detection (McConnell et al. 2020).

A lot of research is being carried out for the development of these point-of-care biosensors to improve sensitivity and specificity, where the presence of an analyte causes the generation of signals, which in case of optical sensors can be measured as well as visible to naked eyes (Gahlaut et al. 2019a; Kalyani et al. 2020). Optical sensors compromise of a biological recognition element (an aptamer or enzyme) to bind a specific analyte (in this case environmental pollutants) and transducer element to generate measurable signal upon interaction of analyte with recognition element (Long et al. 2013; Bai et al. 2015; Chronopoulou et al. 2019). Optical sensors involve methods like colorimetry, where change in the colour can be monitored, and fluorescence methods, where the presence of analyte causes increase in fluorescence intensity, SERS and chemiluminescence (Kumar et al. 2015; Chatterjee et al. 2020). In this chapter, we will discuss various fluorescence methods utilizing aptamers to detect environmental pollutants for point-of-care applications.

A fluorescence-based sensor is an optical sensor that requires a fluorescent compound "fluorophore" and "quencher" for quenching fluorescence. This type of sensor has been very successful in identifying and quantifying environmental contaminants such as heavy metal ions, pesticides, drugs and toxins. Several basic schemes have been reported to transduce the binding of aptamer-ligand to fluorescent signals, like double-stranded structures with complementary sequences, molecular beacons and competitive laser-based flow assays (Fig. 11.1). The best known

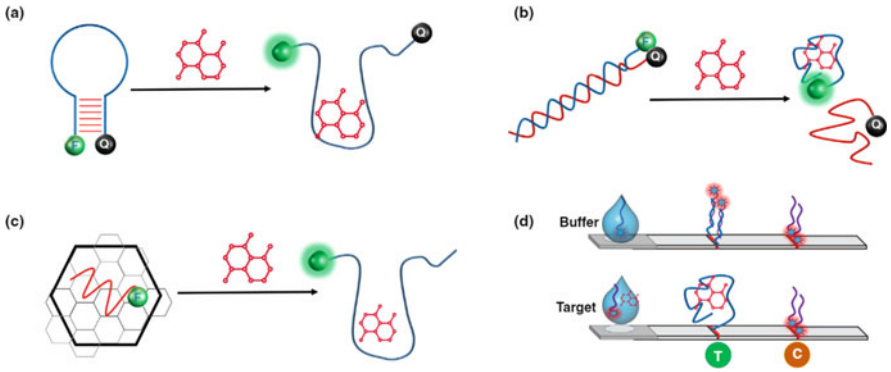

Fig. 11.1 Illustration of detection strategies of fluorescent sensor: (**a**) molecular beacons, (**b**) double-stranded structures with complementary sequences, (**c**) graphene-/CNT-based aptasensor and (**d**) competitive laser-based flow assays. Reprinted with permission from Nguyen and Kwon (2017). Copyright ©2022 Elsevier Ltd.

and most common method for fluorescence detection is the binding of aptamer to ligand by molecular structure-switching (Chen et al. 2012, 2014; Zhao et al. 2013). Fluorescence-based aptameric sensors can be categorized into two types: (1) signal-on and (2) signal-off, where increase or decrease of the fluorescence signal, respectively, can be observed after binding of aptamer to the target analyte. Both signal-off and signal-on fluorescent sensors have been shown to be successfully utilized to detect environmental pollutants (García-Gutiérrez et al. 2017). In case of signal-on biosensors, initially the aptamer is organized such that the fluorophore stays very close to the quencher, which creates a weak fluorescence signal. The interaction between aptamer and target molecule alters its conformation and causes separation of the fluorophore from the inhibitor, which in turn increases fluorescence signal. In signal-off sensors, if the target molecule is present in solution, the fluorophore and the inhibitor are coupled which leads to reduction of the fluorescence signal, which otherwise remains strong (Akki and Werth 2018) as shown in Fig. 11.1.

11.2 Environment Pollutants

11.2.1 Pesticides

Pesticides are a class of chemicals used to prevent and exterminate any pests and weeds and in return also aid in good crop productivity and yield. On the other hand, accumulation of these chemicals in land, soil and food and flushing of these chemicals in nearby water bodies are causing potent threat to humans and animals (Chatterjee et al. 2020). Some of these pesticides can be biodegraded in short time into harmless substances, while others have strong residues such as parathion (organophosphate) and dichlorodiphenyltrichloroethane (organochlorine) (Stewart et al. 1971; Tanabe et al. 1994). Thus, regular monitoring of the amount of pesticides

in environment as well as in food is required and that too in smaller concentration with precision and accuracy.

11.2.2 Antibiotics

The antibiotics are widely used (tetracycline, kanamycin chloramphenicol, ampicillin and oxytetracycline) for their broad-spectrum activity, cost-effectiveness and excellent action (Gahlaut et al. 2019b). In veterinary area, antibiotics are one of the most commonly used medicines and may lead to inordinate residues in animal products. Due to the widespread use of antibiotics for animals, their residues and metabolites can highly contaminate soil, food and water. Due to this, it is harmful for human health and results in antibiotic resistance, nephrotoxicity and allergic reactions, mainly in children (Kalyani et al. 2021a).

11.2.3 Mycotoxins

Toxins produced by microorganisms are a type of food contamination that causes severe damage to the health of humans. In general, they are divided into two types: bacterial toxins and mycotoxins. In foodborne diseases, the presence of mycotoxins, especially in water and food, is an important cause. Mycotoxins, a toxic chemical produced by fungi that can contaminate agricultural products before or after harvest (Marin et al. 2013). Mycotoxins are shown to be highly nephrotoxic, immunotoxic and even carcinogenic under prolonged exposure at low concentrations and are considered a major food contaminant worldwide (Guo et al. 2020; He et al. 2020b; Wu et al. 2014). Mycotoxins include aflatoxin B1 (AFB1), aspergillus (toxin T-2), patulin (PAT), ochratoxin A (OTA) and zearalenone (ZEN), and, among these, OTA and AFB1 are two most toxic and widespread in food products (Vidal et al. 2013). Considering the severe toxic nature and health hazards, the need to devise methods for their rapid detection with sensitivity and specificity becomes important (Wang et al. 2014; Ha 2015).

11.2.4 Heavy Metals

Heavy metals can pollute the environment and endanger human health worldwide. Generally, there are various sources of heavy metals that can be dumped into natural systems like rivers or soil. If the concentration of heavy metals in the soil is high, plant growth is inhibited. In addition, some heavy metals are difficult to decompose and get accumulated in the environment (Su et al. 2012), posing a serious threat to the human health (Li et al. 2013). Therefore, fast and effective methods with high accuracy and sensitivity for detection of heavy metals are very important.

11.2.5 Other Pollutants

Phenolic compounds (like bisphenol A (BPA), polyphenols and chlorophenols) and melamine (MA) are several other chemical contaminants that adversely affect human health. Bisphenol A, a widely used raw material for the production of polycarbonate, is a major endocrine disruptor. BPA is released at high pressure and high temperature, and special attention needs to be paid to its presence in drinking water (Toppari et al. 2002). Therefore, a sensitive and rapid PoC devise for BPA detection is required.

11.3 Fluorescence-Based Sensors for Environmental Pollutants

There are three main ways to modify fluorescence: the introduction of fluorescence resonant energy transfer (FRET), total fluorescence quenching and internal fluorescence filtration (IFE). FRET takes place between two fluorophores, one of which acts as fluorescence donor and another one as receiver, when present at acceptable distances. Fluorescence donors include quantum dots (QDs), various dyes, metallic nanoparticles (MNPs), carbon nanomaterials and carbon dots (CDs). Many nanomaterials, e.g. graphene oxide, gold nanoparticles (GNP), magnetic nanoparticles (MNP), carbon nanotubes (CNT) and MnO_2 layers, can be used as fluorescence inhibitors, with the potential to adsorb nucleic acid molecules (Wang et al. 2018b) and nanocomposite materials. Various combinations of nanomaterials have been developed to design numerous aptasensors with high sensitivity and performance with multiplexing capability (Xie et al. 2022). Metal nanoparticles such as GNP, AgNPs, and Ag nanoclusters are found to exhibit excellent fluorescence signal amplification properties (Yin et al. 2019; Zhu et al. 2019). It should be noted that some aptamer sensors rely on competition between the target and complementary strand. When the target is present in the sample, it binds to the aptamer and causes structural changes, and the complementary strand passes through a hybridization complex to form a variety of fluorescent signals (Yang et al. 2022).

As shown in Fig. 11.2, the complementary strand is bound to GNP to seize aptamers attached with fluorescent molecules. The core-shell upconversion nanoparticles conjugated with aptamers on graphene oxide in the presence of enrofloxacin to created a fluorescent resonant energy transfer (FRET) system. The graphene oxide with or without GNP-aptamer modification was the most fluorescent supports for aptamer sensors. GNP differentially binded with aptamers and fluorescence quenching; further target-induced release of the aptamer leads to restoration of fluorescence. When using fluorescent aptameric sensors, substances introduced by the food matrix and production technology should not create similar fluorescence and interfere with the detection of molecular fluorescence on the detection platform. Preliminary testing of 13 fruits and vegetables for many pesticides proved the feasibility of using fluorescent sensors to detect real sample's contaminants, and the recovery results were satisfactory (Zhang et al. 2020).

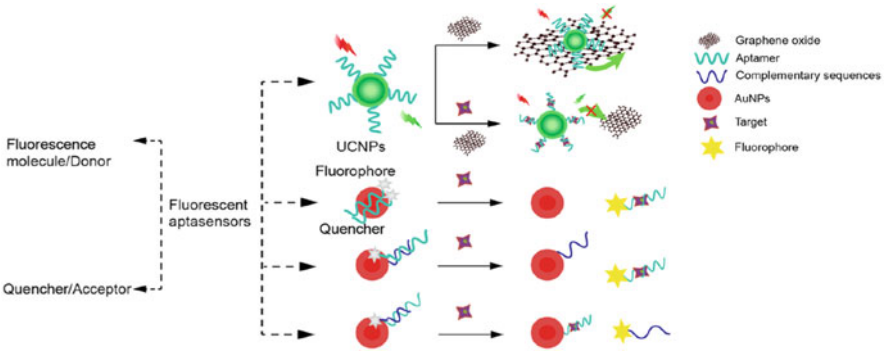

Fig. 11.2 Aptamer-based optical biosensor schematic illustration, including fluorescent biosensors. Reprinted with permission from Zhang et al. (2020). Copyright ©2022 Elsevier Ltd.

11.3.1 Fluorescent Aptasensors for Pesticides

A number of aptameric sensors for the detection of pesticide residues have been devised with high precision and increased sensitivity of the test. Table 11.1 summarizes some of the most recent pesticide studies published.

Acetamiprid is a broad-spectrum insecticide and acaricide with relatively low toxicity and is widely used as a substitute for organophosphorus substances. Detection of acetamiprid is important for sensitive and selective analysis of contaminants in food. In one study, three different complementary strands (CS) are used in the fluorescent aptamer sensor to improve its sensitivity and selectivity. With no acetamiprid present, the aptamer binds to CS1, causing FAM-labelled CS2 to bind specifically and indirectly to GNP by hybridizing with CS3 immobilized on the GNP, causing the GNP fluorescence quenching. As the target dissolves, it binds to a special aptamer and creates a strong fluorescence intensity, forming CS1-CS2-FAM (Bahreyni et al. 2018). Acetamiprid has a detection range of 5 to 50 nM with an LOD of 2.8 nM.

Based on the effect of internal filtration (IFE) of GNP on carbon points (CD), Wang et al. demonstrated a detection scheme that does not need modification of either aptamer or nanoparticles (Wang et al. 2018b). They tested the type and size of GNP to quench fluorescence of CD with internal filtration effect and to effectively adsorb the S-18 aptamer to inhibit agglomeration. The S-18 aptamer is bound to the target and significantly restored the fluorescence of the aptamer sensor in the presence of acetamiprid. The detection limit is only 1.08 g/L and the detection range is 5–100 g/L.

Simultaneous Multiplex Detection of Pesticides

Since organophosphorus pesticides are often mixed in agriculture, the latest aptamer detectors can simultaneously detect several organophosphorus pesticides, including isocarbophos, profenofos, phorate, omethoate, and other combinations. Three organophosphates have been quantified in vegetables using complementary

Table 11.1 List of aptasensors for the detection of pesticides using fluorescence-based methods

Target	Aptamer principle	LDR	LOD	References
Acetamiprid	CS1/CS2-FAM/ CS3-GNPs/ Aptamer	5–50 nM	2.8 nM	Bahreyni et al. (2018)
	IFE: GNPs toward to CDs	5–100 μg/L	1.08 μg/L	Wang et al. (2018c)
	FTIR spectra: Cationic carbon dots	1.6–120 nM	0.3 nM	Saberi et al. (2019)
	IFE: GNPs and UCNPs	0.025–1 μM	0.36 nM 5.73 nM	Yang et al. (2019a)
	N-methyl mesoporphyrin IX/G-quadruplexes/graphene oxide	20–500 nM		Zhao et al. (2021)
Malathion	FRET: Negatively charged upconversion nanoparticles and positively charged GNPs CdTe@CdS QDs	0.01–1 μM	1.42 nM	Chen et al. (2020)
Isocarbophos	Multi-walled carbon nanotubes and G-quadruplex/N-methyl mesoporphyrin IX	10–500 nM	10 nM	Li et al. (2018)
Carbendazim	Rhodamine B; aggregation of GNPs	2.33–800 nM	2.33 nM	Su et al. (2020)
Diazinon	Rare-earth-doped upconversion nanoparticles and graphene oxide	0.05–500 ng/mL	0.023 ng/mL	Rong et al. (2020)
Trichlorfon Glyphosate Malathion	FAM-Apt/NH_2-MNPs/mercapto-CS	0.0001–10 mg/L	72.20 ng/L 88.80 ng/L 195.37 ng/L	Jiang et al. (2020)
Chlorpyrifos Diazinon Malathion	"Turn-on": QDs-AuNSs/ aptamer-based lateral flow biosensor	100 pg/mL–10 μg/mL	0.73 ng/mL	Cheng et al. (2018)
		100 pg/mL–10 μg/mL	6.7 ng/mL	
		100 pg/mL–10 μg/mL	0.74 ng/mL	

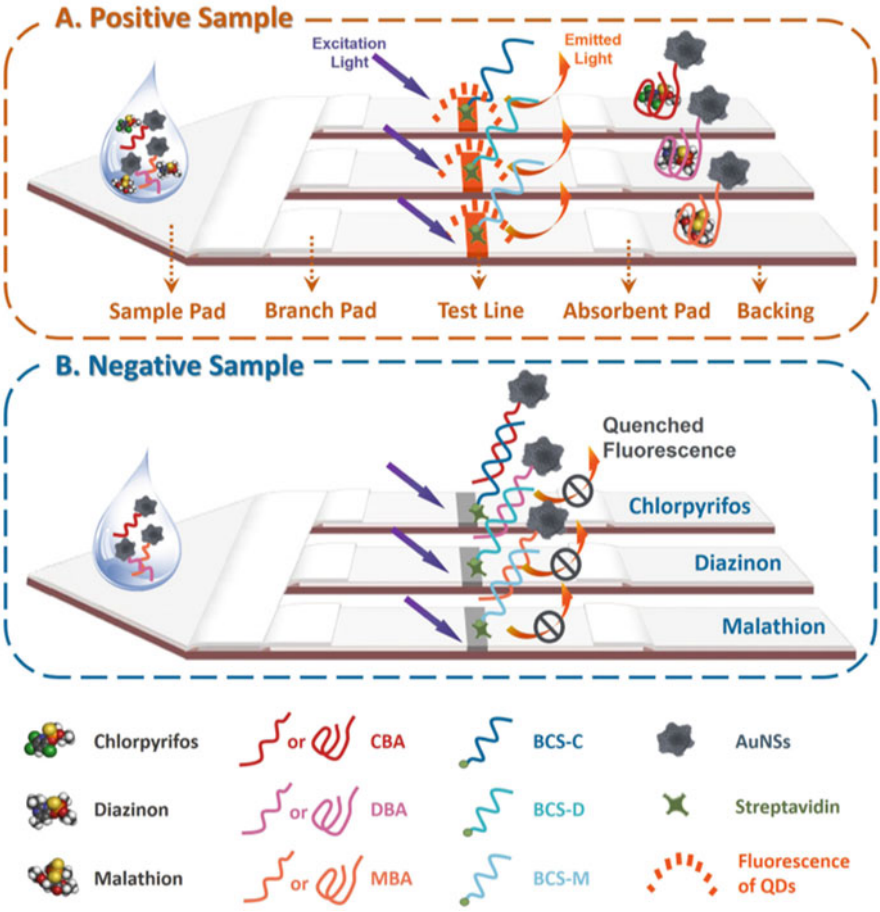

Fig. 11.3 Schematic depicting the lateral flow biosensors for multiplexed detection of different pesticides using aptamers. Reprinted with permission from Cheng et al. (2018). Copyright ©2022 Elsevier Ltd.

strand-coated sulfhydryl NH_2-MNP, as shown in Fig. 11.3 (Jiang et al. 2020). A lateral flow biosensor is developed for chlorpyrifos, diazinon and malathion. Aptamers were used as recognition element and interact with the target. In the absence of target, the fluorescence is high, and when target is present, the fluorescence is quenched.

11.3.2 Fluorescent Aptasensors for Antibiotics

Many nanomaterials and nanocomponents have been used in aptamer sensors to amplify sensor signals. Core-shell upconversion nanoparticles have been used as energy donors to detect enrofloxacin and graphene oxides as energy acceptors for

fluorescence resonance energy transfer-based detection (Zhang et al. 2020). To amplify the signal, a porphyrin-zirconium MOF (PCN-222) was used as a fluorescence quencher that effectively detected chloramphenicol and significantly attenuated fluorescence to increase fluorescence changes in the presence and absence of the target (Liu et al. 2020). Ma et al. have reported an innovative fluorescent-based detection of chloramphenicol method, where an exonuclease I-catalysed reaction was coupled with a hybridization chain reaction to get amplified fluorescent signals. The aptamers undergo conformation change to bind with chloramphenicol, releasing the initiator of hybrid chain reaction. Exonuclease I then helps to cleave the aptamer, releasing chloramphenicol, that continues to attach to the blocked aptamer to determine the free initiator with an LOD of 0.3 pM (Ma et al. 2020). A number of aptameric sensors for the detection of antibiotics residues have been devised with high precision and increased sensitivity of the test. Table 11.2 summarizes some of the most common antibiotics studies published.

11.3.3 Fluorescent Aptasensors for Toxins

In recent years, many attempts have been made to develop nanoparticle-based fluorescent aptamer sensors for the detection of mycotoxins in food (Table 11.3). For instance, graphene oxide-based aptasensor has been proposed to detect ochratoxin A (OTA) by fluorescence quenching of FAM-labelled aptamers (Sheng et al. 2011). In the absence of OTA, the adsorption of aptamer labelled with FAM on graphene oxide through π-π stacking force leads to quenching of the FAM fluorescence via transfer of energy from the dye to graphene oxide (Fig. 11.4). When OTA is added, the aptamer undergoes structural change to form G-quadruplex antiparallel. The antiparallel G-quadruplex then gets released from the graphene oxide surface and restores fluorescence. Under optimized condition, the OTA can be detected within 2–35 μM concentration, and LOD of 1.9 μM is reported. In another study, fluorescent aptasensor based on single-walled carbon nanoparticles (SWCNT) was studied, where SWCNTs were used for the quenching of fluorescence FAM-labelled OTA aptamers. A low limit of detection of 24.1 nM was observed with a range of detection of 25 nM to 200 nM (Guo et al. 2011).

However, the abovementioned biosensing strategy displayed binding of aptamer to one target, affecting the sensitivity of the biosensor. In recent years, DNAzymes or exonucleases have been employed to increase sensitivity through target retrieval. For example, an aptamer sensor for OTA detection based on a nanographite/aptamer hybrid has been reported (Wei et al. 2015). The study represents using nanographite as FAM fluorescence quencher and DNase1 for signal amplification. In the presence of OTA, the G-quadruple structure of the OTA aptamer will be generated, and FAM fluorescence will be recovered. Following which, DNase I is added to carry out the cleaving of the OTA-aptamer complex, releasing OTA. The released OTA must then absorb other devices on the nanomodeled surface and stimulate target recycling, resulting in continuous release of the aptamer, which greatly increases the fluorescence signal. The high sensitivity with an LOD of 20 nM was observed.

