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Gorachand Dutta
Arindam Biswas *Editors*

Next Generation Smart Nano-Bio-Devices

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Next Generation Smart Nano-Bio-Devices

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Preface

Over the years, there are increasing needs for the development of simple, cost-effective, portable, integrated biosensors that can be operated outside the laboratory by untrained personnel. However, to reach this goal, a reliable sensor technology based on printed electronics has to be developed. Our book aims to develop and establish the concept of the field of biosensor and electrochemistry for bench-to-bedside diagnosis. Our goal will be the use of redox cycling-based wash-free electrochemical technique in a portable electronic device that can be operated outside the laboratory for simple and effective point-of-care testing for early-stage disease diagnosis (i.e., cancer, malaria, dengue, tuberculosis, and HIV/AIDS).

In this book, we address these challenges for the development of a point-of-care test platform. The proposal also describes printed chip-based assay (Lab-on-a-Chip, Lab-on-a-PCB) for rapid, inexpensive, biomarker detection in real samples. The main challenges of point-of-care testing require implementing complex analytical methods into low-cost technologies. This is particularly true for countries with less developed healthcare infrastructure. Washing-free, Lab-on-a-Chip, Lab-on-a-PCB techniques are very simple and innovative for point-of-care device development. The redox cycling technology will detect several interesting targets at the same time on a printed chip. The proposed areas are inherently cross-disciplinary, combining expertise in biosensing, electrochemistry, electronics and electrical engineering, healthcare, and manufacturing.

This title would focus on recent advances and different research issues in the nanobiotechnology-enabled biosensor technology and would also seek out theoretical, methodological, well-established, and validated empirical work dealing with these different topics. The title covers a very vast audience from basic science to engineering and technology experts and learners. This could eventually work as a textbook for engineering and biomedical students or science master's programs and for researchers. This title also serves common public interest by presenting new

methods for data evaluation, medical diagnosis, etc. to improve the quality of life in general, with a better integration into society.

Kharagpur, India — Dr. Gorachand Dutta
Asansol, India — Dr. Arindam Biswas

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Editors and Contributors

About the Editors

Dr. Gorachand Dutta is Assistant Professor at School of Medical Science and Technology, IIT Kharagpur. He received M.Sc. degree in Chemistry from Indian Institute of Technology, Guwahati, India. His research interests include the design and characterization of portable biosensors, biodevices, and sensor interfaces for miniaturized systems and biomedical applications for point-of-care testing. He received his Ph.D. in Biosensor and Electrochemistry from Pusan National University, South Korea, where he developed different class of electrochemical sensors and studied the electrochemical properties of gold, platinum, and palladium-based metal electrodes. He completed his postdoctoral fellowships in the Department of Mechanical Engineering, Michigan State University, USA, Department of Chemistry, Pusan National University, South Korea, and Centre for Biosensors, Bioelectronics and Biodevices at University of Bath, UK. During his research tenure in USA and South Korea, Dr. Dutta invented an enzyme-free, disposable miniaturized immunosensor chip using micropatterned electrode and wash-free method for the development of mobile phone-based platforms for fast and simple point-of-care testing of infectious and metabolic disease biomarkers. He has expertise on label-free multichannel electrochemical biosensors, electronically addressable biosensor arrays, aptamer- and DNA-based sensors, and surface bio-functionalization.

Dr. Arindam Biswas received M. Tech. degree in Radio Physics and Electronics from University of Calcutta, India, in 2010 and Ph.D. from NIT Durgapur in 2013. He has worked as Postdoctoral Researcher at Pusan National University, South Korea, with prestigious BK21PLUS Fellowship, Republic of Korea 2015, DST-JSPS Invitation Research Grant Award in 2020, and DST-ASEAN S&T Development Grant Award 2021. He was Visiting Professor at Research Institute of Electronics, Shizouka University, Japan. He has been selected for IE(I) Young Engineer Award : 2019–20 from Institute of Engineers (I), India. Dr. Biswas has 12 years of experience in teaching research and administration. Presently, Dr. Biswas is working as Associate