Table 11.2 List of fluorescent aptasensors for the detection of antibiotics

Target	Aptamer principle	Linear range	LOD	References
Chloramphenicol	FRET: FAM-Apt/PCN-222 Hybridization chain reaction	0.1 pg/mL–10 ng/mL 0.001–100 nM	0.08 pg/mL 0.3 pM	Liu et al. (2020) Ma et al. (2020)
Tetracycline	Triple-helix aptamer probe	5.0–100 nM	1.6 nM	He et al. (2020a)
Oxytetracycline	PicoGreen	2–800 nM	0.15 nM	Bahreyni et al. (2021)
Ampicillin	Polyethylene terephthalate: GNPs and 3,4,9,10-perylenetetracarboxylic acid diimide	100–1000 pM	29.2 pM	Esmaelpourfarkhani et al. (2020)
Florfenicol	Atto647N-labelled aptamer and graphene oxide	5–1200 nM	5.75 nM	Sadeghi et al. (2018)
Enrofloxacin	Graphene oxide and native Fluorescence of enrofloxacin FRET: CSUNPs and Graphene oxide	5–250 nM 0.976 ng/mL–62.5 ng/mL	3.1 ng/mL 0.47 ng/mL	Dolati et al. (2018) Zhang et al. (2020)
Ofloxacin	SYBR Green I and aptamer	1.1–200 nM	0.34 nM	Yi et al. (2019)
Kanamycin	Surface plasmon-enhanced energy transfer: Ag nanoclusters and GNPs	5–50 nM	1.0 nM	Ye et al. (2019)
Multiple drug detection Chloramphenicol Oxytocin Kanamycin Sulfadimethoxine Kanamycin Ampicillin	FAM, ROX, Cy5, two-dimensional metal organic frameworks and Cu-tetrakis(4-carboxyphenyl) porphyrin FRET: Cy3, FAM, Cy5 and graphene oxide	0.05–50 nM 0.008–50 nM 0.003–30 nM 10–50 ng/mL 10–50 ng/mL 10–50 ng/mL	1.5 pM 2.4 pM 1 pM 1.997 ng/mL 2.664 ng/mL 2.337 ng/mL	Yang et al. (2019b) Youn et al. (2019)

Moreover, the development of highly sensitive semiconductor quantum dot strategies based on the fluorescence quenching principle has attracted a lot of attention. A dual-emission fluorescent aptamer sensor is recommended for CdTe QD-based OTA detection (Zhang et al. 2023). CdTe acts as a green-emitting QD

Table 11.3 List of fluorescent aptasensors for detection of toxins

Target	Aptamer principle	Linear range	LOD	References
OTA	AgNCs Single-walled carbon nanohorns PVP-coated graphene oxide Nanographite Single-walled carbon nanotubes MBs	0.01–0.30 ng/mL 20–500 nM 2–35 μM 0.02–0.4 μM 25–200 nM 0.1–1 ng/mL	2 pg/mL 17.2 nM 1.9 μM 20 nM 24.1 nM 20 pg/mL	Chen et al. (2014) Lv et al. (2016) Sheng et al. (2011) Wei et al. (2015) Guo et al. (2011) Zhang et al. (2013)
AFB1	CdTe QDs; graphene oxide AgNCs; MBs	0.003–320 μM 0.001–0.05 ng/mL	1.0 nM 0.3 pg/mL	Lu et al. (2014) Chen et al. (2014)
T-2 toxin	Apt-AgNCs:MoS_2	0.005–500 ng/mL	0.93 pg/mL	Khan et al. (2018)

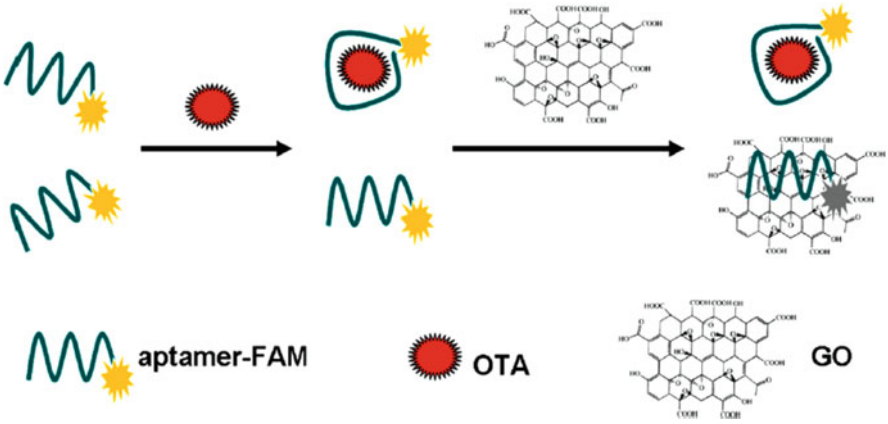

Fig. 11.4 Schematic diagram of the graphene oxide detection platform for OTA detection. Reprinted with permission from Sheng et al. (2011). Copyright ©2022 Elsevier Ltd.

which functions as a donor and is used to label the aptamer. GNPs adhere to the silica surface and act as receptors and bind the complementary DNA (cDNA). If the aptamer hybridizes with its cDNA, the green-emitting quantum dot-GNP pair will come close, allowing the FRET to occur. And in the presence of OTA, FRET does not happen. With this strategy, LOD of up to 1.67 pg/mL is reported. Red-emitting CdTe quantum dots were used as reference signals. With increase in OTA concentration, the change in colour from red to green was visible to the naked eye.

11.3.4 Fluorescent Aptasensors for Heavy Metals

Among all optical aptasensors, fluorescent and colorimetric methods have shown to be widely used for detection of heavy metals (Table 11.4). Li and others developed

Table 11.4 List of fluorescent aptasensor for the detection of heavy metals

Target	Aptamer principle	Linear range	LOD	References
Hg2+	GNPs functionalized with Mercuric ion-specific DNA	0.02–1.0 μM	16 nM	Tan et al. (2013)
	GNPs functionalized with Thiol-DNA	20–90 nM	8 nM	Wang et al. (2018a)
	Graphene oxide	0.1–200 nM	30 pM	Huang et al. (2014)
	Carbon dots; graphene oxide	5–200 nM	2.6 nM	Cui et al. (2015)
Pb2+	Multi-walled carbon nanotubes	20–150 nM	20 nM	Qian et al. (2015)
	Graphene quantum dots; graphene oxide	9.9–435 nM	0.6 nM	Wang and Si (2013)
Ag+	Multi-walled carbon nanotubes	20–150 nM	18 nM	Wang and Si (2013)
	Graphene oxide	0–150 nM	5 nM	Wen et al. (2010)

graphene oxide-based unlabelled fluorescent aptamer sensor for the determination of Hg (Li et al. 2013). Owing to its strong photoluminescence properties, ease of surface modification and significant water solubility, graphene oxide was chosen as a fluorophore (Loh et al. 2010). The ssDNA-rich thymine (T) aptamer was used for specific and high-affinity binding with Hg^{2+}. When the Hg^{2+} is present, the aptamer and Hg^{2+} forms T-Hg^{2+}-T bond which results in the formation of flocculated structure between them which in turn reduces the distance between Hg^{2+} and the surface of the graphene oxide leaf, leading to the disappearance of graphene oxide fluorescence. A fluorescent biosensor that uses hybrid chain reaction to detect Hg^{2+} has been reported by Huang et al. (Loh et al. 2010). The hybrid chain reaction was originally proposed by Dirks and Pierce and is widely used to detect targets with amplified signals. When Hg^{2+} is absent, two hook probes (HP1 and HP2) and an additional DNA molecule were absorbed on the surface of graphene oxide leaf and graphene oxide turned off FAM-labelled HP1 fluorescence (Dirks and Pierce 2004). The presence of Hg^{2+} drives the initiation of hybrid chain reaction between HP1 and HP2. Hg^{2+} forms the T-Hg^{2+}—T structure with aptamer and opens the structure of HP1, allowing the rest of the sequence to hybridize with HP2 partly, which in turn opens HP2, resulting in the hybridization of the rest of HP2 with HP1. The reaction continues and forms a long complex ring which can be easily separated from the graphene oxide, thus increasing the fluorescent signal. The LOD of 0.3 nM was achieved making it suitable for practical use.

In some studies, graphene quantum dots have also been used as fluorophores to produce heavy metal optical sensors. For example, Qian et al. reported that reduced graphene quantum dots are used as a fluorophore and graphene oxide as a fluorescence inhibitor (Qian et al. 2015). As shown in Fig. 11.5, the aptameric probe reduced graphene quantum dots are absorbed without Pb^{2+} on the graphene oxide

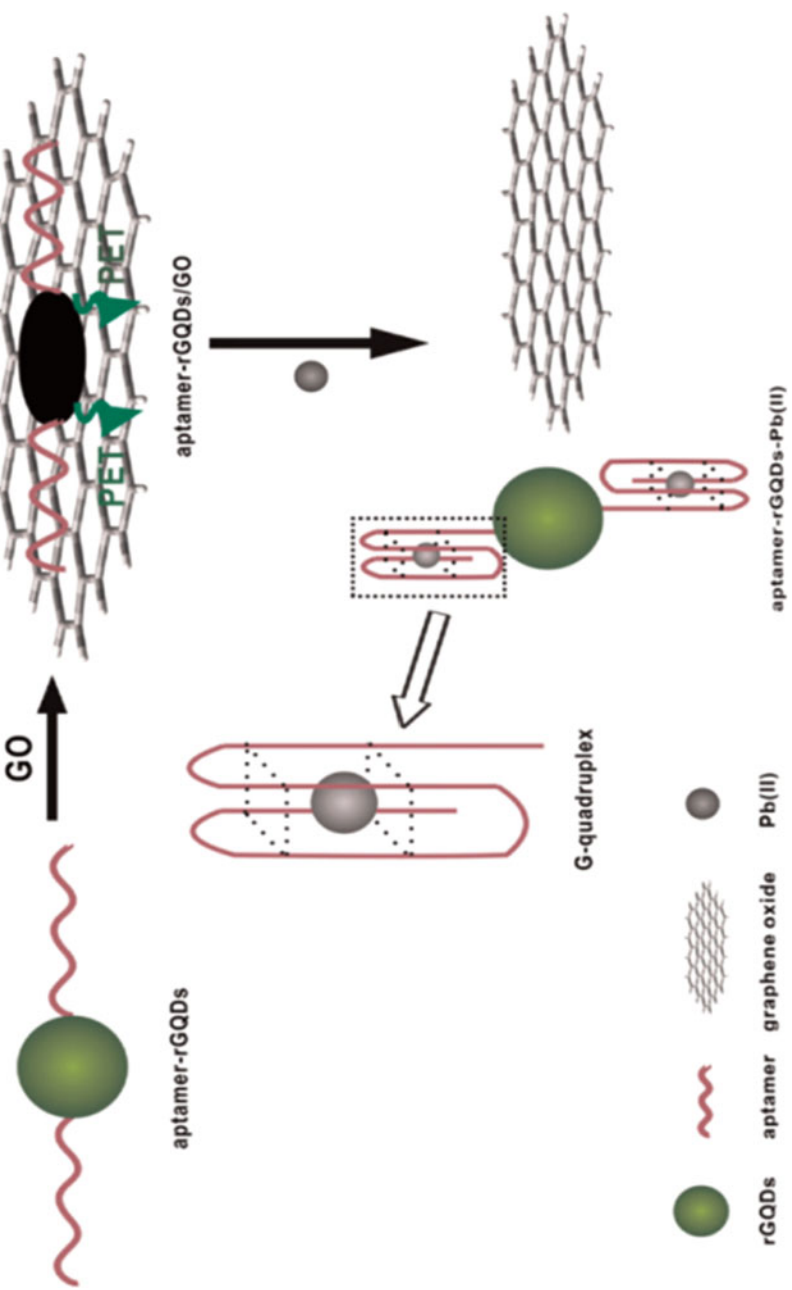

Fig. 11.5 Schematic diagram of Pb^{2+} detection via photoinduced electron transfer. Reprinted with permission from Qian et al. (2015). Copyright ©2022 Elsevier Ltd.

surface, causing deactivation of reduced graphene quantum dot fluorescence. In the presence of Pb^{2+}, the aptamer-Pb^{2+} binding occurred, thus forming the G-quadruplex complex aptamer-reduced graphene quantum dots-Pb^{2+}. The obtained complexes can disrupt the interaction between aptameric reduced graphene quantum dots and graphene oxide, thereby isolating the graphene oxide complex and increasing the fluorescence. This method can be used to detect Pb^{2+} with an LOD of 0.6 nM.

11.3.5 Fluorescent Aptasensors for Other Pollutants

Mae and others proposed an unlabelled GNP-based aptameric sensor for bisphenol A (Mei et al. 2012). In the absence of bisphenol A, aptamers get adsorbed on GNP and protect it from forming aggregates. After the addition of bisphenol A, aptamer and bisphenol A combines and leads to GNP aggregation. A simple strategy was reported with LOD of 0.1 ng/mL, and range of detection was reported to be from 1 to 10,000 ng/mL (Ragavan et al. 2013). In their experiments, they also suggested the use of fluorescent biosensors for detection for bisphenol A. Using the method based on the principles of FRET, the linear range was observed from 0.1 to 10,000 pg/mL and LOD of 0.01 pg/mL.

11.4 Paper-Based Fluorescent Aptasensors

He et al. immobilized one portion of the aptamer on GNPs and the other portion of the aptamer on the cellulose filter. The presence of cocaine caused the formation of functional unit of aptamer by fragmentation, which dampens the luminescence of nanoparticles converted to GNP (He et al. 2016).

The specificity of test was demonstrated by using two important metabolites of cocaine: quinone methyl ester and benzoquinone. These metabolites did not show a significant response. This work demonstrates that the design of split aptamers should be done very critically and the "split points" on the aptamers should be studied very carefully to ensure the formation of an intact secondary structure at the target site. This study shows that the use of single aptamers can significantly prevent the formation of background signals and that it can only be created or combined into a functional object when the target is present.

Natural paper-based inhibitors which include multi-walled carbon nanotubes and graphene oxide can also be used to avoid attaching the inhibitor to the end of the aptamer. In one study, MWCNTs and graphene oxide were shown to detect the norovirus. When virus is present in the sample, the aptamer gets released from multi-walled carbon nanotubes and graphene oxide and in turn binds to norovirus. The limits of detection of virus with graphene oxide and multi-walled carbon nanotubes are 3.3 ng/mL and 4.4 ng/mL, respectively (Weng and Neethirajan 2017).

11.5 Lateral Flow Assay (LFA)-Based Fluorescent Aptasensors

Another paper-based sensor known as lateral flow assay or strip test is closely related to colorimetric detection, although fluorescence measurements have also been shown. Here, the basic principle of fluid movement is capillary action, which depends upon moisture, pore size and substrate microstructure (Mark et al. 2010). This technique is used for both detection and quantitative or qualitative analysis of targets using a portable target reader. The aptamer sensor platform is based on general lateral flow analysis and consists of sample buffer, binding buffer, assay buffer, controlled reagent line, absorption buffer and support buffer (Fig. 11.6). The sample buffer carrying the target analyte is passed through the binding buffer. The binding particles in the binding buffer contain capturing agents (primary aptamers) to form complexes with the analytes. The analyte-particle-aptamer-primary conjugate complex then interacts with a specific secondary capture object (secondary aptamer) on the test area to form a band or coloured band. There are two main types of LFA: sandwiches and competitive trials.

LFA has been utilized to detect bacteria directly. In this, two anti-*Vibrio fischeri* aptamers are selected with Cell-SELEX and utilized in a sandwich analysis test, where one of the aptamers serves as a capture probe and another aptamer as a detection probe. The linear range of detection of *Vibrio fischeri* target cells is from 4×10^1 to 4×10^5 CFU/mL, and the correlation coefficient of linear regression (R2) is approximately 0.9809 (Shin et al. 2018). This study shows the potential of using aptamers to directly detect cells without pretreatment or extraction.

11.6 Smartphone-Based Fluorescent Aptamer Sensors

Smartphones are one of the widely accepted and popular hand sensors, and, due to their size, they can be found almost everywhere. Being decked with batteries, visual displays, fast multi-core processors, digital cameras and intuitive user interfaces, smartphones can be used for real-time measurements. Smartphones can be integrated with a variety of other sensor systems, such as sidestreams, fluorescence systems, colour platforms and handheld detectors, for fast real-time monitoring at the care site. The most common tests for aptamer sensors on smartphones are fluorescent and unlabelled colorimetric analysis.

The smartphone system uses an aptamer-based fluorescence detector, and a fluorescent signal reader detects a signal to transmit a signal generated by the target aptamer complex. The study showed that smartphone detection uses functionalized aptamer nanoparticles to directly and quickly detect bacteria without any cultivation process. The use of fluorescent magnetic nanoparticles functionalized with an aptamer realizes the rapid and uncultured detection of *Staphylococcus aureus*. The aptamer captures the target cells, and the smartphone camera captures the fluorescent signal caused by the aptamer target complex. The lowest detected concentration was found to be 10 CFU/mL. The target cell is recorded by the aptamer, and the fluorescent signal caused by the combination of aptamer and target is recorded by

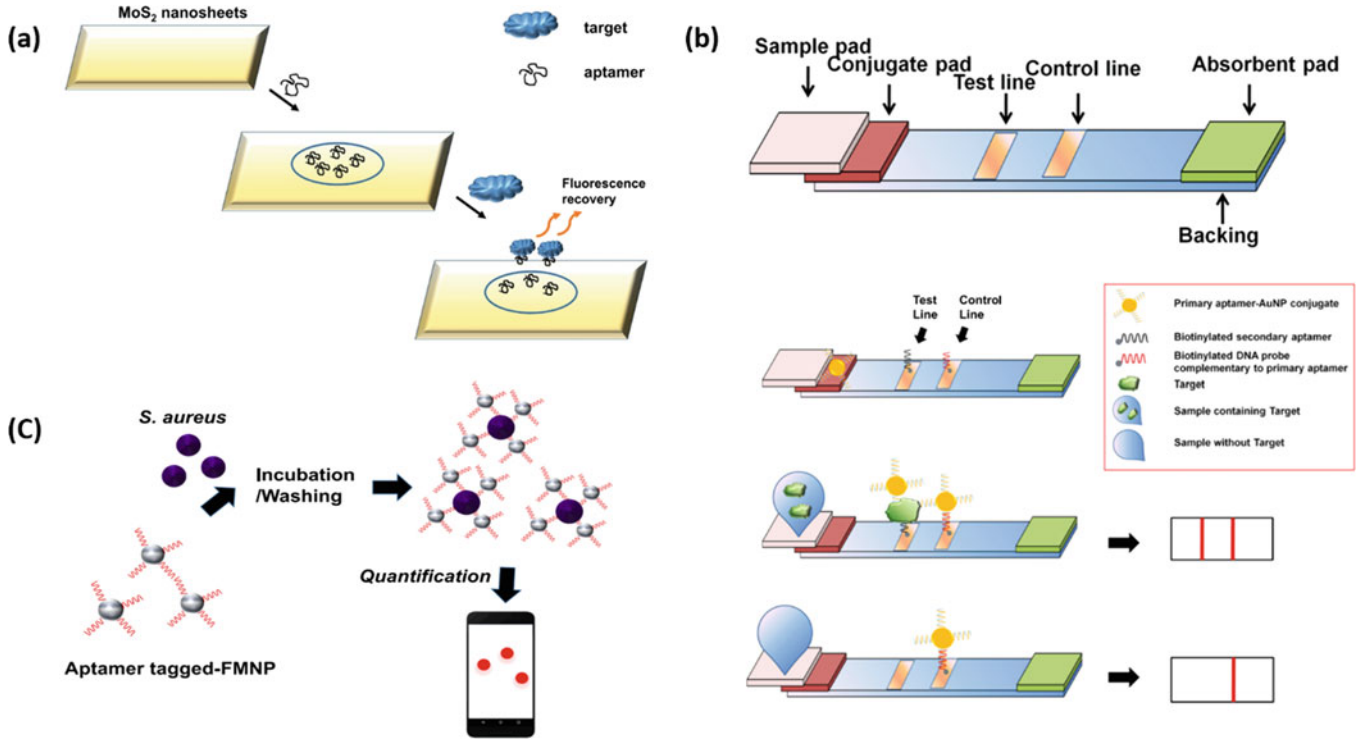

Fig. 11.6 (**a**) Due to the quenching property of the MoS_2 nanosheets, the fluorescently labelled aptamers on the surface of the nanosheets have reduced fluorescence. When the target is present, the aptamer detached from the target binding paper, which restores the fluorescence. (**b**) Schematic representation of the overall composition of the sandwich-based lateral flow test. (**c**) Schematic illustration of smartphone-based (S. aureus) detection using aptamer-bound fluorescent magnetic nanoparticles and smartphone camera. Reprinted with permission from Citartan and Tang (2019). Copyright ©2022 Elsevier Ltd.

the smartphone camera. It was found that the minimum concentration that can be detected is 10 CFU/mL (Shrivastava et al. 2018).

In the triple detection of chlorpyrifos, diazinon and malathion, a sidestream biosensor based on fluorescent aptamers is integrated into the smartphone's spectrum reader. Gold nanostar is used as a nanoquencher. The aptamer sequence was biotinylated and bound to streptavidin before mixing with the QD-nanosphere-BSA conjugate, which was then immobilized on the nitrocellulose membrane. The target-containing sample is added to the gold nanostar aptamer before being applied to the sample cell. When it reached the test line, the deceased gold nanostar aptamer was not captured in the complementary sequence because of the development of a target complex. The lack of AuNS quenching effect leads to fluorescence radiation of CT nanospheres in the test line. With no target, the complex between the gold nanostar aptamer and the complementary sequence is formed which then quenches the fluorescence, leading to loss of fluorescence. The detection limits of chlorpyrifos, diazinon and malathion were 0.73 ng/mL, 6.7 ng/mL and 0.74 ng/mL, respectively (Cheng et al. 2018).

11.7 Summary

Simple assay strategies, portable instruments and ease of use are the essential requirements for point-of-care sensing. Aptamer and aptamer-nanoparticle-based biosensors have been extensively studied and proved its efficacy as optical biosensors for detection and monitoring of environmental pollutants. In spite of many technological advances, there are still many limitations to the on-site detection of environmental pollutants. Nanoparticles owing to their small size and shapes, exhibit unique properties and being used along with aptamers to generate better signals. In recent years, many sensors have been reported that can be used to detect environment pollutants. This chapter highlighted the aptamer-based fluorescent techniques that can potentially be employed for the on-site detection of environmental pollutants. These emerging assays and techniques have the potential to be used as point-of-care applications.

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Nanoenabled Sensing Methods for Pesticide Detection

12

Chumki Praharaj and Seema Nara

Abstract

Pesticides comprise a group of chemical compounds used to control or eliminate pests, insects, or rodents for crop protection. Chemically, pesticides can be broadly classified as organochlorines, organophosphates, carbamates, and pyrethroids. They are known to be neurotoxic to humans and can persist for a prolonged time in the ecosystem. Considering their persistence and toxicity, guidelines have been set in place by different global agencies for limiting their use and defining minimum prescribed limits in diverse sample matrices. Accordingly, sensitive and simple analytical techniques are always in demand to monitor the levels of pesticides in soil, water, or food samples. Nanotechnology is playing a crucial role in overcoming the challenges associated with conventional physical or enzyme-based analytical methods. The unique optical, electrical, magnetic, and catalytic properties of nanomaterials are being smartly used nowadays to detect pesticide residues in trace levels. These new methods are also integrated with smartphones or image analysis software to design point-of-care nanoenabled sensing methods. This chapter presents recent strategies by which nanotechnology is enabling sensitive detection of pesticides along with future ahead.

Keywords

Nanostructures · Pesticides · Biosensors · Aptamer

C. Praharaj · S. Nara (✉)
Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India
e-mail: seemanara@mnnit.ac.in

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12.1 Introduction

Pesticides are the substances that are used to kill insects or organisms which are harmful to cultivated plants or animals. The mode of action of a pesticide is not specific always; they often act against other species or organisms including humans. The estimation of WHO says that every year three million pesticide poisoning cases are seen which includes 22,000 death cases mostly in developing countries (Dinham and Malik 2003). The usage of pesticides not only contaminates our water resources but may also accumulate in food resources at various levels of food chain and could damage the ecosystem. In humans, pesticides increase the production of reactive oxygen species (ROS), as a result of which the defense against oxidative damages goes down in the cellular system. This oxidative stress induces the harmful effects like carcinogenesis, respiratory, cardiovascular, renal, endocrine, and reproductive problems and also disturbs the homeostasis. Developed countries produce pesticides with low risk factor and which specifically controls the pests, rodents, weeds, and diseases with minimum side effects to other organisms. But in developed countries, conventionally formulated pesticides are produced which have adverse effect on the environment as well as human health also (Kaur et al. 2019).

Considering the risk associated with contamination of our ecological resources, it is necessary to strictly monitor the concentration of pesticides in soil, water, and crops/food products. For instance, during vegetable cultivation, the pre-harvesting interval which is the time between the pesticide application and harvesting of vegetables, pesticide levels should be keenly monitored. In this period, the concentration of pesticide should remain below the tolerance level so that the exposure to the consumer remains minimum (Moura et al. 2020). Likewise, the quality of drinking water shall be monitored regularly for the pesticide concentration or any heavy metals before use. In the case of environment, we have to monitor the concentration to check the purity of air to reduce the disease-causing pollutants (Sjerps et al. 2019). Conventionally, several approaches are being used in past decades for pesticide detection which include physical methods like high-performance liquid chromatography and liquid or gas chromatography-mass spectroscopy. Additionally, enzyme-linked immunosorbent assays, colorimetric assays, fluorometric assays, and electrophoretic methods were developed for pesticide detection. However, the use of these sensing methods is limited due to dependency on costly apparatus, requirement of skilled persons, difficulty in pretreatment of the sample, and less robust for various environmental conditions restricts their use in specialized laboratories. Hence, the need for developing more sensitive and easier-to-use methods for pesticide monitoring is still realized. In this context, recent years have witnessed the integration of nanotechnology with biosensing methods to make the process easier, delicate, and precise (Sharma et al. 2021).

The unique chemical, thermal, electrical, optical, mechanical, and magnetic properties of nanostructures can be harnessed to detect and monitor the pesticide residues in trace amounts in air, water, or soil. The nanomaterial-enabled sensors are rapid, sensitive, specific, and used for quick, real-time detection of pesticides at decentralized locations. Portable chemical nano-biosensors are produced by

US-based startup company Razzberry. Another Italian startup company, Nasys Nanosensor Systems, has developed a metal oxide-based nanosensor to detect air pollutants. To detect the harmful contaminants in agriculture, other startups, nGageIT Digital Health, Canada, developed nanosensors to see the fingerprints of medications that are taken inside human body (Sil 2021). This chapter discusses about various classes of pesticides and recent techniques for detection of pesticide using nanotechnology.

12.2 Pesticides and Its Classifications

Pesticides literally mean the substance which kills the pests. Pesticides have been used since ancient times to increase the productivity in agriculture as they show defense against different kinds of pests and rodents and also keep control of diseases. However, in the present scenario, abundant usage of pesticides results in many more negative effects such as hazardous effect on the environment like pollutants in air, contamination in water bodies that are used as drinking water, and increase of disease rates and also mortality rate. The accumulation of various kinds of pesticides in fruits or vegetables more than optimum level also harms the consumer leading to sharp decline in profits of a farmer. There is a major issue in developing countries regarding the usage of pesticides because sometimes expired pesticides are used which impose a threat to the environment. The UN's Food and Agriculture Organisation (FAO) has taken the lead in designing and enforcing the essential measures to address this issue in the developing countries. Pesticides can be classified in a number of ways which gives information about their structure, chemistry, and the end target (Terziev and Petkova-Georgieva 2019). Based on chemical structure, the pesticides can be divided into two groups: inorganic and organic pesticides.

(a) Inorganic pesticides: These chemicals are defined as the simple compounds having the structure like crystalline salt. Inorganic pesticides are stable in different environment conditions and are soluble in polar solvents like water. These particular pesticides were created in the past and made up of compounds containing elements like sulfur, aluminum, copper, mercury, arsenic, and others also includes aluminum phosphide, lime, etc. Generally, inorganic pesticides are toxic in nature and have the capability to persist in natural environment for a prolonged period of time.
(b) Organic pesticides: As the name suggests, these compounds include carbon in their basic molecular structure, and as compared to inorganic pesticides, these are more complex in nature. They are insoluble in polar solvents like water and soluble in nonpolar solvents. Organic pesticides can be subdivided into two categories on the basis of sources they are obtained from, i.e., natural and synthetic organic pesticides. Naturally occurring pesticides are derived from plants, and the synthetic organic ones are made by artificial chemical synthesis.

Organic pesticides are also subdivided into four other groups such as fumigants, organochlorine, carbamates, and organophosphates.

1. Fumigants: Fumigants are small molecules and have the ability to penetrate into materials. These are generally used in sterilization of soil and prevention of stored grains from degradation. Generally, fumigants include carbon tetrachloride, methyl bromide, ethylene dibromide, etc. Carbon tetrachloride is commonly used as solvent in industrial cleaning and also used as stock to prepare other chemicals. According to the Montreal protocol, this is responsible for depletion in ozone layer and thus restricted to use as feedstock substance. Methyl bromide is totally banned under this protocol. This chemical is produced from bromide salts as a by-product. This is also used in soil sterilization process and finally emitted to the atmosphere at the end. It's a kind of toxic material and very dangerous as it dissipates to the atmosphere quickly. Increases in the level of methyl bromide concentration in the atmosphere result in failure of human central nervous system and respiratory system (Terziev and Petkova-Georgieva 2019).
2. Organochlorines: These are the toxic compounds made up of carbon, hydrogen, and chlorine. The compound takes longer period of time to degrade and remains active in the atmosphere due to the presence of chlorine in it and hence is banned in many countries. These are the compounds mostly used as insecticides for grain storage. DDT is the cheapest and most effectively used organochlorine pesticide worldwide. But due to its persistence in the environment for longer period of time, its use was banned in the USA in 1973. Due to some kind of public interest, DDT was used in malaria-prone regions to kill mosquitoes. Organochlorines are toxic because these stimulate the central nervous system in humans. It is responsible for the hyperexcitability of the nervous system by inhibiting GABA-mediated influx of the chloride ions into the central nervous system (Helou et al. 2019).
3. Carbamates: The compounds are quite similar to the organophosphate molecules. Organophosphates are the derivatives of phosphoric acid, whereas carbamates are the derivatives of carbamic acids. They bear a general formula of R=O where R is the alcohol or phenol and R_1 is the methyl group or hydrogen. These groups of pesticides are generally used for the vector control such as insect pests, bee, wasps, etc. These pesticides are generally used in pest control in households, but they have harmful effects on human health. The mode of action of this pesticide includes carbamylating the active site of the enzyme acetylcholinesterase causing its inactivation. In the presence of such inhibitors, acetylcholinesterase does not catalyze the breakdown of acetylcholine into choline and acetic acid, as a result of which accumulation of acetylcholine takes place which overstimulates the cholinergic receptors throughout the central and peripheral nervous system (Leung and Meyer 2019).
4. Organophosphates: These compounds are the esters of phosphoric acid and some of its derivatives. The general formula includes a phosphorus central atom and derivatives of phosphoric acid, triphosphoric acid, and a leaving group which can be replaced by the oxygen of serine residues in the active site of

acetylcholinesterase (AChE). The organophosphate compounds were first synthesized during the nineteenth century for the protection of crops and livestock. But during the Second World War, these were used as warfare agents. The mode of action behind the toxicity of organophosphate compound is the inhibition of acetylcholinesterase which highly depends on leaving group. In the warfare agents, the leaving groups contain fluorine, but in case of lower toxic organophosphate compounds, the leaving group contains alkyl or aryl groups. Where higher tendency of leaving is seen, it results with higher affinity of the enzyme to inhibitor. So, the rate of AchE inhibition increases, as a result of which a variety of chronic and short-term diseases occur to central and peripheral nervous system (Beketov et al. 2007).

12.3 Nanomaterials Used in Nanoenabled Sensors

Nanoenabled biosensors play an important role in monitoring environmental pollutants (Chandra and Prakash 2020). These sensors present numerous advantages over the traditional methods such as high specificity and sensitivity and selective and precise determination of analyte (Akhtar et al. 2018; Chandra et al. 2010). Different kinds of nanomaterials are used for the detection, degradation, and removal of pesticides. Most common nanomaterials used are nanotubes, nanocomposites, and different kinds of nanoparticles (Fig. 12.1). The chemical and the structural properties of the nanostructures perfectly define the potential of the final device (Kaur et al. 2019). For instance, surface-enhanced Raman scattering (SERS) is a popular technique nowadays and used for the accurate and precise detection of any

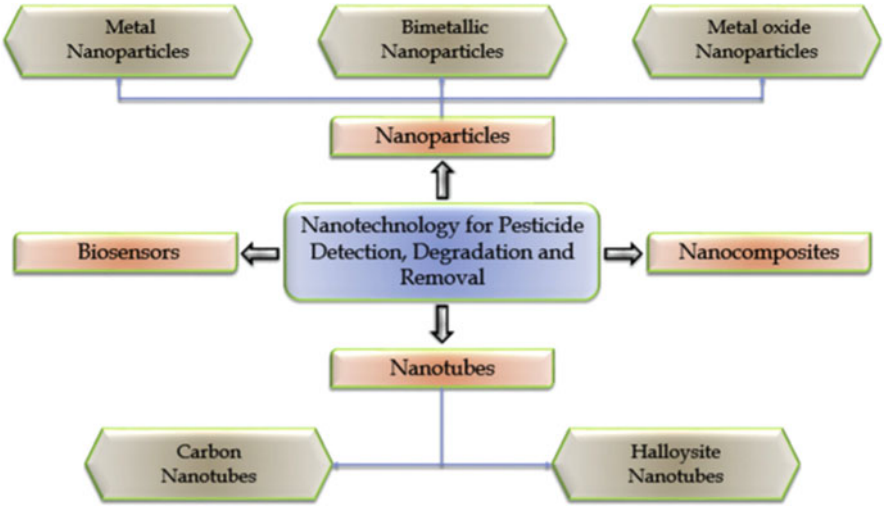

Fig. 12.1 Classification of approaches based on nanotechnology for pesticide detection. Adopted with permission from Rawtani et al. (2018), Elsevier

chemical substance or pollutants. When the target substance with the Raman active modes is adsorbed on the surface of the nanomaterials of some metals like gold and silver, a change in the peak is seen in the Raman spectrum. The target particle can be identified by the difference in fingerprints which gives information about the vibrational modes of the substance. Raman intensity is influenced by the electromagnetic resonance and the size or shape of the nanomaterials. Similarly, the relationship of size of metallic nanoparticles and their surface plasmon resonance can be explored to develop easy and specific colorimetric methods for pesticide detection. Gold nanoparticle (AuNP) coated with ZrO_2 was used for trace element detection in fruit and vegetable (Lee et al. 2018).

Carbon nanomaterials possess excellent electrical conductivity, optical properties, biocompatibility, and the catalytic activities. As a result, carbon nanomaterials (graphene, carbon nanodots, nanotubes) are seen as potential candidate in the construction of biosensors for pesticide detection. Carbon nanostructures are used mainly as two types of detection methods based on enzymes, i.e., (1) with the addition of enzymes and (2) enzyme-free nanoenabled biosensors (Zhao et al. 2018). Fluorescent-based carbon nanomaterials are also used for detecting a trace amount of pesticide like organophosphates and carbamates. The carbon-based metal or covalent, metallic frameworks can be combined with various recognition elements such as antibody, aptamers, molecular imprinted polymers (MIP), and the enzymes to achieve a minimum detection level of pg^{-1}. Also, the carbon nanodots and MnO_2 nanosheet were combined with nanomaterial-based quencher to detect the organophosphate pesticides (Yan et al. 2018).

Mesoporous silica nanoparticle-templated AuNPs were used for the construction of SERS substrate to detect pymetrozine, thiamethoxam, and 2,4-dichlorophenoxyacetic acid in low concentrations (Xu et al. 2020). The large surface area and high sorbent properties of silica nanoparticles allow their use for extraction of pesticides. To increase the extraction potential, silica can be fabricated with the MIPs. By using the electrochemical and optical properties, enzyme- and antibody-based silica sensors have been developed.

Zinc oxide is a versatile semiconductor material which finds application in nanoenabled biosensors. It has been used as the matrix material for immobilization of biomolecules owing to its biomimetic and electron transfer properties. ZnO was combined with glutathione for the construction of a nanosensor to detect organophosphate pesticides (Kumar et al. 2013). A model pesticide thiram was detected by a combination of Au with ZnO assembled nano-urchins. The nano-urchin structure allowed huge electric fields inside the nanogaps present in the branches of urchins and allowed good potential of adsorption on the gold part of the nanostructure (Barbillon et al. 2021).

Electromagnetic nanosensors are used in detection of pesticides by the minute changes in electromagnetic waves. They take the account of quantum phenomena in the nanoenabled biosensors (Javaid et al. 2021). The advantages of nanostructure and the magnetic manipulative properties can be a good candidate for constructing nanosensors. Magnetic nanoparticles are being used as plasmonic sensors in three categories: surface plasmon resonance, localized surface plasmon resonance, and the

surface-enhanced Raman scattering techniques (Ghoorchian et al. 2021). Thus, unique properties of nanostructures can be harnessed to design sensitive detection methods for pesticides.

Sensing methods reported so far in the literature for detection of pesticides using nanostructures can be broadly divided into two categories:

1. Methods relying on pesticide-biomolecule interaction: This category makes use of any biomolecule such as antibody or aptamer that can specifically recognize and bind to a target pesticide, or it incorporates a natural enzyme like AchE, whose catalytic action is inhibited after interaction with pesticide. The nanostructures are used in these methods either for the purpose of immobilizing biomolecules on sensor surface or for amplification of signal intensity.
2. Methods relying on nanostructure-pesticide interaction: This category of sensors does not incorporate any biomolecule for pesticide detection. Here, some unique property of nanostructures is influenced in the presence of a particular pesticide, and the change in that property is directly monitored using a suitable sensing platform. The following section discusses the nanoenabled pesticide detection methods under these two categories.

12.3.1 Methods Relying on Pesticide-Biomolecule Interaction

Pesticides can be detected by monitoring its interaction with a specific enzyme, an antibody, or an aptamer. This affinity-based recognition can be used to develop optical, electrochemical, or other biosensing modules.

(a) *Enzyme-Based Sensors*

AChE enzyme is one of the most commonly used enzymes for pesticide detection in different types of biosensors. Organophosphate compounds inhibit the function of AChE enzyme by blocking the active serine covalently. Hence, AChE is immobilized on sensor surface to specifically detect the organophosphate pesticides. Recent approaches focus on using novel nanostructures for immobilizing AChE enzyme to fabricate the sensing surface or for signal generation. For instance, Singh et al. (2020) modified gold electrode with reduced graphene oxide (rGO), zinc oxide nanoflowers (ZnONF), and chitosan (CHIT) for immobilization of AChE and sensing of organophosphate pesticides (Fig. 12.2). rGO makes one-atom-thick sheet to form a planar structure with high electrical conductivity; ZnONF is used as matrix due to its biomimetic, catalytic, enzyme loading and electron transfer properties; and CHIT is used for good adhesion, film forming, biocompatibility, and enzyme immobilizing properties. The sensor was able to detect a broad class of organophosphate pesticides in the detection range of 0.2–0.5 nM/g with 0.1 nM limit of detection. The sensor had a lower detection limit of 7.9×10^{-15} M for malathion, 7.9×10^{-14} M for chlorpyrifos, and 8.6×10^{-15} M for parathion methyl (Ma et al. 2018). Acid phosphatase (ACP) enzyme is also inhibited by OP pesticides. Using this

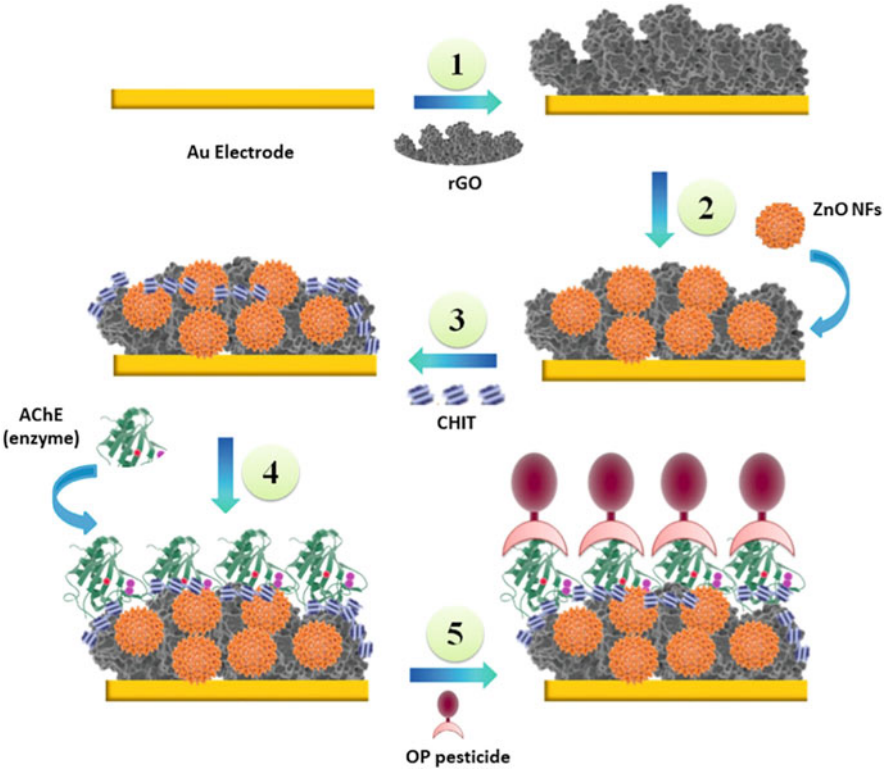

Fig. 12.2 Scheme for the development of nanomaterial-modified biosensor using rGO and ZnONF-electrodeposited Au surface and AChE immobilized by glutaraldehyde cross-linking for OP detection. Adopted with permission from Singh et al. © (2020) Elsevier

criterion, a nanosensor was prepared for a pesticide Dufulin, which is an inhibitor of ACP. Here a stimuli responsive nanocarrier Zr-terephthalate metal framework with doxorubicin dye incorporated in it (DMOF) was synthesized. The fluorescence of dye was quenched by the metal organic framework. ACP converted its substrate L-ascorbic acid-2-phophatase to ascorbic acid which further reduced the azobenzene of DMOF nanocarrier. As a result, MOF was degraded to release the dye to fluoresce again. However, in the presence of Dufulin, the florescence recovery is blocked as pesticide blocked the enzyme action. The lower limit of detection of this sensor detected was 2.9 ng/ml (Liu et al. 2022). Tyrosinase enzyme was also used for detection of atrazine and carbamate pesticides because its activity is inhibited by the OPs. The major advantage of these biosensors is that it is stable at high temperature but is not specific (Bucur et al. 2018).

Atrazine is a main triazine group herbicide inhibits the function of tyrosinase. Martinazzo et al. reported a cantilever-based sensing of atrazine using tyrosinase, where the sensor surface was modified with a self-assembled monolayer

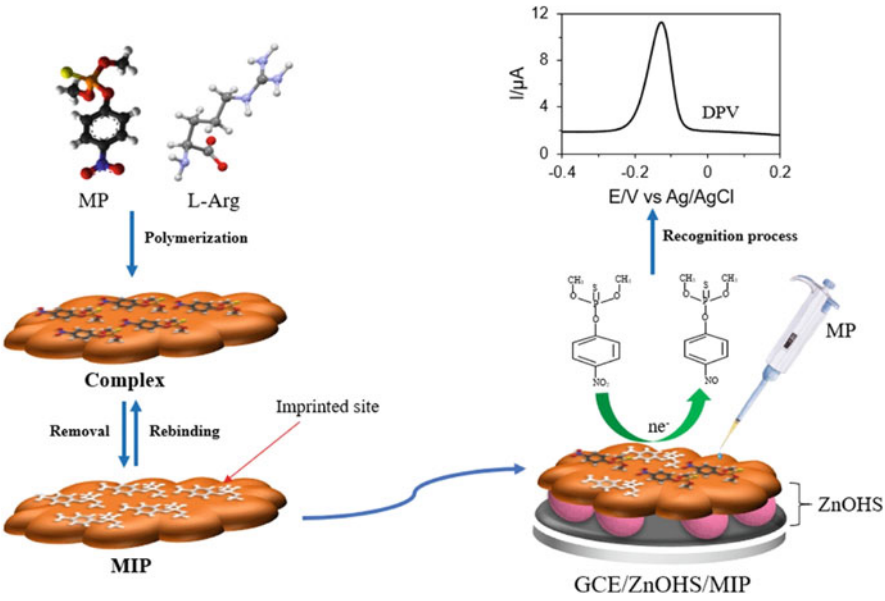

Fig. 12.3 Scheme of electrochemical MIP-based pesticide sensor development using a ZnOHS-modified GCE electrode. Reused with permission from Daizy et al. © (2021) Elsevier

(SAM) layer of AuNP. The compression tension increased with increasing concentrations of atrazine, and the performance was observed by change in voltage response. The sensor showed a LOD of 7.754 ppb (parts per billion) and can detect a low concentration of 22.792 ppb. This sensor displayed unique properties like biomolecular interaction measuring properties, specific recognition, miniaturization, and sample pretreatment-free properties.

(b) *MIP-Based Sensors*

Recently, the MPP-based biosensors gained more popularity in the area of sensing applications due to their high specificity and thermostability. MIPs are budget-friendly and take less time for the synthesis. These are the synthetic or artificial materials which are cross-linked with the molecular imprinted cavities. They act just like natural receptors having the plastic antibodies (Ab). MIPs are electrochemically synthesized by polymerization of functional monomers in the presence of a linker and the target molecule as template. The basic mode of action is similar to the Ag-Ab interaction (Zhang et al. 2014). Figure 12.3 shows an example of MIP-based sensor development, where the MIP film was formed onto the surface of zinc oxide hollow sphere (ZnOHS)/GCE by electropolymerization of functional monomer, l-arginine, and template molecule. ZnOHS was used in the sensor for its high conductivity, which improved the sensitivity of the sensor. Various kinds of pulse voltammetry were used for the evaluation of detection efficiency. The sensor achieved a detection range of 5×10^{-9} to 0.1×10^{-4} mol/L and a lower detection limit of 0.5×10^{-9} mol/L

and sensitivity of 571 nA/μmolL^{-1} cm^{-2} (Daizy et al. 2021). Perovskite quantum dots have high brightness property, but their use is limited in analytic applications because of their instability and sensitivity toward moisture and oxygen. To address this, Tan et al. (2019) used $CsPbBr_3$ perovskite quantum dots and injected them inside molecularly imprinted polymer of organosilicon monomer having various functional groups (phenyl and two hydrogen bond donor) by solgel process. These modified MIPs were used to detect phoxim which specifically quenched the fluorescent signal of the sensor. The fluorescence signal of MIP/QD sensor was linearly related with phoxim concentration in the range of 5–100 ng Ml^{-1}, and the sensor has the lower detection limit of 1.4 ng Ml^{-1}. This MIP sensor detected phoxim successfully from potatoes and soil sample having the recovery rates ranging from 86.8% to 98.2%.