Professor in School of Mines and Metallurgy at Kazi Nazrul University, Asansol, WB, India. He has 53 technical papers in different journals, 35 conference proceedings, 7 books, 8 edited volumes, and 6 chapters with international repute. Dr. Biswas received research grant from Science and Engineering Research Board, Government of India, under Early Career Research Scheme for research in terahertz-based GaN source. He has also received research grant from Centre of Biomedical Engineering, Tokyo Medical and Dental University in association with RIE, Shizouka University, Japan, for study of biomedical THz imaging based on WBG semiconductor IMPATT source for two consecutive years 2019–20 and 2020–2021. Presently, Dr. Biswas has served as Associate Editor of Cluster Computing, Springer (SCI Indexed). Dr. Biswas has produced 05 Ph.D. students in different topics of applied optics and high-frequency semiconductor device. He has organized and chaired different international conferences in India and abroad. His research interest is in carrier transport in low-dimensional system and electronic device, nonlinear optical communication, and THz semiconductor source. Dr. Biswas acted as Reviewer for reputed journals, Member of the Institute of Engineers (India), and Regular Fellow of Optical Society of India (India).

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Chapter 1
Aspects of Biosensors with Refers to Emerging Implications of Artificial Intelligence, Big Data and Analytics: The Changing Healthcare–*A General Review*

P. K. Paul

Abstract Biological sensor in short biosensor is an analytical machine that is basically combined with the biological detecting system and also a transducer. As far as this sensor is concerned here the element (i.e., biological element) may be an enzyme or nucleic or simply an antibody. This is particularly useful in analyzing and finding chemical substance that may be integrating both bio-related equipment and on the other hand physicochemical detector. In regard to other than devices biosensors are more advanced, intelligent and with the features of sensitivity. This is a powerful element and mechanism dedicated in biological sensing in different aspects and areas such as environment and ecology, healthcare and medicine, drug discovery, food processing and safety, homeland security, agricultural sectors. In developing biosensor nanotechnology, electrochemistry is also playing a leading role due to cost-effective and increasing applications and role. Biosensors are analytical devices dependent, and it converts a biological response into a quantifiable signal. The core components of a biosensor are *bioreceptor* and *transducer*. Gradually different tools, techniques and technologies are integrated in biosensors for its productivity, development, etc. The rising applications of biosensor result effective results in healthcare sectors. Information technology applications in the field of biosensor are immense and increasing rapidly including artificial intelligence with ML and DL, big data and analytics, Internet of Things (IoT), etc. Better ICT applications in biosensors are directed in better analysis and result in diagnosing, monitoring and even in preventive health. This paper is theoretical and conceptual in nature which is discussed on biosensors including its evolution, rising applications and emergence of ICT and computing for better biosensor results.

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1.1 Introduction

Clark and Lyons in the year 1962 have invented and first introduced us the biosensor for the purpose of analyzing glucose within biological entitles and the way of electrochemical detection of the oxygen. The development of the biosensor is widely noticed in recent past, and such aspects can be noted in the advanced healthcare sensors [1–3]. Though, the recent developments of biosensor in the pollutions and environmental systems, agriculture and horticulture, food and allied areas are also important to note. With the biosensor the bio-elements basically communicate by analyzing and being checked and changed into the electrical signal using transducer. In general different types of biosensor can be seen such as resonant mirrors, immune, chemical canaries, optrodes, bio-computers, glucometers and biochips and various biologically connected or derived contents, viz., tissue, cell receptors, antibodies, nucleic acids, enzymes, etc. The growth of biosensor leads to the development of its applications in diverse areas (also refer Fig 1.1) such as:

- In basic healthcare scrutiny
- In measurement of the metabolites

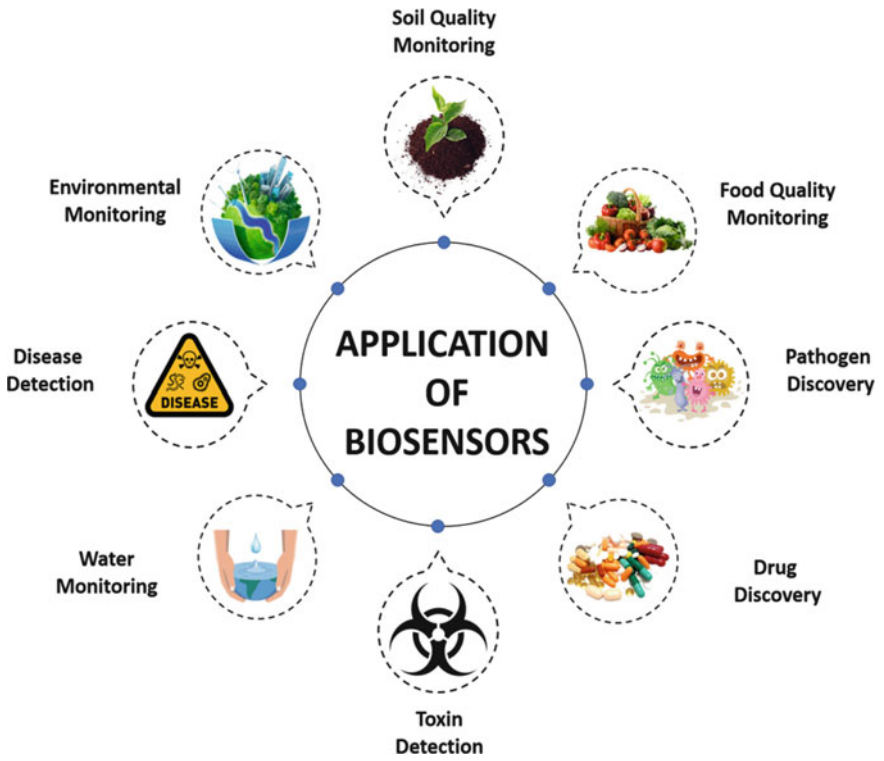

Fig. 1.1 Some of the potential and emerging application areas of biosensor

- In analyzing and finding sickness
- Insulin treatment
- In diagnosis of the disease and psychotherapy
- In agricultural, veterinary areas
- In pharma areas like drug improvement including offense detection
- In ecological management and monitoring
- In quality control, process control in the industries
- In the areas of bioterrorism applications
- In clinical analysis and clinical research
- In biosensors applications in stretchable electronics
- In recognition receptors in biosensors
- In proper biodetection
- In pharmaceuticals manufacturers and organ replacements.

Biomedical Engineering is a valuable subject in advancement of the biosensor. Nanotechnology and nanofabrication are also important regarding biosensor development [4–6].

Therefore biosensor is becoming more and more interdisciplinary in nature. The biosensor substrate holds three electrodes, and these are reference electrode, working electrode and counter electrode. The biosensing now becomes an important technology due to its wider applications such as healthcare, agriculture, food processing and nutrition, environmental monitoring [7, 8], And therefore here IT and allied technologies are widely increasing, viz., cloud computing, big data, smartphone and telecommunications, machine learning, Internet of Things (IoT), and so on. In traditional areas of Biological Science as well biosensor is actively useful, viz., clinical diagnosing, health and clinical monitoring. In preventive health, physiologic functions also biosensor is noticeable and increased its wider scope. Biosensor is constituent with sensor, transducer and associated electrons.

1.2 Objective

The present paper entitled, 'Aspects of Biosensors with refers to emerging implications of Artificial Intelligence, Big Data, & Analytics: The Changing Healthcare–*A General Review*' is theoretical and conceptual work and planned to rich agendas such as:

- To know about the basics of biosensors such as evolution, nature in brief.
- To get about the components of biosensors including general applications of biosensors in diverse areas.
- To learn about the biosensing technologies including application areas of Information Technology.
- To know about the artificial intelligence, big data analytics, cloud computing including its basic features, characteristics and services.

- To get the knowledge on applications of the artificial intelligence, big data analytics, cloud computing in brief.

1.3 Methods

This research work is theoretical in nature as depicted its title on 'Aspects of Biosensors with refers to emerging implications of Artificial Intelligence, Big Data, & Analytics: The Changing Healthcare–*A General Review*', and furthermore it is review based. Hence various secondary and primary sources are being used in preparation of this work. For the completion of the work web review and website review of the biotechnology-related company are also studied in order to get data related to rich theme. All gathered knowledge is analyzed and further here is reported in various sections.

1.4 Biosensors: Fundamentals and Types

As discussed before biosensor is having two major components like biological components and physical components. Regarding biological components important are cell, enzyme, *whereas* amplifier and transducer can be suitable example in physical components. Here it would be worthy to note that, biological components are dedicated in identification and communicate by analyzing and generating signals which are able to be sensed by the transducer. This is properly immobilized over the transducer and useful in remaining and future as long as possible.

At biosensor transducer is responsible for generating signal transformed keen on a detectable response in the biological part [9]. And this is considered as critical component in a biosensing device. Detector is another component in biosensor which amplifies and dedicatedly processes signals by the electronic display system. As biosensors are essential in compliances in various kind of *sensitive biological element* like tissue, microorganisms, enzymes, antibodies, etc., therefore it is a kind of biomimetic component, and various types of biosensor fulfill each of its categorically requirements [9–11].

The rising role and implications of the biosensors in different sectors are just increasing like medicine, healthcare, food processing in international markets. Traditional engineering and sciences principles and methods are widely applicable in better analysis of biological systems, in addition to the materials sciences and nanotechnology. Healthy biosensing practice is required regarding stand-off detection of various components which must be interconnected as depicted in Fig. 1.2.

The technological needs are not always same, and there are always changing applications areas, viz., Biomechanics and allied subjects, ubiquitous devices needed in biodetection, agriculture and production engineering, genetic engineering, food quality and safety. In public healthcare also biosensor applications are rising, and

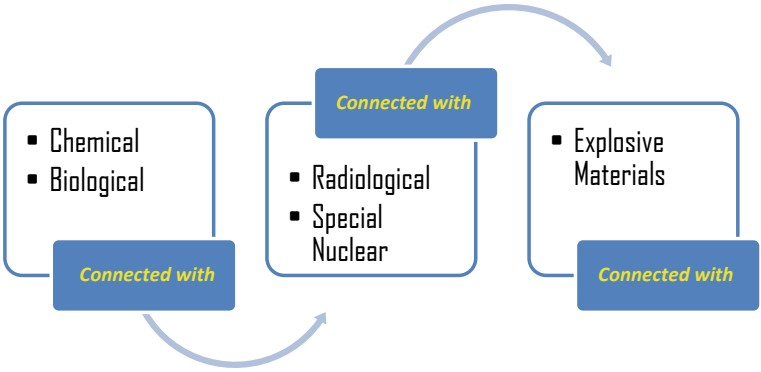

Fig. 1.2 Healthy biosensor practices depend on various interconnected facets as depicted

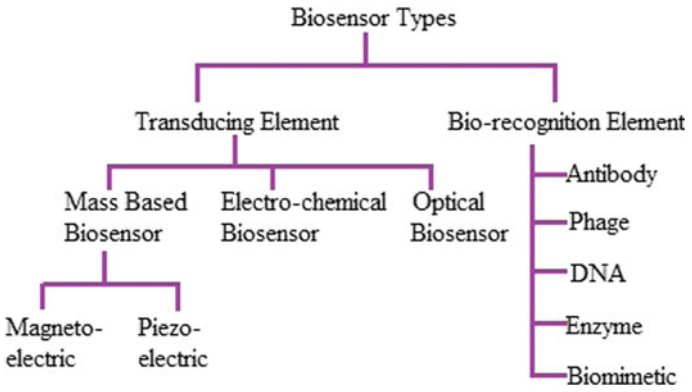

Fig. 1.3 Some of the major types of biosensor

therefore various technologies are important in this regard like artificial intelligence, cloud computing, IoT, etc. Different types of biosensor are briefly mentioned below with their proper classification (also refer Fig. 1.3 in this regard).