(c) *Aptamer-Based Sensors*

In the 1990s, the concept of a specific sequence of nucleic acid binding to a target species with very high sensitivity was developed (Klug and Famulok 1994). The newly developed technique was known as SELEX (systematic evolution of ligands by exponential enrichment) method, while the specific DNA or RNA oligonucleotides were coined as aptamers. Aptamers are made up of 30 to 40 nucleotide sequences, short fragment structure. Various sensors used aptamers that specifically recognize a particular pesticide as capture agents and immobilized them on the electrode surface. Fu et al. reported a Cu-rGO-based aptamer-modified working electrode to immobilize for OP detection (Fu et al. 2019a, b). In the sensor, Cu-like metal nanoparticles exhibit greater electrical conductivity while rGO exhibits decreased charge transfer resistance. DPV and SEM were used for the characterization and evaluation of aptasensors against OPs. Particularly four targets, viz., profenofos, isocarbophos, phorate, and omethoate (Ops), were detected. The sensor was able to detect all four pesticides in the subnanomolar range with very high sensitivity. The developed aptasensor was shown to detect phorate in vegetables and could be potentially applied to other fields due to simple preparation and modification process. Madianos et al. reported a nanomaterial-modified aptasensor for acetamiprid and atrazine detection, where the SiO_2 substrate was modified with a SAM layer of Pt nanoparticles (Madianos et al. 2018). The pesticide-aptamer interaction altered the charge resistance properties of the sensor surface and can be monitored to develop a calibration curve for the detection of pesticides. The use of the nanoparticle film in the aptasensor significantly improved the sensing performance of the electrode. The nanomaterial modification helped the sensor to detect the acetamiprid and atrazine with a LOD of 0.6×10^{-11} M and 0.4×10^{-10} M, respectively.

12.3.2 Nanosensor Without Having any Capture Moieties

Pesticides can also be detected using the inherent property of the pesticide molecules and using the enzymatic property of novel nanomaterials.

(a) *SPR-Based Nanosensor*
Plasmonic nanoparticles have unique optical properties so they have benefits of detection of environmental pollutants. When pesticides come in contact with the colloidal nanoparticle solution displaying SPR phenomenon, a visible shift in the SPR wavelength shift along with a color change of solution is observed. This change in SPR wavelength is due to pesticide-induced aggregation of colloidal nanoparticle solution and depends on the structure and the concentration of pesticide. Dissanayake et al. (2019) prepared three different nanoparticles, viz., Au, Ag, and Au-Ag, used for the detection of OP pesticides, and the sensor could detect as well as differentiate various OPs: ethion, fenthion, malathion, parathion, and paraoxon methyl. The localized surface plasmon resonance of the nanoparticle depends on the size and dispersity. The LODs of Ag NPs were found to be 9 ppm, 11 ppm, 18 ppm, and 44 ppm for ethion, fenthion, malathion, and parathion, respectively. The Au NPs showed LODs of 58 ppm, 53 ppm, 139 ppm, and 3203 ppm with ethion, fenthion, malathion, and parathion, respectively. Ag-Au NPs could detect ethion, fenthion, malathion, and parathion as low as 228 ppm, 231 ppm, 1189 ppm, and 1835 ppm, respectively. Kant (2020) developed an SPR-based fiber-optic sensor using silica fiber film to detect fenitrothion, a nitro-aromatic organophosphate compound. The sensing surface consisted of tantalum oxide nanoparticle which has high refractive index supported on reduced graphene oxide matrix. The sensing mechanism was based on the interaction between sensor surface and pesticide that resulted in a change of refractive index of the sensing surface. The spectral sensitivity was 24 nm μM^{-1}, and the limit of detection was 38 nM with a response time of 23 s.

(b) *Electrochemical Sensor*
Tian et al. used CuO-TiO_2 nanocomposites for electrochemical detection of methyl parathion. Titanium dioxide (TiO_2) nanomaterials have enhanced stability and strong affinity to phosphate group and possess high surface areas ideal for biosensor development. The role of CuO in this sensor was essential, as CuO interacts with MP forming a coordination effect, and that led to the decrease in the current response in DPV. In most of the electrochemical sensors, the current response increases with increase in target concentration. However, in this work, due to the coordination of CuO-MP, the current response gradually decreased. Using this method, the sensor was able to detect MP with a range of 0–2000 ppb and a LOD of 1.21 ppb (Tian et al. 2018). Sakdarat et al. fabricated a pesticide sensor by electrodepositing CuO nanorod on a copper foil substrate (Sakdarat et al. 2021). The designed electrodes could detect multiple pesticides such as chlorpyrifos, parathion, paraoxon, and pirimiphos with an enhanced sensitivity though CV (cyclic voltammetry). The LOD for sensing all pesticides ranged from 0.29 to 0.61 μM showing the ability to detect ultralow concentration. This nonenzymatic CuO nanorod sensor could be a promising sensing device for quick, low-cost, and on-site pesticide detection.

(c) *Fluorescent-Based Nanosensor*
Panda et al. developed a carbon dot-based luminescence probe for the detection of the flumioxazin through alkyne azide reaction method (Panda et al. 2018).

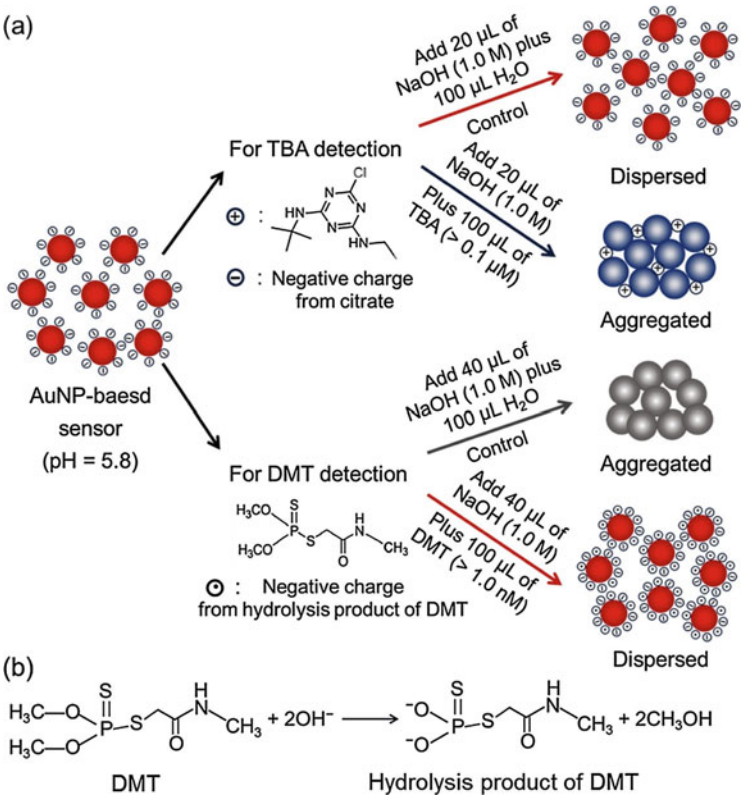

Fig. 12.4 Scheme for the detection of TBA and DMT using a AuNP-based colorimetric assay and (b) the reaction mechanism behind the sensing. Reused with permission from Chen et al. © (2018) Elsevier

The sensor was sensitive toward azide due to the presence of copper (II) which causes the flumioxazin selectivity even in the presence of different classes of pesticides. This nanosensor was mostly used in the field of agriculture to detect and monitor harmful flumioxazin pesticide. Carbon quantum dots (CQD) were prepared by a hydrothermal route and surface functionalized with N3 groups of 4-azidoanilne through amide bond. The alkyne end of flumioxazin was cycloadded with azide-modified carbon dot in the presence of Cu (II) and ascorbic acid. The fluorescence increase factor (F/F0) for flumioxazin was linear in the range 0–35.4 µg L^{-1} and can detect with a LOD of 0.027 µg L^{-1}. Chen et al. developed a colorimetric gold nanoenabled fluorescence sensor for terbuthylazine (TBA) and dimethoate (DMT) with high sensitivity and specificity (Chen et al. 2018). The detection mechanism for TBA and DMT is shown in Fig. 12.4, where the DMT can be detected by change in the aggregation of AuNP-based nanomaterials. The DMT, on other hand, causes the aggregation of the sensing materials. Citrate-stabilized AuNP was used in the sensor

development, where a shift in gray to red or vice versa was monitored, and the sensor achieved a detection range of 0.1–0.9 μM and 1–40 nM for TBA and DMT, respectively. The detection of DMT or TBA was insensitive by any other chemical or pollutants present in the environment. This nanosensor was cost-effective and can be used widely for field applications.

Similar to these developments, there are many techniques used for pesticide detection using biosensing methods. SERS is nowadays commonly used in the detection of trace amount of pesticide residue. Mu et al. reported a SERS system which can be combined and integrated with cell phone for the detection. The SERS chip can be integrated directly in the mobile phone and can measure the pesticide residue detection by one click (Mu et al. 2018). Here the researchers successfully developed SERS for 12 kinds of different pesticides having the lower limit less than 12 ppm. The technique is proved to be very rapid, on-site detection and can be used in point-of-care detection in daily life. Fu et al. reported a cost-effective and easy-to-handle platform for OP detection. The dipstick was modified with AChE enzyme, and it can convert the red color reagent (indophenol acetate) to blue (acetic acid and indigo). In the presence of OP, this activity was hindered, and the inhibition of catalysis can be used for sensing by monitoring the change in color. They used a smartphone-based ambient light sensor (ALS) for the quantification of the OP residues, and the colorimetric sensor was able to detect OPs with a detection limit of 3.3 μg/mL (Fu et al. 2019a, b). The sensor performance was comparable to standard Ellman's assay.

12.4 Conclusion and Future Prospective

Nowadays, pollutants are increasing day by day in our environment. This leads to a portable, rapid, and specific detection of pollutants or pesticides present in the agricultural field, atmosphere, and water bodies. Most of the traditional techniques which are used for the detection of pesticides are expensive and difficult to handle and need specialized and skilled persons. Nanoenabled biosensors are portable, less expensive, and rapid and do not need skilled persons and give specific and selective results. However, certain constraints are there with incorporation of recognition elements in the nanosensor. For instance, the cost of enzyme and antibodies is high due to cumbersome enzyme production and purification process, and the final product formed is not stable for a longer duration of time. This carries only the maximum efficiency at optimum temperature and pressure condition. On the contrary, fabrication process of MIPs is quite difficult and time-consuming. The system has also limitations in using aqueous medium and leakage of template molecules. Hence, nanoenabled sensors independent of biorecognition elements could have high potential for designing cost-effective, on-site devices for pesticide detection. However, it is still required to enhance their specificity and integrate them with portable reading devices to capture the end signal. Although various such biosensors are reported in the literature, very few are commercialized and available in the

market. Fabrication of materials and devices and its subsequent commercialization is a great challenge despite a well-demonstrated proof of concept. Hence, it is needed to explore the nanostructure-pesticide interaction more with the help of in vitro sensing methods and computational tools also. Further, work in the direction of simplification of signal monitoring units and their integration with biosensing surface would result in simplified and sensitive detection of pesticides.

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Smartphone Interface and Wearable Biosensors for on-Site Diagnosis

13

Guang Liu, Yanli Lu, Chen Cheng, Jie Xu, and Qingjun Liu

Abstract

With the rapid development of next-generation manufacturing, communication, and display technologies, smartphone has been widely integrated with multifunctional modules, such as sensor chips and handheld detectors for biochemical detections. Owing to the merits of high computing speed, high-resolution image analysis, and user-friendly human-computer interface, smartphone-based point-of-care testing (POCT) devices for personalized healthcare provide an affordable and accessible way for on-site diagnosis without using sophisticated and expensive instruments. The conventional biochemical analytical instruments are always bulky, not portable, and especially expensive; therefore, further applications are rather limited. The smartphone-based sensors and electronics have been developing rapidly, playing an increasingly important part upon the challenges confronting medical service, food industry, and public safety. This chapter presents smartphone interface and wearable biosensors for on-site analysis and takes our team's work as examples to elaborate. The sensing mechanism, design principle of smartphone-based portable and wearable sensing system, and implementation of biosensing strategies were discussed. For better insights into the important and valuable smartphone-based portable and wearable sensing devices, several examples were carefully discussed of device designing, application scenarios, analytical targets, and future applications. In the smartphone-based portable system, optical sensing, electrochemical sensing, and photoelectrochemical sensing were introduced with specific exciting and sampling circuit implementation and on-site diagnosis applications. In the

G. Liu · Y. Lu · C. Cheng · J. Xu · Q. Liu (✉)
Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, P. R. China
e-mail: qjliu@zju.edu.cn

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smartphone-based wearable system, we summarized the representative applications of the perspiration analysis system, implantable system, ingestible system, and wound monitoring system. Due to the wide-range permeability rate of smartphone in our lives, it can be expected that smartphone interface and wearable biosensors will gradually complement and dominate the existing health management approaches.

Keywords

Smartphone · Biosensor · POCT · Wearable electronics · On-site diagnosis

13.1 Introduction

With the growth need of point-of-care testing (POCT) for on-site diagnosis of analytes and pathogens in a variety of clinical conditions, there has been a transformation from the centralized biomedical analysis to easier, cheaper, and more reliable health monitoring at home or anywhere (Liu et al. 2019; Xu et al. 2018; Vashist et al. 2015; Tran et al. 2019; Jung et al. 2015). The conventional analytical instruments are bulky and more expensive and require professional operator, although they are sensitive and more authoritative. The strictly controlled laboratory conditions and time-consuming also limits large-scale household use. Thanks to the advances in electronics, functional material, nanotechnology, and micromachining technology, constructing biosensor on a variety of substrate provides an alternative solution to this problem (Turner 2013; Kuila et al. 2011; Kanchi et al. 2018). From a typical perspective, sensitive element modification, optimization, and signal sampling and analyzing are necessary steps to build biosensor for POCT devices (Kim et al. 2019; Miller et al. 2020; Perumal and Hashim 2014; Kirsch et al. 2013). In such case, how to effectively process signal, transmit data, process data, and display result is a new issue to be addressed. Designing and fabricating such biochemical sensing system to reach the function mentioned above is the key to fulfill the momentous needs.

Smartphones were initially brought to market by IBM and BellSouth in 1993, and the total number of users reaches a staggering 3.9 billion by 2021 (Huang et al. 2018). By now, smartphones are widely equipped with multicore processor, big memory, large storage, large battery, high-resolution camera, universal serial ports, and stable operating system. The wireless data transmission by 4G or 5G cellular net service for telecommunications, as well as Bluetooth, Wi-Fi, and near-field communication (NFC) for local communication, forms a fast connected network (Purohit et al. 2020). The cameras built into the smartphones have an easy access to contribute to the sensing system to achieve optical detection (Severi et al. 2020; Ardalan et al. 2020). In the meantime, more and more peripheral modules are integrated into the smartphones to realize product iteration, where the sensing system could be developed as stand-alone device with less space consumption and cost (Chen et al. 2021; Sun and Hall 2019). As there have been many interesting, unique,

Fig. 13.1 Illustration of on-site diagnosis with smartphone-based portable and wearable system

and applicable smartphone-based POCT devices, it is necessary to summarize recent advances in designing and developing of such devices.

Therefore, this chapter described the smartphone-based biosensors from two aspects, (1) portable system and (2) wearable system (Fig. 13.1), and the content is a selection of classic and recent research advances. In the field of portable system, spectroscopy detection (optical, surface plasmon resonance, and electrochemiluminescence), electrochemistry detection (amperometry, potentiometry, and impedimetric), and photoelectrochemical detection on smartphone were summarized and discussed with device design principles and sensing mechanism. As for the wearable biosensors, epidermal, implantable, ingestible, and integrated wearable devices for drug delivery were introduced with detailed technical circuit designing and fabricating, and their application in combination with smartphone-based devices has been briefly discussed.

13.2 Smartphone-Based Portable Detection System for On-Site Diagnosis

As the high computing speed and camera technology advance rapidly, smartphones have been providing a portable detection method that can be applied in a variety of scenarios. In this section, smartphone-based optical, electrochemical, and photoelectrochemical systems were summarized in the following part.

13.2.1 Smartphone-Based Optical Sensing System

Smartphones are widely used in biosensing system for optical detection with the built-in capabilities of high resolution and image acquisition (McCracken and Yoon 2016; Geng et al. 2017). The first smartphone-based optical sensor was reported by Martinez et al., in which the analysis of glucose and proteins was conducted by a paper-based microfluidic chips with smartphone (Martinez et al. 2008). Compared with the high cost of large laboratory instruments, which cost time and need special operators, smartphone-based platforms for optical sensors have lower costs and more simplified operation requirements (Wang et al. 2016; Zhang and Liu 2016). In this section, smartphone-based spectroscopy system, surface plasmon resonance (SPR) system, and electrochemiluminescence system were summarized with device designs and imaging analysis.

13.2.1.1 Spectroscopy Sensing

The urgent need of good health management is accelerating the development of health monitoring devices combined with smartphone sensing technologies. At the same time, a variety of smartphone-based platforms including smartphone units and integrated circuits for optical analysis of biological samples, such as saliva, urine, and serum were developed (Li et al. 2018a). Compared with the traditional laboratory analytical instruments, the acquired microscopic images can be analyzed based on the smartphone camera and processor with the features of the target components, such as the color, bulk, and brightness (Zhu et al. 2011; Smith et al. 2011). To acquire the image and spectra of micron-scale regions, a microspectroscopy/imaging system consisting of a portable spectrometer as an optical sensor and a compact homemade microscope was proposed by Guang et al. (Chen et al. 2020). In their work, the quantification of protein concentration was demonstrated with the smartphone-based proposed microspectroscopy/imaging system to analyze the spectrometer signals which highlighted the possibility for adopting the smartphone device for building a microspectroscopy/imaging system for point-of-care testing. With the development of the detection technology, colorimetric and fluorescence biosensors are gradually applied based on microscopic imaging. A colorimetric paper-based analytical device with high sensitivity was reported by Huy et al. for the detection of tetracycline by applying the green fluorescent carbon nitride ($g\text{-}C_3N_4$) nanoparticles using a smartphone for imaging (Huy et al. 2020). However, the sensitivity of smartphone-based colorimetric sensing system still needs to be

improved. To address this, nanomaterial-enabled colorimetric detection integrated in the smartphone-based point-of-care platform was developed by Xia et al. with high sensitivity and selectivity for the detection of the avian influenza virus (Xia et al. 2019). The modification of nanomaterials and the smartphone-based imaging system with giant data acquiring and processing ability improved the limit of detection, which reached down to 8×10^3 EID_{50}/mL.

Fluorescence microscopic imaging improves spatial resolution, specificity, and sensitivity for biosensing, which greatly promotes the bioanalysis of proteins, nucleic acids, and cells (Wei et al. 2013). Compared with conventional fluorescence equipment, the smartphone-based fluorescence sensing is limited by the camera and processor of smartphone itself as the cost is also lowered down. To surmount these shortcomings and realize molecule-level detection for on-site diagnosis, a Forster resonance energy transfer (FRET)-based nanoprobe combined with a smartphone RGB camera was proposed by Severi et al. for detection of nucleic acids (Severi et al. 2020). The nanoprobe response of nucleic acids can be detected in time as low as 10 pM in true color and in red-to-green ratio images using the RGB camera. The FRET-based biosensor can be easily applied using a smartphone coupled to an appropriate optical setup, expanding the method to high sensitivity and low cost for point-of-care analysis. Despite the hardware differences between smartphones, the software and high-speed computing of the smartphone itself can compensate for the shortcomings of this method to some extent (Chen et al. 2021).

13.2.1.2 Surface Plasmon Resonance (SPR) Sensing

SPR is a physical phenomenon which resonates under the excitation of incident light at the interface of materials with different conductivity (Karlsson 2004). When this phenomenon happens on the surface of nanoscale metal structures, such as gold and silver, it is also called local surface plasmon resonance (LSPR) (Zhang et al. 2015a; Bian et al. 2019). Due to its merits of interface effect, label-free, real-time, and eco-friendly, LSPR has attracted extensive attention. At the same time, with the rise of mobile health (mHealth) concepts, smartphones have gradually developed into an effective platform for LSPR-based biochemical sensing detection (Li et al. 2018b). Seemesh et al. reported a template-free plasmonic material of Ag-Soret colloids (Ag-SCs) on the surface plasmon-coupled emission (SPCE) platform to realize more than 100-fold enhanced fluorescence which enabled real-time monitoring of glutathione (GSH) (Bhaskar et al. 2020a). The proposed biosensing system was more sensitive and selective, while had lower cost for on-site detection of biomarkers. With the further researches in SCs from various nanomaterials and nanocomposites, the smartphone-based analytical and photonic devices for the next generation will help the system reach to a higher level. Meanwhile, a lateral flow plasmonic biosensor consisting of gold-viral biomineralized nanoclusters (AuVCs) for on-site GSH determination was developed by Pang et al. (2020). The sensing system reached a limit of detection (LoD) of 9.80 μM with the linear in the range of 25–500 μM. At the same time, Seemesh et al. also demonstrated a label-free smartphone-based SPCE biosensor for the detecting spermidine with Au-decorated SiO_2 nanoparticles (Bhaskar et al. 2020b). The smartphone platform can capture the

multifold hotspots that were rendered by the nanohybrids assisted in augmented electromagnetic (EM)-field intensity, which made it promising for portable and economical biosensing system in such area.

13.2.1.3 Electrochemiluminescence (ECL) Sensing

ECL is widely used in bioanalysis for point-of-care diagnosis, which combines the merits of chemiluminescence (CL) analysis without background light noise and easy control of reactions by applying a voltage to the electrode (Cao et al. 2020; Li et al. 2019a; Qi and Zhang 2020). As an analytical technique, due to its versatility, it can have a simpler optical setup than photoluminescence (PL), better time, and space control than CL. Zhu et al. proposed a handheld device capable of ECL analysis, which used smartphone as optical signal reader for the discrimination of 3-nitrotyrosine (3-NT) (Zhu et al. 2021). The device consisted of printed circuit board for ECL stimulation and a flexible antenna was designed coupled with an NFC for data transmission. In the design of smartphone-based ECL system, the high-definition cameras and programmed software in smartphones can capture luminous images (Rahn et al. 2020). Therefore, the integration of smartphone and ECL is a preferable method for optical analysis as it meets the need of on-site biosensing for point-of-care diagnosis with simplified system and powerful capabilities (Nie et al. 2019).