1.4.1 Electrochemical Biosensor

This kind of biosensors depends on reaction of the enzymes catalysts which generate electrons or consume as well. Counter, reference and working type are three electrodes considered as substrate of this kind of biosensor. Electrochemical biosensors are basically four types, viz., amperometric, potentiometric, impedimetric, voltammetric.

Amperometric biosensor is a kind of self-contained incorporated devices. This type of biosensors has reaction times, energetic ranges as compared to potentiometric. *Potentiometric biosensor* offers logarithmic reply by means of a high energetic range. This biosensor basically measures electrical potential of an electrode at the moment when there is no current present. It depends on three additional types of sensors. As this is a kind of potential chemical sensor therefore here concentration of the analyte in the gas or solution phase is considered as important and valuable. *Impedimetric biosensor* is sensitive indicator with different chemical and physical properties. This type of biosensor used is widely increasing throughout. The techniques and methods of this type of biosensor are also executive in other areas and to examine catalyzed responses of enzymes, etc. In other words, impedimetric biosensor is a sensitive technique which is required for the analysis of the interfacial properties related to biorecognition events. *Voltammetric biosensor* is basically built with a carbon glue which is additionally adapted with hemoglobin including four prostatic groups of them, i.e., *voltammetric* biosensors basically detect analyte by determining the amount of changes in the current as a function of applied potential [12, 13].

1.4.2 Physical Biosensor

Physical biosensor is another major type of biosensor; this is considered as most basic and widely used sensor. This categorization is basically happened regarding the inspection of the human minds. The physical biosensors are classified into two types, namely piezoelectric biosensors and thermometric biosensors. Physical can be able to sense a biological event with the changes in physical phenomena, and among these important are mass, resonance frequency, refractive index, fluorescence, etc. There are two types of physical biosensor, viz., piezoelectric biosensor and thermometric biosensor.

Piezoelectric biosensor is a collection of analytical devices based on the principle of affinity interaction recording. In other words, this kind of biosensor depends on the law of 'affinity interaction recording'. Here principle of oscillations is considered as worthy and important for the purpose of mass bound on the piezoelectric crystal surface. Piezoelectric biosensor is a kind of devices that are useful in managing and effecting piezoelectric effects to compute and measuring changes in the following, viz.:

- Pressure
- Temperature
- Force
- Strain, etc.

Piezoelectric biosensor is with the modified surface with the antigen, antibody, etc. The piezoelectric material lies on three main operational modes, viz., transverse, longitudinal, shear. Several biological reactions are associated with the release of

heat. *Thermometric biosensors* measure the temperature change of the solution, and there are many biological reactions connected with invention of heat, and this refers to the thermometric biosensors [9, 14]. This is used in serum cholesterol.

1.4.3 Optical Biosensor

This kind of biosensor is being used with the optical measurement principle. Here fiber optics and optoelectric transducers are being used [9, 15, 16]. Optrode combines with optical and electrode to find out and involving with antibodies and enzymes. This kind of biosensor has increased its uses in different areas; it is due to its ample benefits such as higher speed, sensitivity and robustness. Moreover optical biosensors are small in size and very much cost-effective over the traditional systems. According to market expert optical biosensors are useful due to its scope in direct, real-time and label-free detection of many chemical substances and biological substances. Direct optical detection biosensors and labeled optical detection biosensors—these two are major types of optical biosensor.

These are some of the important types of biosensor as mentioned above. However based on different criteria and features biosensor can be additionally with few more variants and some of them are mentioned below.

Wearable biosensors: this is a kind of electronic device used to wear, i.e., human body by various means such as:

- Smartwatches
- Smart T-shirt or shirt
- Tattoos
- Shoes
- Thumb, etc.

Such types of wearable biosensor are responsible in getting blood glucose, blood pressure, rate of the heartbeat, and so on. Some of the wearable biosensors are depicted here in Fig. 1.4.