A smartphone-based ECL device was designed for the biochemical sensing of nicotine and trinitrotoluene (TNT) with the on-site fingerprint mapping (Fig. 13.2a) (Li et al. 2019b). The electrochemical excitation for ECL and optical analysis were integrated in a handheld smartphone-based system with a specially designed device. The bioanalysis of typical enhanced luminescence for nicotine and quenched luminescence for TNT was demonstrated for proof of concept of the smartphone-based ECL sensing system. In addition, this system could also conduct multimode imaging analysis on smartphone for fingerprint biochemical mapping, such as color, gray, and RGB extraction (Fig. 13.2b). At last, in situ analysis of exogenous substances on human hands, such as nicotine and TNT, was realized through the smartphone-based ECL device where the fingerprint's morphology and grain were clearly exhibited on the optical images (Fig. 13.2c). The fingerprints are widely used for unlocking terminal equipment such as smartphones and laptops. Thus, the combination of fingerprint analysis and smartphone-based sensing platform will contribute to the development of personal health monitoring as well as public safety. In addition, the proposed sensing platform can also provide additional information of diet habit, drug intake, and personal medicine. The excellent performances of smartphone-based ECL system can be further applied in various fields of biochemical detection and imaging analysis.

13.2.2 Smartphone-Based Electrochemical System

Nowadays, the electrochemical biosensors which take advantage of the specificity of biological element, such as enzyme, antibody, and cell, are widely researched and

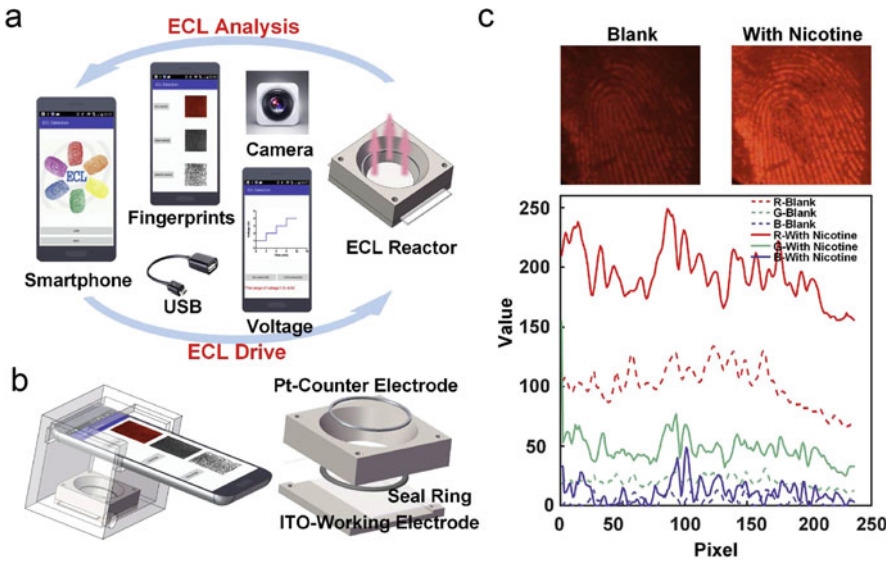

Fig. 13.2 Smartphone-based ECL system for fingerprint mapping and biochemical analysis. (**a**) Schematic of the smartphone-based ECL system with the universal serial bus-based electrochemical excitation, the camera-based imaging analysis, and the user interface. (**b**) The concept of reaction cell and electrodes (platinum and ITO). (**c**) In situ analysis of nicotine on fingerprints. Reproduced from Li et al. (2019b). Copyright 2019 Elsevier

partially commercialized (Maduraiveeran et al. 2018; Cesewski and Johnson 2020). However, traditional lab-based analysis methods always require complicated instrumentation like electrochemical workstation and stringent operating environment. With multiple functional modules, smartphone has been significantly improving the portability of electrochemical detection (Zhang and Liu 2016). To reach a full combination of smartphone and electrochemical methods, an external miniaturized potentiostat applying electrochemical excitation on the surface of the electrode is needed. In addition, the integrated circuits for data sampling, amplifying, and communicating always require individual designs. Besides, the electrochemical electrode is another essential part for the bioanalysis. With the advances in additive and subtraction manufacturing, various kinds of electrode can be fabricated on soft and hard substrate such as paper, polyethylene terephthalate (PET), and polyimide (PI) through the screen-printing technology, laser-based manufactory, etc. Recently, multifold electrochemical sensing methods have been integrated with smartphones, including amperometry (Ji et al. 2017, 2020), potentiometry (Xu et al. 2019; Yao et al. 2019), and impedimetry (Zhang et al. 2016; Rosati et al. 2019). These detection systems extend the potential of electrochemical biosensor for applying in real-time biochemical analysis and on-site diagnosis.

13.2.2.1 Amperometry Sensing

Integrated with smartphone technique, the amperometry-based biosensing is commonly applied in numerous detection fields (Zhang and Liu 2016; Garcia et al. 2018). As the excitation potential applied, the current is related to the oxidation or reduction of the electrical mediating species during the amperometry, which is proportional to substrate concentration. For smartphone-based amperometry system, these extraordinary methods, such as chronoamperometry (CA), cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV), could effectively improve the sensitivity of the biosensor system. Due to the good simplicity and excellent adaptability, it became the first reported of smartphone-based biosensor application (Zhang and Liu 2016; Lillehoj et al. 2013). Among various detecting techniques, CV and CA with more practicability for the quantitation of analytes attract much attention. Recently, a smartphone-based CV system was developed by Ji et al. with simplified electrode modification to perform electrochemical detections (Ji et al. 2017). The detecting system consisted of electrochemical modified electrodes, the designed detector, and the smartphone with software. Compared with the standard analytical equipment, the proposed CV-based system had lower cost and smaller size and could perform electrochemical test in real time. To further apply the bioanalysis ability of smartphones, an electrochemical device based on the paper fabrication was proposed by Caratelli et al. for the detection of butyrylcholinesterase (BChE) activity in whole blood (Caratelli et al. 2020). The development of an analytical method for easy self-testing of BChE inhibitor activity in whole blood on a paper-based lab-on-a-chip with lower cost and user-friendly operation made a good contribution to personalized administration of drugs along with Alzheimer's disease.

In addition to cyclic sweeps, pulsed potential excitation such as SWV, DPV, and differential pulse amperometry (DPA) is also a widely used electrochemical method, which could be employed for biochemical sensing, such as nucleic acids, peptides, and heavy metal ions (Ji et al. 2018, 2020; Shi et al. 2020). For example, a rapid detection system combined with a smartphone-based electrochemical detector was constructed for monitoring of levodopa, which can be applied for Parkinson's disease prevention and treatment, while the conventional treatment is to supplement levodopa so as it could overcome blood-brain barrier and synthetic dopamine (Ji et al. 2019). The system contained these main parts: a disposable screen-printing electrode, a handheld detecting circuit, and a smartphone with programmed software (Fig. 13.3a). The detecting circuit was powered by lithium ion battery, and the electrochemical excitation can be applied to measure the subsequent current change on the surface of electrode. Finally, the potential and current information were transferred to smartphone via Bluetooth module. This proposed system exhibited good performance in the detection of levodopa from 0 μM to 200 μM and with a limit of detection as low as 0.5 μM in human serum, in which the modified sensor could also distinguish levodopa from representative interference (Fig. 13.3b). To implement the smartphone in the nucleic acid detection, Low et al. demonstrated a detector using smartphone for electrochemical sensing of circulating microRNA-21 (miR-21) biomarker in saliva as a proof of concept (Shin Low et al. 2020). The

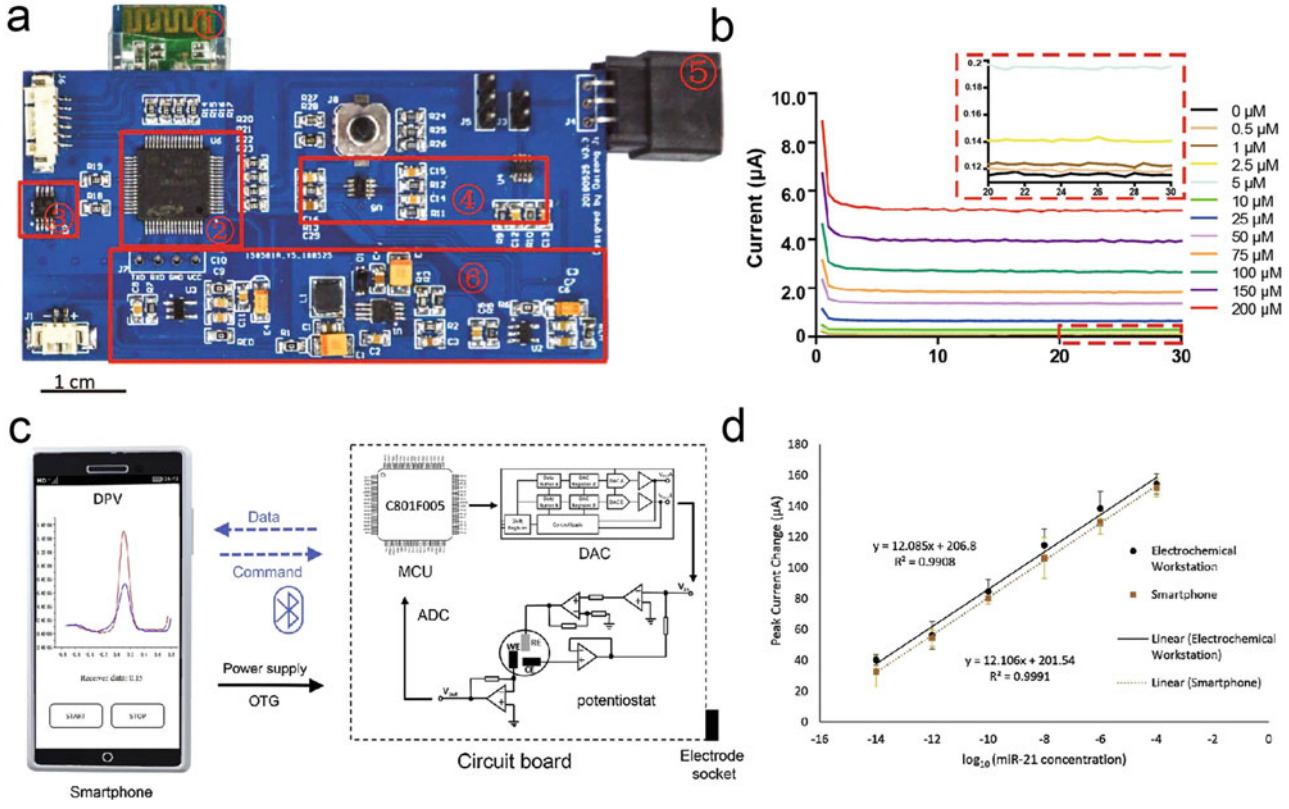

Fig. 13.3 Smartphone-based electrochemical system. (**a**) Smartphone-based DPA system (the Bluetooth module, controller unit, digital analog converter, potentiostat module, electrode socket, and power management module are in the red box from 1 to 6). (**b**) The diagram of DPA measurement using the system for levodopa detection at different concentrations. Reproduced from Ji et al. (2019). Copyright 2019, Elsevier. (**c**) Schematic diagram of smartphone-based DPV system. (**d**) Corresponding peak current change of the smartphone-based sensing system and standard analytical equipment in miR-21 gradients. Reproduced from Shin Low et al. (2020). Copyright 2020, Elsevier

electrochemical detecting system consisted of commercial screen-printing electrode, which was modified with reduced graphene oxide/gold nanoparticles (rGO/Au), a detector, and a smartphone with programmed Android software (Fig. 13.3c). The salivary miR-21 was detected as a proof of concept with concentration level of 1 pM to 100 μM with correlation coefficient of 0.99 (Fig. 13.3d). Furthermore, with the huge demand for virus testing and family practice, this smartphone-based sensing system is capable to provide timely, low-cost, rapid on-site diagnosis, which satisfied the testing needs and reduced the squeeze of medical resources.

13.2.2.2 Potentiometry Sensing

The potentiometry is easier to implement in smartphone-based electrochemical sensing system but as important as amperometry, which is widely used to determine the potential change or biochemical activity (Zhang and Liu 2016; Yao et al. 2019). The mechanism of potentiometry is to detect the cumulative charge resulting in potential change in the dielectric layer, which is commonly applied in the detection of electrolyte ion and other biomarkers (Zhang and Liu 2016; Zhang et al. 2015b, c). Therefore, ion-selective membranes are usually modified on the electrode surface for the trace detection using potentiometry (Dimeski et al. 2010). For example, a wearable and battery-free epidermal electrochemical system was reported by Xu et al. with NFC technology and screen-printing electrode. The wearable detection system got rid of the rigid batteries, and it can adhere to the skin surface and detects electrolyte ions in sweat (Xu et al. 2019). The potentiometry can be applied for molecular imprinting or enzyme-based biosensing in the same way. Yao et al. proposed an enzymatic aptasensor using freestanding graphene paper for wirelessly detecting kanamycin (Yao et al. 2019). A nuclease was used to assist to amplify, and it realized an ultrasensitive biosensing platform with the smartphone recording real-time data. This work paved the way for implementing of paper-based material and enzyme-based biosensing, which provides a broader application for smartphone-based potentiometry sensing.

13.2.2.3 Impedimetric Sensing

To measure the impedance of an electrode system, the electrochemical impedance spectroscopy (EIS) is set with the scanning from low frequency to high frequency, which was usually from 0.1 Hz to 1 MHz. The EIS could exhibit the change of electrochemical properties, such as electrode capacitance changes and the resistance caused by electron transfer (Chang and Park 2010). Through a sinusoidal voltage applied, the ratio of alternating voltage and current change at different frequency was measured, where the corresponding electrochemical signal detection can be realized. Due to the high resolution of frequency, smartphone-based impedimetric sensing systems are widely used in the detection of small molecules, proteins, nucleic acid, and gases. The first reported application of smartphone-based impedimetric system was developed by Broeders et al. in 2013 (Broeders et al. 2013). Later, Zhang et al. reported a smartphone-based wirelessly controlled biosensing system developed with electrochemical impedance spectroscopy for detecting proteins along with the data transferring via Bluetooth (Zhang et al. 2016). In addition to the solid/liquid

phase biosensing, the volatile organic compounds (VOCs) are usually measured by impedance. For example, a smartphone-based impedance system was developed for monitoring VOCs in expiratory gas by Liu et al., which provided a platform for early diagnosis for pulmonary biomarkers (Liu et al. 2017). Recently, an electronic nose with small size and low cost was developed by Arroyo et al. with a mobile programmed terminal for classification services (Arroyo et al. 2020). Based on frequency resolution, impedance analysis methods can make a unique contribution to material detection especially in selectivity.

13.2.3 Smartphone-Based Photoelectrochemical (PEC) System

Based on the photoexcitation of photoelectric compound under exogenous excitation, PEC analysis is an emerging analytical method with potential application for on-site diagnosis recently (Zhao et al. 2017; Zhou and Tang 2020). The exogenous excitation facilitates the separation of surface charges in photoelectric compound and to stimulate the electrochemical reaction. As the excitation source and electrochemical current/potential response are separated, the PEC-based analysis system has lower background interference and improved sensitivity (Zhang et al. 2017, 2019). Conventional PEC analysis depends on the large excitation source and standard measuring equipment, such as high-power xenon lamp and electrochemical workstation, which hinders PEC analysis for portable and on-site rapid testing. Recently, the rapid development of integrated electronics, the processing, and fabrication of miniaturized devices also provides opportunities for the application of PEC analysis method (Zhang and Liu 2016). At the same time, combining terminal equipment such as smartphones with the designed analytical devices can achieve lower cost and faster testing.

Combined with the advantages of smartphone and PEC analysis, a portable and universal biosensing platform was constructed for rapid on-site diagnosis (Fig. 13.4a) (Zhang et al. 2021). The portable system consisted of the electrodes, detecting circuit board, and a smartphone. The miniaturized detecting circuit board and battery provided photoexcitation; then, the photocurrent was measured by the sampling module. Smartphone was used as commander and data display via Bluetooth control. The graphitic g-C_3N_4 was used as photoelectric compound, and the gold nanoparticles (AuNPs) were applied to immobilize matrix metalloproteinase-2 (MMP-2) for specific cleavage peptide. The small light-emitting diode (LED) excited g-C_3N_4 to generate photocurrent, and the hydrolyzed and cleaved process of MMP-2 for peptide was evaluated by photocurrent (Fig. 13.4b). The photocurrent varies linearly with the concentration of MMP-2 ranging from 1 pg/mL to 100 ng/mL (Fig. 13.4c). This system reached a limit of detection buffer and 0.55 pg/mL in artificial serum. These results verified the effectiveness of the portable smartphone-based PEC device, which provided a potential solution for miniaturized and portable on-site diagnosis.

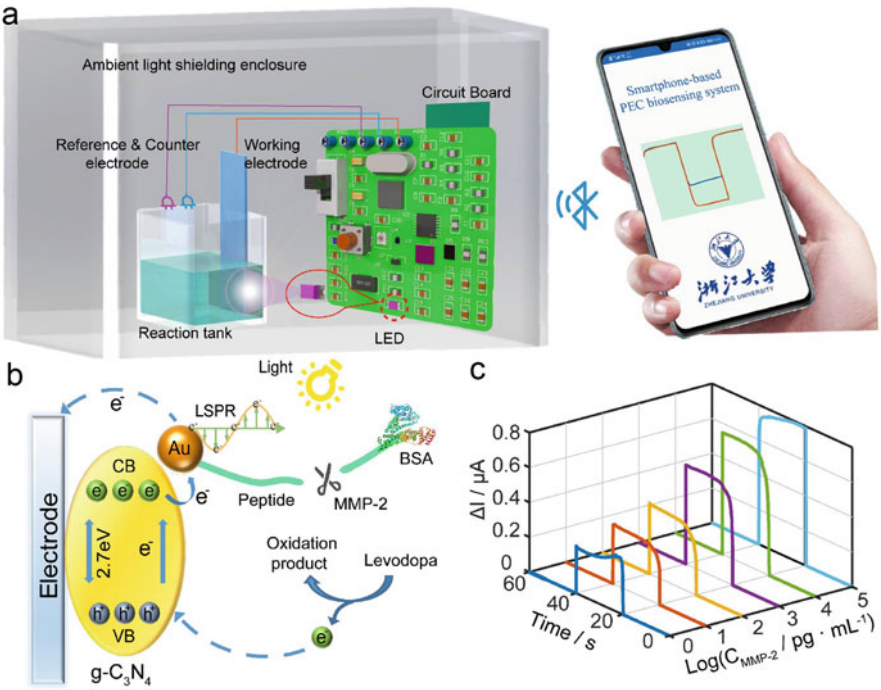

Fig. 13.4 Smartphone-based PEC system. (**a**) Illustration of the smartphone-based PEC detection system, which is composed of a reaction tank, the electrodes, detecting circuit board, and a smartphone. (**b**) The electron-hole transfer mechanism in the proposed PEC system. (**c**) Photocurrent response of MMP-2 gradients. Reproduced from Zhang et al. (2021). Copyright 2021, Elsevier

13.3 Smartphone-Based Wearable System for On-Site Diagnosis

Over the past few decades, with the rapid development of microelectronics technology and materials science, wearable electronics have achieved tremendous development (Gao et al. 2016; Nyein et al. 2016). Here, smartphone-based biosensors for epidermal application, implantable application, ingestible application, and integrated wearable drug delivery system were summarized in the following section.

13.3.1 Epidermal Biosensor System

The epidermis covers most of the human body, which is the largest organ capable of protection, absorption, sensation, metabolism, and immunity. The biological fluids in epidermis, such as sweat and interstitial fluid, contain plenty of health information, which can be analyzed in real time by epidermal biosensors (Menon 2002). In the above example, smartphone-based electrochemical sensors and biosensors are

mainly employed to measure small molecules and ions in sweat, such as lactic acid, glucose, sodium ion, potassium ion, hydrogen ion, calcium ion, chloride ion, etc. These sensors can measure trace molecules or ions accurately, and they exhibited outstanding sensing performance. However, much biochemical analysis has not been able to establish a clear correlation with body condition or specific diseases. For instance, the correlation of sweat glucose and serum glucose has not yet been studied clearly, which is still under research stage (Bandodkar et al. 2016). The concentration of sodium and potassium ions is related to the state of exercise metabolism. However, there is no clear research to explain its mechanism (Corrie et al. 2015). Chloride ions in sweat have been used to indicate cystic fibrosis (CF), but this disease is relatively rare (Bariya et al. 2018). However, with the giant health information in sweat, developing a wearable device to monitor biomarkers is important for preclinical diagnosis of specific diseases (Nimse et al. 2016). To find a solution, Gao et al. reported a fully integrated wearable and flexible sweat analysis device in 2016 (Gao et al. 2016). The devices executing embedded programs can run various electrochemical detection methods with flexible and bendable circuit. The development of microelectronics technology enables miniaturized circuit systems to be designed in the size of a single wristband. Later, Xu et al. reported a battery-free and flexible detection patch with wireless communication based on NFC technology for the more stretchable and comfortable sweat analysis (Xia et al. 2019; Xu et al. 2019).

Detection of sweat substances like ions, glucose, and lactic acid only reflects electrolyte loss and exercise health. However, the hormones and sterols might contain more health information in body regulation. In regard to this consideration, a flexible skin-attached detection device that could monitor the concentration of cortisol in sweat was developed (Fig. 13.5a) (Cheng et al. 2021a). Secreted by the adrenal cortex, cortisol was an adrenal cortex hormone, whose concentration in serum and sweat indicates the mental state of the human (Katsu and Baker 2021). Cortisol concentration in sweat was associated with clinical psychiatric disorders such as anxiety, depression, and post-traumatic stress disorder (PTSD). Detecting cortisol in sweat could aid in the diagnosis and treatment of these diseases. Based on the flexible substrate, the proposed detection device contained a signal processing module and NFC module, which can be bent and folded to fit the surface of the human skin closely without causing discomfort (Fig. 13.5b). The detection device adopted DPV as the detection technology, which could be applied to measure the target substance quickly in real time. The detection device was compared in experiments with a commercial large-scale electrochemical workstation, in which the testing results showed similar performance. The device took advantage of electrochemical immunoassay technology with the detection range that could reach 0 nM to 500 nM for cortisol (Fig. 13.5c). Further, the device also tested sweat samples of the volunteers in vivo. During 7 days, the volunteers' sweat was collected in the morning and evening for cortisol analysis. These data reflected the circadian rhythm of cortisol content in subjects over 7 days (Fig. 13.5d). The detection system proposed by Cheng et al. established an integrated, small, flexible

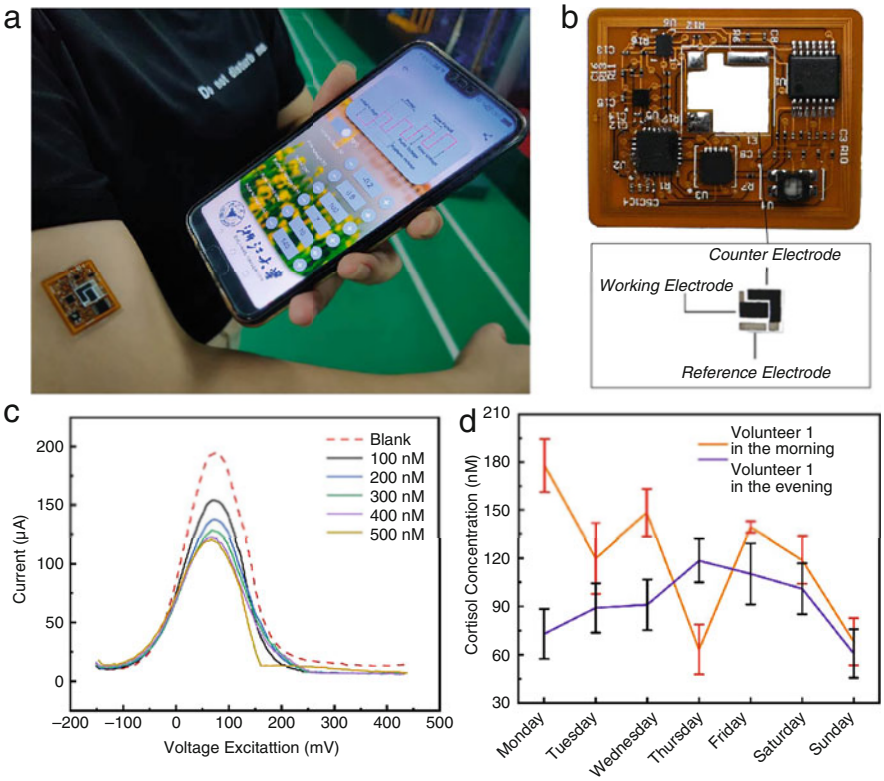

Fig. 13.5 Smartphone-based epidermal biosensor system. (**a**) Using a smartphone to read data from a sweat cortisol analysis device. (**b**) Photograph of the flexible and wearable analysis system. (**c**) Cortisol detection with DPV from 0 nM to 500 nM. (**d**) Sweat cortisol detecting results during 7 days. Reproduced from Cheng et al. (2021a). Copyright 2021, Elsevier

detection platform for the sweat cortisol monitoring, which had potential value for the health management of human mental state.

13.3.2 Implantable Biosensor System

The urgent need for continuous monitoring of body condition with the purpose for solving the problem to the routine requirement of hospitalization and supervision of the patients drives integrated biosensing system field growing fast. Hence, much research aims to explore and develop skin-integrated and implantable medical devices (Bobrowski and Schuhmann 2018; Ashammakhi et al. 2021; Oh et al. 2021). In these devices, the common monitoring signals are heart electrocardiogram (ECG), blood pressure, pulse rate, blood glucose level, and respiration efficacy. Except for physiological or biochemical sensing, external excitation for tissues is

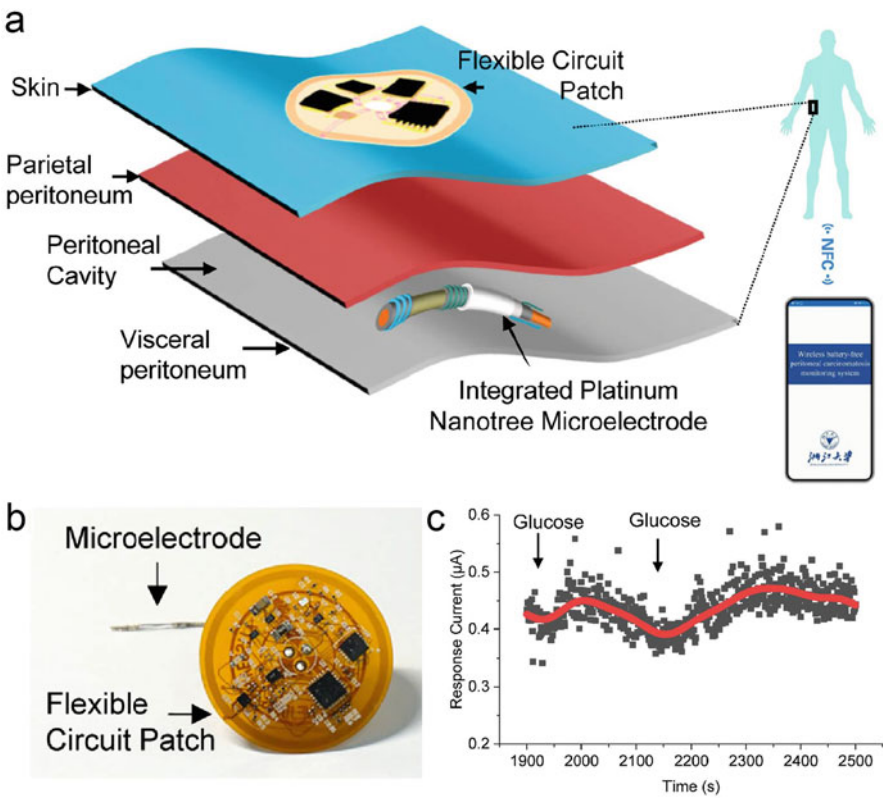

Fig. 13.6 Smartphone-based implantable glucose sensors. (**a**) The explosive view of the battery-free and implantable electrochemical system. (**b**) Image of microelectrode and the flexible circuit patch. (**c**) The variation of response currents after glucose injections. Reproduced from Xu et al. (2021a). Copyright 2021 Elsevier

also a research hotspot for implanted devices. With the rapid development in biocompatible material, miniaturized design, wireless communication, and power supply technology, the electrochemical detection device is converted from rigid and wired designs to wireless, battery-free, and flexible system, which makes in vivo, in situ, and real-time physiological data monitoring available (Boutry et al. 2018).

Smart devices such as smartphones with NFC function are suitable for implantable applications, because they could be designed to be smaller and thinner without the requirement of battery, along with data transmitting outside of body wirelessly. Based on this, an implantable, wireless, and battery-free electrochemical system was proposed (Fig. 13.6a) (Xu et al. 2021a). It could perform detection of the glucose concentration in the peritoneum while a patient had peritoneal cancer. The electrochemical detection system was mainly composed of a flexible circuit patch and an integrated platinum nanotree microelectrode. The circuit patch consisted of a potentiostat for electrochemical excitation and NFC module. The developed system

was capable of fast detection of glucose concentration, wireless data transmission, and power supply (Fig. 13.6b). The modification of highly sensitive platinum nanostructure and Nafion on implantable electrode enhanced the performance for in vitro and in vivo test in rat peritoneal cavity. In addition, results from the biochemical analyzer with the implantable and wireless patient ascites monitoring system were compared. The reliability of monitoring system was demonstrated in Fig. 13.6c. The implantable electrochemical system for detecting peritoneal glucose owned the characteristics of less invasiveness and fast feedback and provided a hassle-free method for the monitoring of peritoneal cancer.

13.3.3 Ingestible Biosensor System

The rapid development of microelectronics and materials science has promoted the emergence of small-sized, low-power consumption and ingestible biomedical electronic devices (Abramson et al. 2021). The ingestible electronic devices have the feature of small size which is enough to be swallowed into the gastrointestinal (GI) tract without anesthesia. They are not available in previous semi-invasive devices. Hence, the development of ingestible devices has attracted much attention in recent few years. However, designing competent ingestible biomedical electronic devices faces many challenges. For instance, the volume of the device needs to be small enough, and shape of the device must conform to the characteristics of the GI tract. These allow it to smoothly pass through the narrow parts of the GI tract without causing additional damage such as perforation, intestinal obstruction, ulcers, etc.

Based on wireless communication and electrochemical sensing method, a capsule-based ingestible sensing system was developed with IrOx modified for monitoring the pH of the GI tract in real time (Fig. 13.7a) (Cheng et al. 2021b). The capsule system uses IrOx-deposited screen-printed electrodes (SPE) featuring high sensitivity as the pH sensor. The capsule detection circuit is designed with rigid-flexible composited printed circuit board (RFPCB) technology and can be loaded with the pH sensor (Fig. 13.7b). Capsule structure was fabricated by 3D-printed technology. The capsule detection circuit can be bent and folded into a small volume and assembled into the capsule structure. Polydimethylsiloxane (PDMS) was covered on the outer surface of the capsule structure to improve its biocompatibility and ensure its reliability. The data and control command of the capsule detection circuit is wirelessly transmitted by the Bluetooth module to the external receiving device, where the external receiving device could be smartphone, personal computer (PC), or programmed auxiliary circuit. At last, the capsule system was applied to monitoring the pH value of artificial digestive juice as an in vitro simulation. For in vivo evaluation, the pH value of the GI tract of canine was detected. The results of the detection process were compared with commercial pH sensors, which exhibited excellent performance (Fig. 13.7c). The proposed method could be used to evaluate the performance of the capsule system for monitoring or drug releasing and even laid the groundwork for further clinical trials.

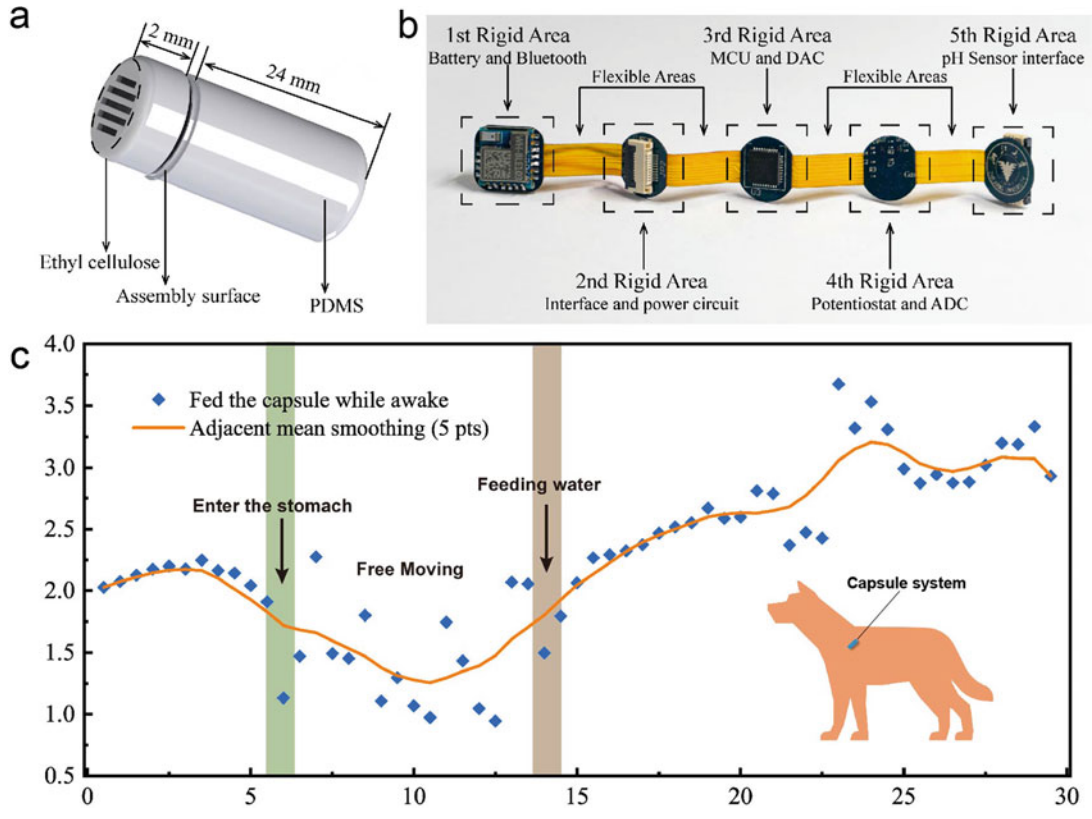

Fig. 13.7 Smartphone-based wireless capsule sensing device for GI pH detection. (**a**) The view of smartphone-based ingestible capsule. (**b**) The capsule detection circuit designed by rigid-flexible composite printed circuit board (RFPCB) technology consists of four flexible substrates and five rigid substrates. Among them, the flexible substrate only plays the role of connection, and the five rigid substrates are respectively placed: lithium ion battery and Bluetooth module, download interface and power circuit, microcontroller (MCU) and digital-to-analog conversion (DAC) module, potentiostat and analog-to-digital conversion (ADC) module, and electrical interface of pH sensor. (**c**) The results of real-time detection of pH in the GI tract of beagle dogs by capsule system. Reproduced from Cheng et al. (2021b). Copyright 2021, Elsevier

13.3.4 Integrated Wearable Drug Delivery System

The acute illnesses on skin are usually treated by hypodermic injection or oral administration of drugs, while the chronic diseases require an integrated wearable drug delivery system for long-term treatment and protection (Mirvakili and Langer 2021). The destruction of the intact skin or subcutaneous tissue by sharps, burns, and blows usually causes chronic wounds, which increases the pain and long-term stress for patients. Wounds could be infected by pathogens if without proper care where the infection may cause slow healing and further pain to the patient (Liang et al. 2021; Farahani and Shafiee 2021). Chronic wound and a series of complications such as tissue damage, organ failure, and septicemia could be caused by severe wound infections. Hence, biochemical monitoring and medical treatment of wounds are vital for wound recovery. In recent years, with the development of intelligent materials and structures that can respond to various external stimuli, wound treatment can be realized through various kinds of wearable smart wound dressings (Tang et al. 2021; Tu et al. 2021). Thus, constructing a biocompatible and wearable wound diagnosis and treatment device to monitor wound status by integrated biosensor and provide treatment by smart wound dressing is quite important.

To address this, a fully integrated, battery-free, and wireless smart wound dressing was proposed, which had the functions of wound infection monitoring and on-demand drug delivery (Fig. 13.8a) (Xu et al. 2021b). The proposed wound dressing established a closed-loop monitoring and drug delivery platform, in which the system showed its electrical controllability and stability. Through miniaturized circuits and integrated smartphone application, the smart wound dressings with integrated NFC modules can realize wireless energy harvesting, data transmission, on-site signal processing, and on-demand drug delivery control (Fig. 13.8b). To assess wound condition, the sensors of the dressing synchronously detected three indicators including wound temperature, pH value, and uric acid. In the meantime, through electronically controlled antibiotic delivery, the drug delivery electrodes in the dressing could provide on-demand treatment for wound infections. In situ animal studies showed that the integrated wearable drug delivery system can effectively promote wound healing, which fully validated to be effective in the wound treatment (Fig. 13.8c, d). Utilizing the advantages of NFC technology and flexible electronics, the battery-free and integrated design of sensing and treatment provides a promising solution for the development of a closed-loop biomedical system integrating monitoring, diagnosis, and therapy in single device.

13.4 Conclusion and Future Perspective

In summary, this chapter focuses on the smartphone interface and wearable biosensors for on-site diagnosis. According to the way of application, the development of biosensor technology on smartphone can be divided into two parts: portable and wearable system. As for smartphones portable biosensors are concerned, the optical, electrochemical, and PEC-based sensing system were summarized. Among

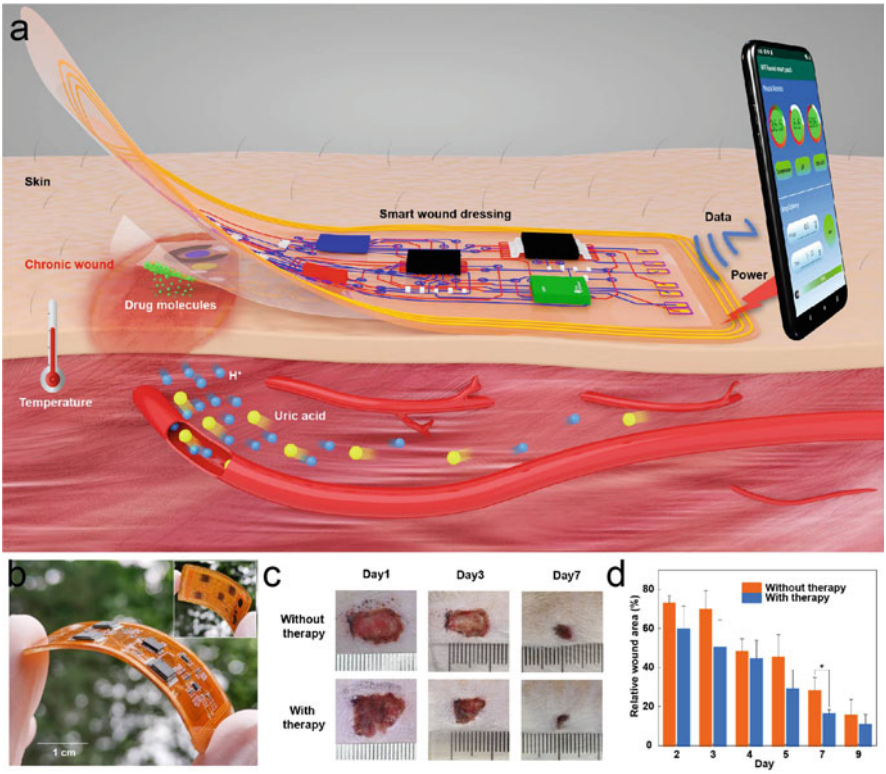

Fig. 13.8 Smartphone-based integrated wearable drug delivery system. (**a**) Schematic diagram of the smart dressing, which could monitor temperature, pH, and uric acid simultaneously when attached to the wound and provide feedback treatment through the electronically controlled release of drug molecules according to the monitoring results. (**b**) Side view of the wearable drug delivery patch. (**c**) Experiments in rats to assess the effect of dressings on wound healing. (**d**) Image of relative wound area over time during wound healing. Reproduced from Xu et al. (2021b). Copyright 2021, Wiley

them, smartphone-based optical biosensors have been making good use of the integrated camera imaging and optical intensity measurements. These designs are characterized by simplicity, high miniaturization, and multifunction. However, the complex operation and quantitation of optical biosensors were not as accurate as electrochemical methods. The electrochemical biosensors rely on the interface or external device to excite redox reactions and to sample signal with wireless communication via Bluetooth, radio frequency (RF) antenna, etc. Among them, the application of smartphone-based PEC system for the on-site diagnosis could be expanded to more fields especially in nucleic acid detection. As for the wearable biosensors, trends of flexible electronics in building next-generation biosensors for in situ detection have emerged. The wearable biosensors take advantage of smartphone to achieve wireless communication and power supply for integrated and miniaturized

sensing system. The implantable and ingestible biosensors have attracted more researchers for the capability to capture more direct biochemical information. With the integrated wearable system, the transdermal drug delivery to human body would greatly reduce the pain of hypodermic injection, increase the comfort and adherence of patients, and finally enhance the efficiency of drug administration.

With the revolutionary invention of smartphone, the evolution of electronics technology has led to decreasing the size but increasing the diverse functions. These devices can also integrate various sensors and biosensors, making them portable and wearable with high-resolution image processing and fast computing ability. As mentioned with above examples, the smartphone-based devices in portable sensing system could be designed to the size of the palm of hands, and the wearable system could be miniaturized to a microneedle size or electronic tattoo. Researches on wearable sensing devices have been widely developed on smartphones for biomedical applications. But in most application, smartphone serves only as a control and display analysis device. As the materials and electronics developed, customizing sensor, special function hardware, and integrated smartphones will finally meet the growing need for personalization of health management. In addition, due to the complexity of ergonomic design and the obstacles to energy supply, these devices still face enormous commercialization challenges. In the meantime, plenty of clinical trials should be carried out, and collected samples can be used to prove the availability, biocompatibility, and the anti-interference properties of these biosensors previous to the next generation of wearable sensors entering in commercial application. By then, the whole smartphone-based sensing system can be closely connected with people's healthcare for sustaining physiological monitoring.

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Nanomaterial-Based Lateral Flow Assays for Point-of-Care Diagnostic Tests

14

Arnab Ghosh, Arpita Banerjee, and Rohit Srivastava

Abstract

Point-of-care tests (POCTs) have become increasingly popular recently because of their advantages like user compliance, quick results, reproducibility, ease of monitoring and bedside availability, and relatively lower cost than laboratory-based tests. Despite its wide popularity, the precision factor limits the use of POCT in many instances. Nanotechnology-enhanced lateral flow assays (LFAs) can increase precision in existing LFA technologies and improve test outcomes. The development of lab-on-a-chip system that includes miniaturization, micromachining, and nanotechnology is a promising advance in this field. LFAs identify biomarkers such as hormones (serum/urine HCG, TSH), glycosylated hemoglobin, cardiac troponin I, C-reactive protein, and interleukins and many infectious diseases like dengue, SARS-CoV-2, HIV, and many others. Gold nanoparticles (GNPs) are preferred over other NPs in LFA development due to their ease of synthesis, stability, protein grabbing capabilities, direct quantification approach, and higher sensitivity and specificity. Besides, carbon nanotubes, carbon nanoparticles, quantum dots, and nanozymes are also frequently used for the same purpose. The various limitations of LFA can be eliminated through a variety of signal amplification procedures, including pre-concentration, enzymatic color enhancement, modification of the size and

A. Ghosh (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai, India

Medical Specialist, Indian Navy, New Delhi, India

A. Banerjee · R. Srivastava
Department of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai, India

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shape of GNPs, and the addition of other types of dye-loaded chromatic agents that impart the LFA strips with exceptional color, magnetic, or fluorescent properties. Despite several unsolved concerns, it remains one of the most promising tools for future POCT systems, owing to its simplicity, portability, user-friendliness, and affordability.

Keywords

Point-of-care · PoCT · Lateral flow assay · Nanoparticles · LFA · Nanotechnology · Gold nanorods · Carbon dots · Detecting probes · Affordable · Rapid diagnosis · Healthcare

14.1 Introduction

An analyte in a sample can be identified using lateral flow assay (LFA) technology, a paper-based, point-of-care strip biosensor. LFA has grown in popularity due to its low cost, ease of use, portability, and speed in detecting analytes in industries such as agriculture, food, biomedicine, healthcare, etc. Despite its simplicity of use, it lacks accuracy compared to standard gold testing. The precision mechanism has been considerably enhanced by using metal nanoparticles, carbon-based nanoparticles, quantum dots, lanthanides, up-converting phosphor, and many more. To understand the prospect of nanoparticles in LFA technologies, we will first review the pros and cons of point-of-care tests and compare LFA with other point-of-care testing (POCT) techniques. Amidst various LFA techniques, the relative advantages of nanoparticle-based LFA and its challenges will be discussed in this chapter. The background of POCT, principles, components, and techniques of LFAs are vast topics and have been covered briefly for comprehensive review.

A POCT is the comprehensive examination of patient specimens conducted outside a clinical laboratory, at or near the site of patient care, and is typically performed by healthcare personnel who do not necessarily have laboratory experience. Laboratory medicine is regarded as an important component in differential diagnosis in clinical practice since extensive laboratory analyses help in accurate diagnosis in more than half of all diseases and adequate drug therapy monitoring in various conditions (Luppa et al. 2011). Early diagnosis is typically possible by eliminating unnecessary pre- and post-analytical delays. The turnaround time for laboratory data might be more than 60 min, compared to 10–15 min for POCT (Kankaanpää et al. 2018). It can also incorporate self-monitoring by the patient and thus offers immediate findings close to the patient, allowing it to be addressed immediately (Mahato et al. 2016). This is a great advantage compared to laboratory-based tests where decision-making is delayed (Florkowski et al. 2017) due to sample collection, transport, analysis, and interpretations.

Discovery of POCT The term "on-site patient care" was first introduced in England in 1955 in a hematology testing manual (England et al. 1995). In 1972, Dr. Kost

Table 14.1 Clinical applications of POCTs

Clinical application	Parameter
Critical care and general medicine	Arterial blood gas analysis pCO_2, pH, pO_2
Biochemistry	Electrolytes like sodium, potassium, magnesium, calcium, Ca, and urine microalbumin
Metabolic parameters	Lipid profile, renal function tests like urea, creatinine, serum lactate, uric acid, and bilirubin
Serum enzyme analysis	γ-GT alkaline phosphatase, AST, ALT, amylase, CK, CPK, and CKMB
Hematology	Blood counts, hemoglobin and formed elements of blood
Coagulation profile	D-dimer, PT, aPTT, INR, and bleeding time
Hemoglobin fractions	Carbon-monoxide oximetry
Cardiac biomarkers	Troponin-T, troponin-I, CK-MB, myoglobin, and BNP/NT-pro-BNP
Acute-phase reactants	CRP
Endocrinology	Glucose, HbA_{1c}, hCG, LH, and FSH
Rheumatology	Antibodies against mutated citrullinated vimentin
Toxicology	Therapeutic drugs, alcohol, barbiturates, amphetamines, cocaine, opiates, cannabinoids, benzodiazepines, and methadone
Infectious agents	*Plasmodium falciparum*, *Streptococcus A and B*, influenza A and B, HIV, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and infectious mononucleosis
Urinalysis	Urine strips (pH, protein, glucose, leukocytes, urobilinogen, bilirubin, ketones, nitrite, and NMP22 bladder carcinoma)

conceptualized the new field of "patient-focused" testing in biosensors and monitoring of pH changes under cardiopulmonary stress and shock in vivo (Kost 1977). Dr. Kost invented the phrase "point-of-care testing" in the early 1980s. POC has been critical for promptly providing evidence-based medicine during critical care since then. The POC devices have wide application areas. A few examples are listed in Table 14.1.

As previously stated, LFA is composed of a chromatographic system and an immunochemical reaction and so is also referred to as an immunochromatographic approach. It is based on the sample's passage across the membrane.

14.1.1 Advantages of POCT

Time POCTs result in significant time and cost savings. While it is unavoidable that this would reduce time, speed should not take precedence above accuracy or dependability (St John and Price 2013).

Compliance Patients can self-administer and operate POCTs at home, for example, a glucometer. It gives them independence and confidence and reduces hospital visits, travel expenditures, and time. Self-testing has been shown to improve adherence to

diagnosis and treatment regimens. A patient-centric approach through POCT has been effective in many follow-up regimes, for instance, determining the time required for blood to coagulate after taking warfarin (Gubala et al. 2012) or weekly self-monitoring and self-dosing of oral anticoagulation (Oertel and Libby 2010).

Cost The cost criteria applicable to point-of-care diagnostics are unique from those applicable to routine laboratory analysis (Nichols et al. 2007). Readers (instruments) are more compact and specialized than laboratory equipment, making them less expensive while performing a single or a few tests. Since samples are not in direct contact with the reader, self-cleaning subsystems are superfluous. POC chips, strips, or cartridges are disposable devices containing the sample but are not intended to be cleaned or reused (S. Chen and Shamsi 2017). They may have onboard fluidics, reagents, dyes, optics, electrodes, and even temperature control. Due to the greater complexity and functionality of the POC device than a blood-draw tube, it is more expensive, and tests offered in high numbers get the majority of their revenue from the consumables (Gubala et al. 2012). Indirectly, POC diagnostics have the potential to drastically lower medical costs. On the other hand, complex test cartridges are more expensive than ordinary sample tubes, and when the cost of large laboratory equipment is amortized across hundreds of thousands of samples, the cost per test can be decreased.

Convenience It is called "test and treat" since the tests, assessment, and possible therapy are done in one visit.

Accessibility POCT in a clinic, hospital OPD, or pharmacy may be more accessible than a pathology collection center for those who do not live in urban or suburban regions with good access to pathology testing facilities.

Clinical Benefits Clinical advantages vary depending on the test, but they may include:

- Earlier diagnosis or ruling out a diagnosis.
- More specific treatment.
- Better treatment results.

With POCT of some tests, certain doctors can better manage their patients' healthcare, especially if they require findings soon. Because of this, GPs in rural and isolated areas are eager to utilize POCT.

14.1.2 Disadvantages of POCT

Device Qualities Standards for laboratories are ensured by legislation and accreditation processes but not for POCT devices and testing. Not all POCT devices available for purchase are of sufficient quality to be used in patient care, which

must be considered (Müller et al. 1999b). With the rise in the knowledge of this risk, more POCT providers are only using devices that have been evaluated to be appropriate for patient care.

POCT Know-how Blood samples are applied to a strip or cartridge, and the instrument reads the results. However, there is more to POCT than this simple procedure. Even though many POCT devices are simple to use, the tests must be controlled to verify that a POCT device delivers the correct answer. A practice nurse or a POCT operator is the best individual to handle all of these operations (Müller et al. 1999b). The time taken to conduct a POCT and its effect on the practice of healthcare professionals must be considered while running the practice.

Improper Handling and Maintenance In many instances, POCT results are not at par with routine laboratory results because of inadequate or even absent calibrations. Environmental factors like temperature, humidity, and contamination are often variable and can affect the outcomes (Müller et al. 1999a).

Quality Controls Unlike labs, the POCT kits, after reaching to market, do not guarantee a foolproof system which conventional labs do. There are regular calibrations and quality checks in the latter case, which is not possible in the case of a POCT device after it is released to the consumer (Müller et al. 1999a).

Economy Though the cumulative cost of logistics is very promising in POCTs, the cost-effectiveness is a function of the number of users in that particular demographic zone. The cost to customers lessens in bulk procurements. Often the POCT strips or consumables are device-specific. There is always a chance of monopoly by the manufacturer or seller in pricing and supply chain.

14.2 Lateral Flow Assays (LFAs) in POCT

LFAs have attracted substantial attention because of their user-friendly forms, short test times, low interferences, and affordability. Unskilled medical staff can also use them easily. Biochemical interactions between antigen and antibody or probe and target DNA are at the heart of this method.

14.2.1 Components

The standard LFA is made up of four parts: a sample pad where the sample is poured, a conjugate pad where labeled tags are combined with biorecognition elements, a reaction membrane (typically nitrocellulose) where the test and control lines for target DNA-probe DNA hybridization or antigen-antibody interaction are located, and an absorbent pad for waste (Sajid et al. 2015). In commercial contexts, LFAs primarily employ colloidal gold particles for diagnostic purposes, such as food

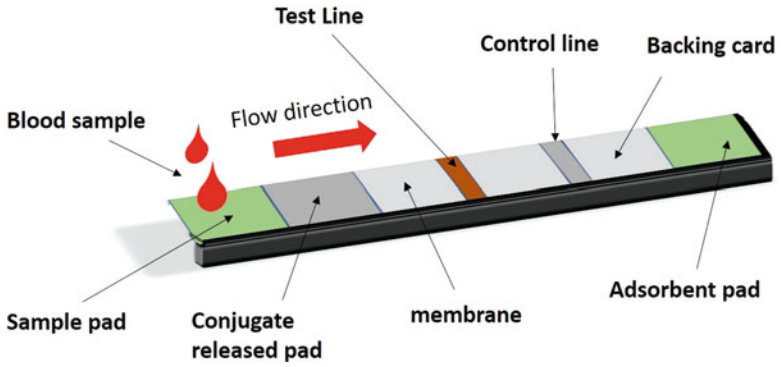

Fig. 14.1 LFA strip components and process: First, two anti-IgG antibodies are attached to a membrane. Since the antibodies are attached to the film, nothing changes. To prevent nonspecific antigen or antibody attachment, the membrane's free sites are blocked with chemicals. Capillary action draws the sample containing the analyte toward the strip's end. The analyte antigen binds to two antibodies on the membrane, forming a line. Performance depends on sample flow and antigen-antibody interaction.

monitoring, drug screening, and identifying infectious organisms found in the environment that cause illnesses, such as *Enterobacteriaceae* in food and water. The standard LFA involves several steps and is shown in Fig. 14.1.

14.2.2 Characteristics of LFA Strips

There are four main components to LFA strips. Below is a brief description of each, and Fig. 14.1 shows a visual representation.

Sample Application Pad It is made of cellulose and/or glass fiber. Samples are put on this pad to start the assay. It moves the sample to another lateral flow test strip (LFTS). Sample pads should move the sample in a smooth, continuous, and uniform manner and may be used to make the sample suitable to test the microenvironment before sending it forward. This may include separating the sample parts, removing interferences, changing the pH, and so on (Sajid et al. 2015).

Conjugate Pad Biorecognition molecules that have been labeled are given out at this pad. The material of the conjugate pad should quickly release the conjugate when it comes in contact with a moving liquid sample (Koczula and Gallotta 2016). In this instance, the conjugate should hold up throughout its entire shelf life. Any modifications to the dispensing, drying, or release of the conjugate can significantly alter the assay's outcomes. The assay may become less sensitive if the labeled conjugate is improperly prepared. Conjugate pads are primarily cellulose, glass fiber, or polyesters (Tsai et al. 2018). The material used to make the conjugate pad

influences how much labeled conjugate is released and how sensitive the assay is (Sajid et al. 2015).

Nitrocellulose Membrane Lines have been drawn on this membrane to test its functionality. A good membrane should be strong enough to support itself and the probes (antibodies, aptamers, etc.). Because nonspecific adsorption over test and control lines can significantly impact assay results, a good membrane has less nonspecific adsorption in areas where test and control lines are present (Hristov et al. 2019a). The rate at which the nitrocellulose membrane wicks can impact the assay's sensitivity. An assay's sensitivity depends on several factors, including getting the right quantity of bioreagents, drying them, and blocking them.

Adsorbent Pad At the end of the strip, it serves as a sink. Additionally, it aids in maintaining the liquid's flow rate over the membrane and prevents the sample from passing the membrane again. Adsorbents can significantly impact the outcomes of an assay (Koczula and Gallotta 2016; Sajid et al. 2015).

Backing Card These components are all mounted or fixed to a card placed on top. The materials used for the card's back are very flexible because they have no bearing on LFA other than ensuring that all the pieces are assembled properly. In this way, the back of the card is used as a support for the strip (Koczula and Gallotta 2016; Sajid et al. 2015).

Steps in an LFA Technique Major LFA steps include:

(a) Antibody preparation against the analyte of interest.
(b) Labeling preparation.
(c) Biorecognition molecule labeling.
(d) After dispensing reagents at their proper pads, assemble all components onto a backing card.
(e) Using a sample and obtaining results.

14.2.3 LFA Formats

Sandwich Format An antibody or aptamer coated with a label (nanoparticles, enzymes, or fluorescent dyes) is immobilized on a conjugate pad in a typical configuration. This is temporary adsorption that can be eliminated with any buffer solution. Over the test line, a primary antibody or aptamer directed against the target analyte is immobilized. A secondary antibody or probe directed against the labeled conjugate antibody/aptamer is immobilized in the control zone. The sample containing the analyte is placed on the sample application pad before migrating to the remaining areas of the strip. The immobilized labeled antibody or aptamer conjugate captures the target analyte at the conjugate pad, forming a labeled antibody conjugate/analyte combination (Sajid et al. 2015). Capillary action propels this

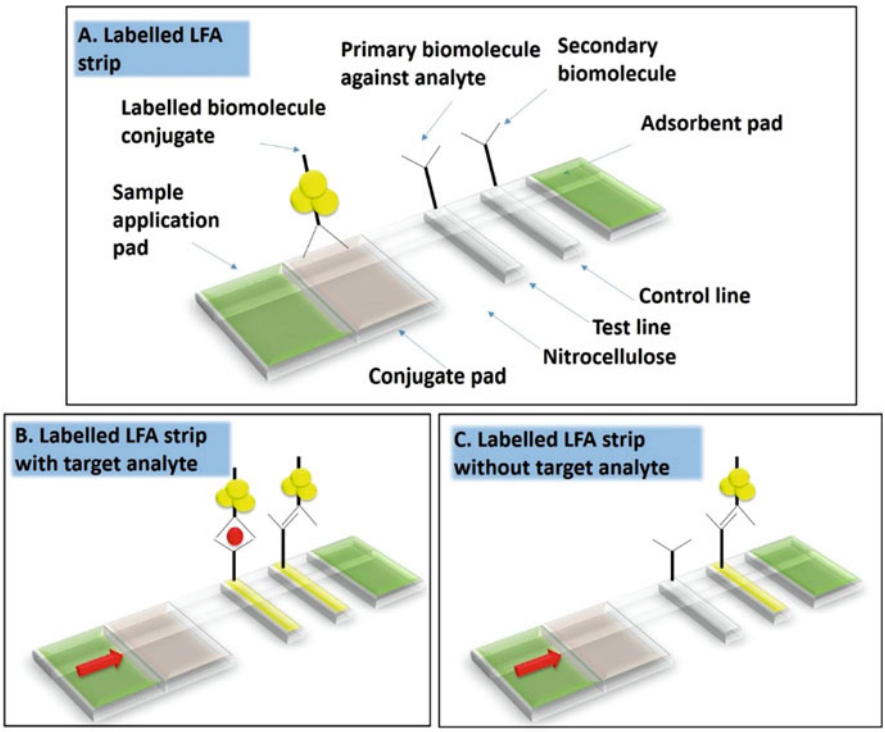

Fig. 14.2 (**a**) Labeled LFA strip; (**b**) Capillary action occurs when a sample containing a target analyte is placed on the sample application pad. The sample flows across the strip, which features colored test and control lines; (**c**) When a sample that does not contain the target analyte is placed on the sample application pad, it shifts and only the test line displays color

complex toward the nitrocellulose membrane. At the test line, a second antibody specific for the analyte captures the labeled antibody and analyte conjugate. In this case, the analyte is sandwiched between two labels and two primary antibodies, forming a "complex."

The secondary antibody will catch any extra labeled antibody conjugates in the control zone. The absorbent pad takes in any extra buffer or solution. There are two ways to figure out how much analyte is in a test strip: with an optical strip reader or by looking at it. For a strip to work properly, there must be color at the control line. Fig. 14.2a–c shows the pictorial representation of a standard sandwich format of LFAs (Hristov et al. 2019b).

Competitive Format Small compounds that cannot bind to two antibodies at once benefit the most from this configuration. While the presence of color at either the test or control lines shows that both lines are positive, the absence of color at the test line indicates the existence of the analyte. There are two main designs:

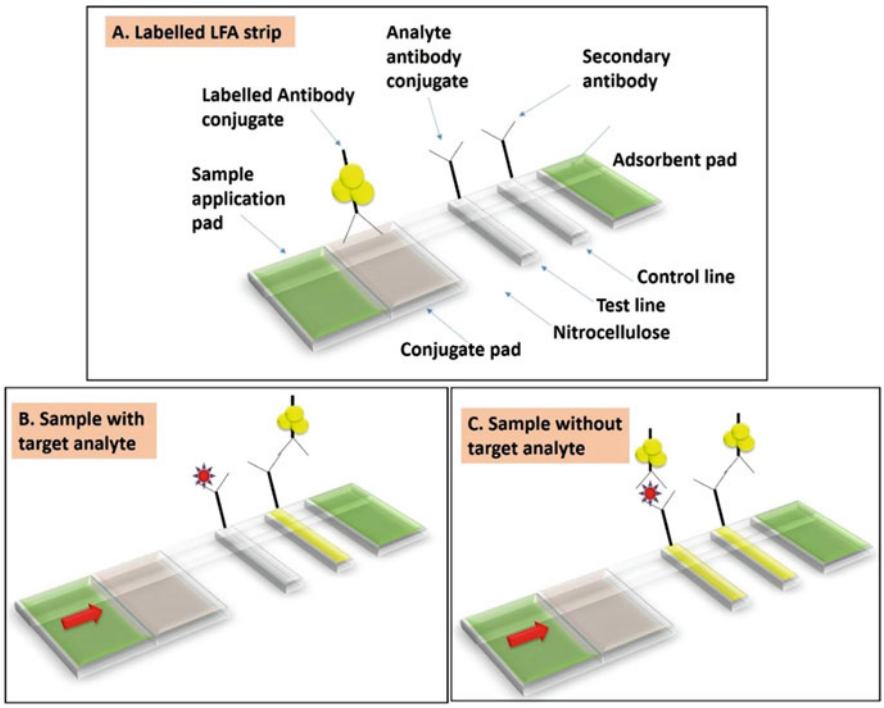

Fig. 14.3 Scheme for (a) a labeled lateral flow strip, (b) when a sample containing the target analyte is applied to the sample application pad it leads to a prominent color emerges at the control line and a thin band of color emergence or no color at the test line because of less available labelled antibodies for the test line since the same are occupied by the target analyte. (c) when a sample devoid of the target analyte is applied to the sample application pad, a prominent color emerges at the test test line and a thin color or no color change appears at the control line since the labelled antibodies are mostly occupied at the test line

(a) A solution containing the target analyte is first applied to the sample application pad. The labeled biomolecule conjugate (antibody/aptamer) becomes hydrated and begins to move with the liquid. ***Test line:*** It contains an immobilized antigen (the same analyte to be detected) that binds in a specific manner to the label conjugate. ***The control line***: It already has a secondary antibody on it that can attach to an antibody conjugate that has been labeled. It occurs if the target analyte is absent from the sample solution or is present in such minute quantities that some spots on an antibody conjugate labeled are empty. Competition between the antigens in the sample solution and the antigen on the test line of the strip is present for the conjugate that is colored the same as the conjugation (Sajid et al. 2015). Fig. 14.3a–c describes a pictorial view of the mechanism.

(b) In a different design, a primary antibody to the analyte is dispensed at the test line, while the analyte conjugate is dispensed at the conjugation pad. The test line is then supplemented with an analyte solution. The analyte and its label compete to bind to the primary antibody at this line. A novel layout with an

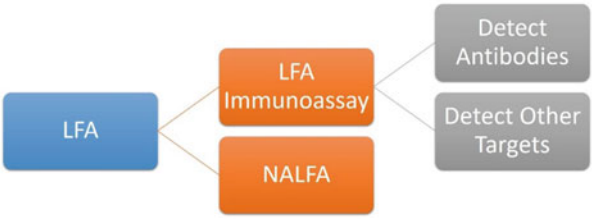

Fig. 14.4 Classification of lateral flow assays

antigen line between the test and control lines has been adopted to prevent errors in detecting low concentration of an analyte.

Multiplex Detection Format To detect more than one analyte, a multiplex detection format is used, in which the experiment is carried out over the number of target species to be researched. Analyzing many samples simultaneously under identical conditions is the ideal scenario. Using a multiplex detection format, many analytes in clinical diagnostics that are interdependent in defining the stage of a disease may be recognized (Panhotra et al. 2005). To generate lateral flow strips, the length and number of test lines on a normal strip can be expanded, as well as extra structures such as stars or T-shapes. The shape of the LFA strip is determined by the number of analytes to be measured. There are various advantages to using microarray-based LFA for multiplex detection of DNA sequences, including lower consumption of test reagents, a smaller sample size, and a better degree of accuracy.

Types of LFA based on biorecognition molecules:

LFAs may be divided into several sorts based on the aspects of recognition they employ (Fig. 14.4). LFIAs, known as "lateral flow immunoassays," are based on immunoglobulin-mediated detection principles. Amplicons that can develop during the polymerase chain reaction can be detected using nucleic acid LFA (PCR).

Antibodies On a lateral flow strip's test and control lines, antibodies are used as biorecognition molecules that bind to the target analyte through immunochemical interactions. An immunochromatographic assay based on the results of this procedure is called a "lateral flow assay" (LFIA). Common pollutants have antibodies accessible, but particular analytes for which antibodies may be produced are also available.

Aptamers Synthetic nucleic acids are known as aptamers. Aptamers have a high affinity for a wide variety of analytes intended to target. Aptamers are the best detection probes for organic compounds with molecular weights between 100 and 10,000 Da. Because of their affinity for the target molecules, interferences may be seen any many instances. They are preferred over antibodies since they can be synthesized quickly, labeled easily, amplified after selection, altered structurally, and have the best stability, repeatability, and application flexibility (Tuerk and Gold 1990).

Molecular Beacons There is a bright and dark end to the molecular beacon, which was first introduced in 1996 (Tyagi and Kramer 1996). A fluorophore and a quencher are added to this specific sort of DNA hairpin. Because of a nearby quencher, fluorophores cannot produce fluorescence without analytes. It only occurs when the analyte has the same DNA sequence. Forcefully opening the stem and loop reveals a fluorescence signal. To discover and attach to certain molecules, such as nucleic acids, poisons, proteins, and more, scientists utilize molecules called "beacons." LFA uses straightforward DNA probes to find DNA sequences associated with various illnesses and hereditary issues. Antibody-antigen complexes, the most prevalent kind of complex in LFTS, generate more quickly than DNA hybridized complexes (Mao et al. 2013).

14.3 Labels of Lateral Flow Tests

There are two ways to label LFA: gold nanoparticles and colored latex beads. Carbon and selenium nanoparticles are also used for the same. Labeling agents like quantum dots and organic fluorophores also enhance the functions. An ideal label should be visible at very low concentrations and keep its properties linked to biorecognition molecules. This conjugation should also keep the identity of biorecognition probes the same. Besides, a good label should be easy to attach to biomolecules and stay there for a long time. Some labels produce an immediate signal (like gold colloidal color) when the assay is done, and some labels need more steps to produce analytical signals (as enzymes produce detectable products upon reaction with a suitable substrate). Because direct labels are faster and less complicated to use when using LFA, they are the most common choices. Different types of LFA labels are listed in Table 14.2.

14.4 Use of Nanotechnology in LFA Kits

Nanotechnology can make the LFAs better, so they can meet the needs of a diagnostic tool (Sadeghi et al. 2021). Lateral flow biosensors, also known as lateral flow tests (*LFTs*), are the most recent and cutting-edge technology in the LFA-based diagnostic tools for POCT that use *LFAs* (*point-of-care testing*). LFTs are paper-based tools that can act like biosensors. These are cheap, durable, accurate, and quick and only need many samples. A good thing about LFT diagnostic tools is that they help patients and personalized medicine. A wide range of LFTs has been documented and used to diagnose various human diseases. Nanozymes (Sadeghi et al. 2021), luminescent nanoparticles, SERS active nanoparticles, and magnetic nanoparticles are some new nanotechnology-based LFTs (Napione et al. 2021).

Table 14.2 Different types of labels for LFA

Nanoparticles	Nanoparticles have been used as a tracer for developing LFAs because they have a lot of unique properties. To make optical signals like fluorescence or color shifts, they put them together and put them together again (Koczula and Gallotta 2016). They have unique nanostructures that make this possible. In the past, they have made LFAs with colloidal gold and silver, as well as carbon nanoparticles and nanotubes, to make them stronger and more durable. Use them for various tests (Bahadır and Sezgintürk 2016)
	Gold NPs
	Colloidal gold nanoparticles are one of the most frequently utilized detection reagents in the LFA strip. They demonstrate how the signals are shown on the strip. Red is a color that aids in the perception of what is occurring. This is not the only characteristic that distinguishes gold nanoparticles. They also have several other distinguishing characteristics, such as being extremely stable in the laboratory and having a large surface area. All of these factors contribute to the speed and reliability of on-site analysis (Singh et al. 2015)
	Luminescent NPs
	Recently, in several researches on LFAs, fluorescent nanoparticles (quantum dots, fluorescent quenching material, lanthanide, up-converting particles, and so on) have been mentioned in place of colorimetric markers. Their detection limits are extremely low (Díaz-González et al. 2020; Paterson et al. 2014)
	Superparamagnetic (MNPs)
	MNPs have been employed as a novel labeling material in manufacturing LFAs. When MNPs are used, the sensitivity can be increased by ten- to 100-fold. MNPs, on the other hand, may generate magnetic signals that remain constant for an extended period. These signals have a very low background noise level because of the absence of magnetic components in the surroundings or the samples used to evaluate them (Akbarzadeh et al. 2012a)
Enzymes	
The literature discusses three distinct types of test strips. One can alter the hue of the paint using enzyme-substrate reactions. The color is visible both visually and on a typical color chart. Fung et al. constructed an enzyme-based LFA that consisted of four components: a sample pad, a peroxidase substrate zone, a test zone, and an absorbent pad. Each of these components had a distinct role. HRP clung to the test zone and consumed H_2O_2 and TMB (a chromogenic substrate). A blue signal was observed following the HRP and H_2O_2 mixture. On the other hand, the vast variety of human judgments may make precision difficult. To resolve this issue, we devised a noninstrumental method of displaying the color signal. The signal is depicted as a ladder bar	
Liposomes	
Sphere-shaped vesicles made out of phospholipid bilayers are called liposomes. They are made up of one or more layers. Because liposomes are small and have both hydrophobic and hydrophilic properties, they are ideal for giving drugs to people. Using liposomes as labels, researchers have been able to look for *Staphylococcus* enterotoxin B (SEB), target DNA, and the peanut protein Ara h1 while making LFAs	

14.4.1 Dual Gold Signal Enhancement Method

Gold nanoparticles (GNPs) can be used to improve signal detection (Chandra et al. 2010; Chandra and Prakash 2020). Among the two types of GNPs, the first GNP

molecule type is mixed with the analyte detection probe. The second GNP is made only to bind to the first GNP molecule. These GNPs are of different sizes and placed in different places to avoid mixing up before the test. Antigen-antibody reactions or other binding systems can be used to get both types of GNPs to stick together. These include binding primary and secondary antibodies, biotin-streptavidin binding, and so on. There are more signals because the two GNP molecules are grouped and mixed twice (Sadeghi et al. 2021). The gold signal enhancement method is generally better because it has more sensitivity, is easy to use and cheap, and can be used quickly (Sadeghi et al. 2021). In Fig. 14.5a, the gold signal enhancement method is described.

14.4.2 CNTs and CNPs

Carbon nanomaterials (CNPs) can be used in place of GNPs because of their lower cost. They are made up of a variety of carbon nanostructured compounds. Carbon molecules have an intrinsic black hue that enhances the contrast of nitrocellulose membranes, increasing the sensitivity of LFTs. Carbon nanotubes (CNTs) and CNPs have been used as labels for LFTs, and they work well. It is not just that they help with sensitivity and visualization, but CNTs and CNPs are inexpensive, have good reactivity, and are stable. It is shown in Fig. 14.5b how the CNP- and CNT-based LFAs work (Napione et al. 2021).

14.4.3 Nanozymes

Enzymes have been in use in LFAs for quite some time. Horseradish peroxidase (HRP) is used in LFAs to help redox reactions at both the test and control lines (Napione et al. 2021). Nanozymes are nanomaterials with enzyme-like properties and are more stable and cheaper than naturally occurring enzymes. Nanozymes have been used in recent years to enhance the sensitivity of the tests. These have all the same properties as natural oxidase, catalases, and peroxidases, but they are made in a lab. Nanozymes with internal peroxidase-like activity, like platinum (Pt) nanoparticles, have been explored extensively. A schematic diagram in Fig. 14.5c describes how nanozyme-based LFAs function.

14.4.4 Luminescent Nanoparticles

LFAs based on fluorescent signals are better than colorimetric markers because they are more sensitive and stable and can measure analytes at lesser concentrations with higher sensitivity. Different luminescent nanoparticles have been studied and used to improve the LFTs that use a fluorescent signal. Quantum dots, up-conversion nanoparticles, and time-resolved fluorescence nanoparticles are some of the most important fluorescents used recently (Napione et al. 2021).

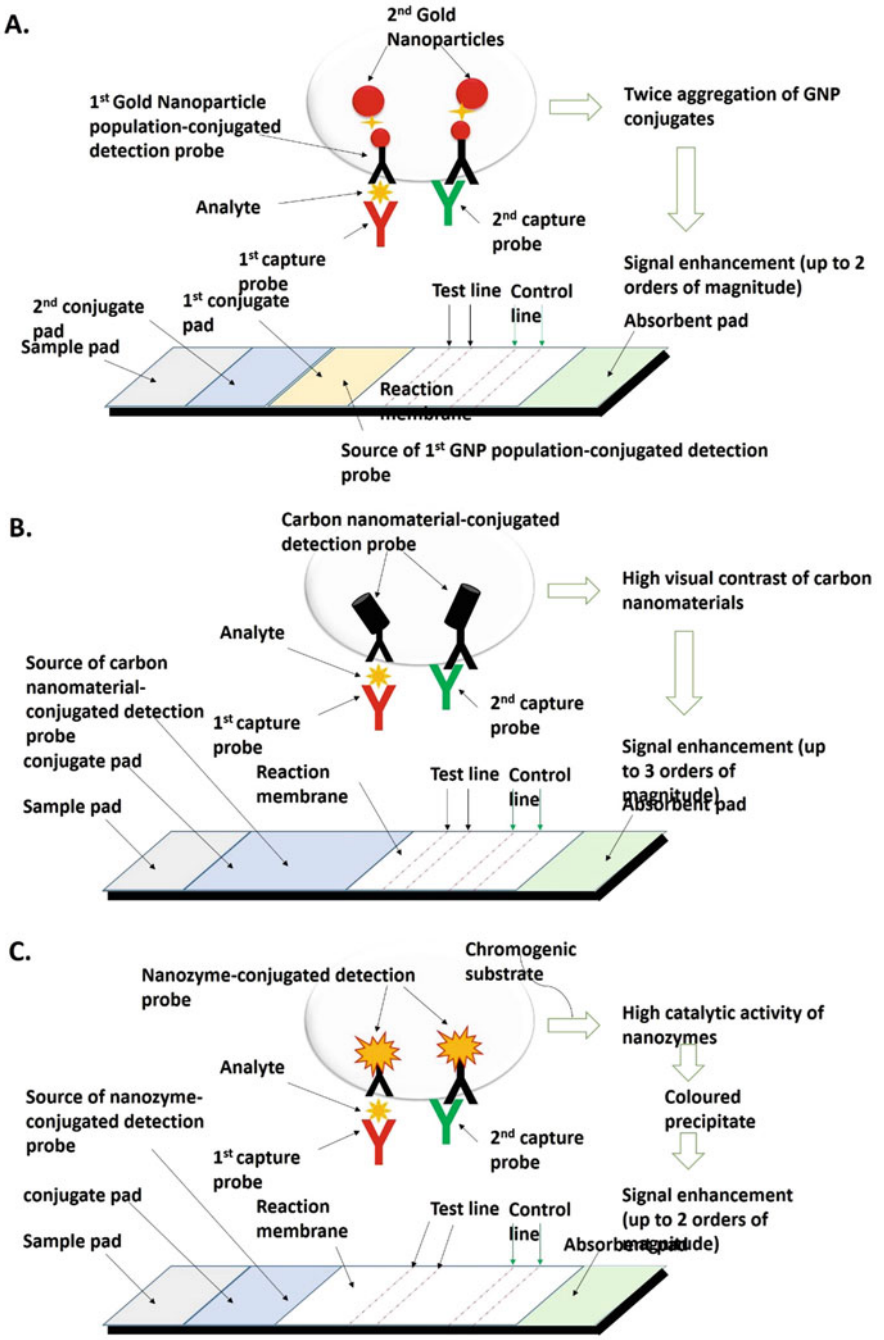

Fig. 14.5 (**a**) Gold signal enhancement method applied to LFTs. (**b**) CNP- and CNT-based LFT. (**c**) Nanozyme- and platinum (Pt)-based LFT

Quantum Dots QDs are a kind of semiconductor nanoparticles composed of molecules belonging to the groups III–V and II–VI (Bera et al. 2010). Since 2010, both qualitative and quantitative POCTs have been conducted using QD-based LFTs. Numerous investigations and research have established that various viruses, nucleic acids, peptides, and biomarkers may be identified with increased sensitivity and specificity (Díaz-González et al. 2020; Wilkins et al. 2018).

Up-Conversion Nanoparticles (UCPs) Luminescent nanomaterials mixed with rare earth elements can change IR light into a visible light. Most of the time, **ytterbium** (Yb) or erbium (Er) is double-doped in LFAs. Yb takes IR lights and sends them to Er, turning them into UV and visible light (Wang et al. 2011). UCPs are better than QDs because they are less toxic, and have less biological interference (Chien et al. 2020).

Time-Resolved Fluorescence Nanoparticles (TRFIAs) Lanthanides are put into nanoparticles, and bifunctional groups are added, making them look different. Lanthanide chelates are used as labels to the TRFIAs due to their brightness and slow decay of fluorescence (Guo et al. 2021). Lanthanide chelates' fluorescence takes about 103 to 106 times longer to die off than conventional fluorescence (Wu et al. 2002). Lanthanide chelate fluorescence is used, with different types of light to get the best results. This reduces background noise and makes the detection more sensitive (Hemmilä 1988).

SERS Active Nanoparticles SERS (surface-enhanced Raman scattering) is a sensitive tool to detect molecules at the molecular levels with much specificity. The method uses Raman scattering spectroscopy, and nanostructured metal surfaces, used as substrates make them even more powerful. A nanogap on the surface of the metal makes more Raman signals than without gaps. This is due to the creation of hot spots arising out of Localised surface plasmon resonance (LSPR). Combining SERS nanoparticles to the standard LFIAs makes them ultrasensitive in detecting a wide range of tiny biomolecules. To make the SERS-LIFA even more sensitive, different shapes of SERS nanotags are explored (Guo et al. 2021).

14.4.5 Magnetic Nanoparticles

MNPs (magnetic nanoparticles) made of iron oxide (Fe_3O_4) is a novel type of nanomaterial developed in recent years. They can be linked to biomolecules such as antibodies and enzymes and utilized as labels in LFIAs due to their great biocompatibility (MLFIAs) (Farouk et al. 2020). LFIAs based on MPs have lower background interference than fluorescent labels and do not require large-scale, expensive detecting hardware (Moyano et al. 2020). On-site quantitative and speedy

detection is feasible by evaluating magnetic strips contained on the test strip in conjunction with a MAR (magnetic assay reader). Because MNPs are paramagnetic, the tagged biomolecule may change in reaction to the external magnetic field, allowing for a more controlled approach. Shell/core configurations have been used to manufacture MNPs to boost sensitivity (Guo et al. 2021). Excessive magnetic enrichment is a major problem for MLFIAs. As MNPs accumulate on the conjugation pad, they constrict the liquid flow channel, prolonging detection time and interfering with the antibody-antigen reaction. Using superparamagnetic NPs as labels is a game-changer in overcoming the abovementioned issue. The phrase "superparamagnetic NPs" refers to a class of magnetic ions with no coercive force or remanence in their hysteresis loop. Unlike normal MNPs, superparamagnetic NPs have a greater surface area and are free of hysteresis (Akbarzadeh et al. 2012b). Magnetic enrichment to an excessive degree is a major concern for MLFIAs. As MNPs accumulate on the conjugation pad, they constrict the liquid flow channel, extending the time required for detection and interfering with the antibody-antigen reaction. Using superparamagnetic NPs as labels is a game-changer in overcoming the abovementioned issue. The phrase "superparamagnetic NPs" refers to a magnetic ion with no coercive force or remanence in its hysteresis loop. Compared to regular MNPs, superparamagnetic NPs have a greater surface area and no hysteresis (Guo et al. 2021).

14.5 Future Scope of LFA

LFAs are simple to use, rapid, inexpensive, and excellent diagnostic tools for various conditions. However, there are many drawbacks of LFAs, especially with the accuracy. There are continuous efforts from researchers to enhance the accuracy and versatility of LFAs; for instance, the identification and sorting of biochemical substances can be used to find targets, like high-affinity molecules (peptides, aptamers, antibodies, ligands, nano-molecules, receptors, etc.) and making changes to avoid or minimize nonspecific binding, such as reducing the number of molecules that can be bound, label blocking, or blocking the membrane (Fig. 14.6).

14.5.1 Approaches to Make Better LFAs

Innovation on Improving Sensitivity The majority of the time, the sensitivity of detection is enhanced by altering the markers, adding shading, upgrading experts, boosting the susceptibility reaction, or combining numerous NPs. Presently, the perceptive awareness and cutoff of recognition of lateral flow immunochromatographic assays (LFICS) may be significantly enhanced by incorporating three distinct types of NPs as an alternative (Fig. 14.7). These include:

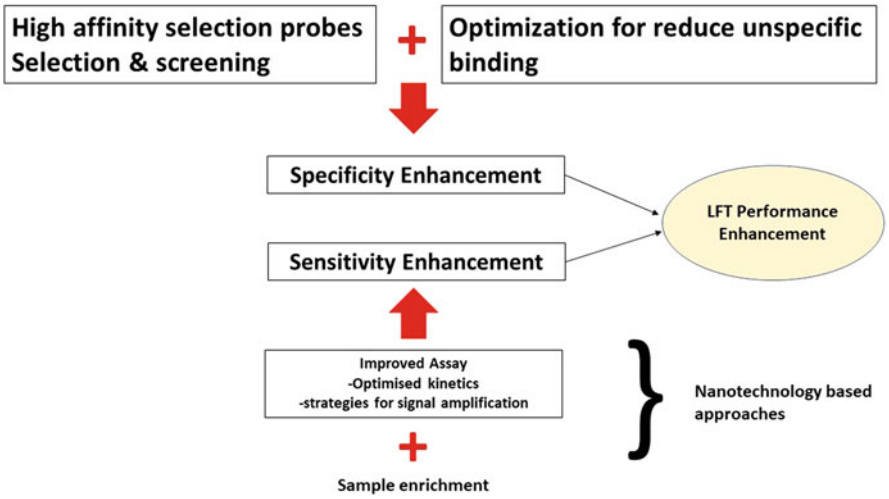

Fig. 14.6 Strategies for LFT detection enhancement

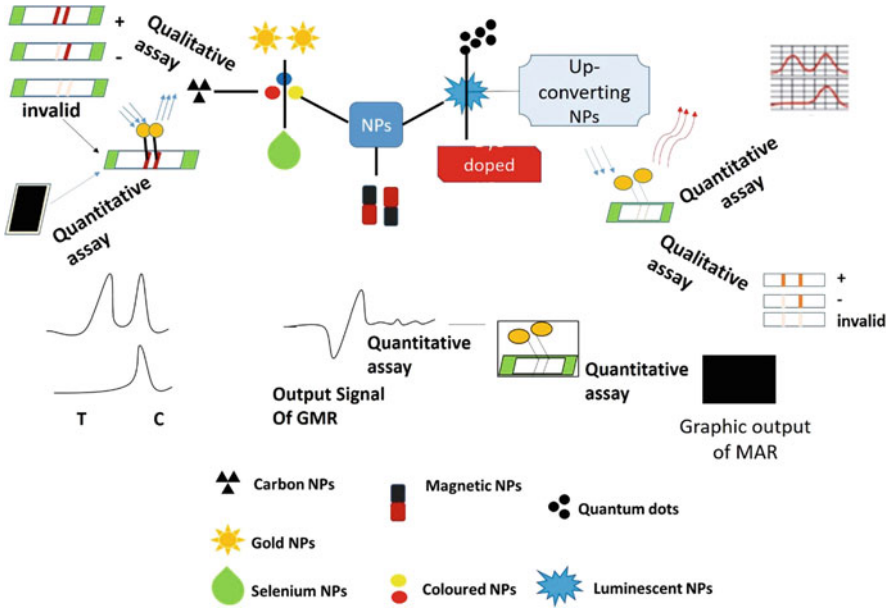

Fig. 14.7 Qualitative to quantitative conversion

(a) *Colored* (e.g., GNPs, CNPs, and colloidal selenium nanoparticles [SNPs]).
(b) *Luminous* (e.g., quantum dots (QDs), up-changing over phosphor nanoparticles (UCPs), and color-doped NPs).
(c) Appealing nanoparticles (NPs) (MNPs).

Horton et al. used colloidal gold when testing mouse IgG. The responsiveness expanded multiple times when they utilized fluid bit to fortify the coloring result (H. J. Chen and Wen 2011). Muller et al. acquainted avidin and biotin with an immunochromatography framework to recognize fetal heart troponin T. One avidin has four restricting locales which can tie with the biotin. So, the sign force could be enhanced enormously (Bhattacharjee et al. 1997). A few analysts used liposomes as marks that offer higher responsiveness. Gred et al. utilized europium microparticles as names in the immunochromatography framework (Liang et al. 2017). The lipocalin can reflect the grouping of eosinophils and neutrophils in the blood. As another material created lately, magnetic nanoparticles have higher responsiveness in immunochromatographic examination through testing magnetism than the ordinary luminescence method (Mao et al. 2013).

Development of Multivariate LFAs Tjitra et al., using immunochromatographic assay, fostered a testing strip that could at the same time examine *Plasmodium falciparum* and *Plasmodium vivax*; it had great awareness, explicitness, and clinical application esteem (Salwati et al. 2011). Buechler et al. utilized one layer splashed with a few T lines and prevailed in concurrently identifying seven prohibited medications in urine (Buechler et al. 1992). To enable multiplex diagnosis of three virally intractable diseases (acquired immune deficiency syndrome, hepatitis C and A), seven different 3D probes based on proteinticles that display distinct viral antigens on their surface have been developed Jong-Hwan Lee et al. (Lee et al. 2015). These probes were synthesized in *Escherichia coli* by self-assembling human ferritin heavy chains that had been genetically modified to include viral antigens at their C-terminus (Lee et al. 2015). An intriguing gadget with a ten-channel LFICS using UCP innovation was additionally utilized for the concurrent discovery of various antibodies against *Yersinia pestis* (Mao et al. 2013).

Development from Qualitative to Quantitative Detection Quantitative research is critical for identifying the most severe cases of sickness and ensuring that large quality-control groups adhere to health regulations. Tippkotter et al. set up a connection between the strip's color values and where the analytes were in a quick test to see if microcystin could be grouped (Tippkötter et al. 2009). In clinical medicine, rapid and simple quantitative research can assist physicians in determining patients' pathogenic states and planning and carrying out therapy, which is critical for treating dangerous conditions such as localized myocardial necrosis. The popularity of quantitative research is not just due to the accessibility of strips. It also has to do with the tool's development. The primary focus of quantitative testing research at the moment is on using photoelectric identification to convert an optical sign to an electrical sign and obtaining an advanced sign from which to obtain the quantitative location result from the test. It could also look into nanoparticle-based LFTS to get more precise results (H. Chen et al. 2021).

IoT and Technological Integration Smartphones have become essential personal devices in the present world. Smartphones can greatly enhance contact tracking and

tracing, patient separation, disease management, and surveillance monitoring during a pandemic, thanks to their connection, Global Positioning System (GPS), and computational capabilities. Smartphones can track infectious diseases by identifying those who have come into contact with a patient. Finally, routine test data analysis using AI has been demonstrated to be a reliable method for disease surveillance by correctly detecting diseased patients. Numerous other diagnostic techniques, including RT-PCR, CRISPR/Cas, chest computed tomography, and paper microfluidic devices, along with lateral flow tests, have been integrated with smartphones and AI for point-of-care testing. These innovations have changed illness diagnosis by providing a quick, precise, affordable, and user-friendly POC testing method. Modern, highly sensitive, and AI-based auto-diagnosis kits, like Covid fast antigen POCTS, are being created using recent advancements in nanotechnology and IoT-based devices.

14.6 Conclusion

LFA is employed in various disciplines, including medicine, sanitation, and environmental monitoring, due to its low cost, fast speed, flexibility, and lack of complicated equipment and specialist abilities. This framework's presentation has to be improved in terms of LOD or awareness, quantitative assurance, and numerous concurrent places. There have also been some novel concepts, such as using distinct NPs for LFA. This article discusses recent advances and achievements in LFA sensitivity augmentation, including sample pretreatment; structural, material, and label alterations; and an objective evaluation. AuNPs are most commonly used to designate LFA. While combining AuNPs with other materials such as silver, gold, enzymes, or catalytic metals may improve LFA sensitivity, this method has limitations in terms of manufacture, purification, storage, and detection. As a result, according to a recent study, there is still tremendous development opportunity for LFA with a smaller demand volume, a faster analysis time, no hook effect, and higher accuracy and sensitivity. Because of their simplicity and ease of integration with standard LFA, optical techniques based on AuNPs and their derivatives are projected to be the most practicable POCT devices among LFA detection technologies. As a result, significant technical efforts should be put toward miniaturizing the entire process to make it affordable and portable in field conditions and resource-constrained environments.

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