Enzyme biosensor is a kind of biosensor which is having the nature of analytical device which is basically used in merging of the enzymes by the use of a transducer [17, 18]. It is worthy to note that enzyme-based chemical biosensors are basically based on biological recognition.

DNA biosensor is an important kind of biosensor which can be identified based on nucleic acid for the purpose of analyzing simple, rapid and economical testing. DNA biosensor is worthy and important in the food analysis, clinical research including environmental protection, etc. However it is worthy to note that for better and efficient detection SAM and SELEX technologies are basically worthy and important. With DNA biosensor accurate and required data in a simpler, cheaper and faster manner can be possible to retrieve.

Fig. 1.4 Major examples of wearable biosensors

1.5 Biosensors and Information Technological Involvement

Biosensor is increasing with the Information Technology applications and integrations. As Information Technology is dedicated in different information activities such as collection, selection, organization, processing, management and dissemination, therefore various subsections and parts such as Software Technology, Web Technology, Networking Technology, Multimedia Technology, Database Technology, etc. involve into the biosensor-related activities. Here among the subfields of IT and Computing Computational techniques, Networking Technologies and Communications played valuable role. Among the types of biosensor optical biosensor is important one and use of Communication Technology is prime example in this regard [12, 19].

Optical sensors are growing rapidly over the years and here uses of optical sensing other than transduction methods are widely useful. In optical biosensor one of the important features is integration of the high sensitivity of fluorescence detection alongside with the high selectivity powered with ligand-binding proteins. The field image processing is dedicated in optimizing the medical fields with utilizations of the computer vision, virtual reality and robotics, and so on. There are certain areas where IT are effectively used as depicted in Fig. 1.5.

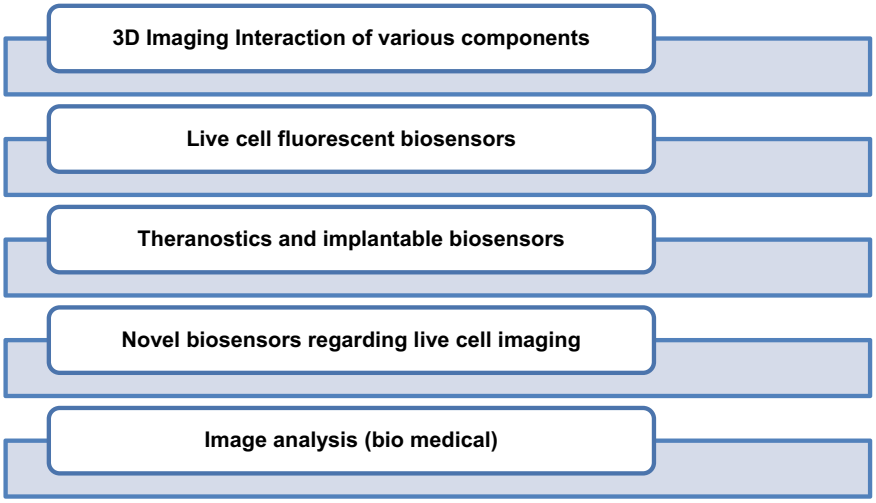

Fig. 1.5 Effective and potential areas of IT in biosensor and healthcare

In the Information Technology segment there are many emerging and potential areas and among this important are Virtualization Cloud and Fog Computing, Data Analysis and Analytics and Big Data Management, Internet and Web of Things, Wireless Networks and Edge Computing, Converged Networking, HCI and Usability Systems, etc. The applications of the Cloud Computing and Internet of Things alongside Artificial Intelligence, Robotics are also rising in the areas, viz., biosensor and medical systems and technologies [20, 21]. In the next sections different aspects and applications of artificial intelligence, big data analytics, Internet of Things first elaborated at first and then basic, emerging and potential applications of these technologies and systems into the biosensors and allied systems.

1.6 Artificial Intelligence Basics with Biosensors and Smart Biodevices

Arthur Samuel coined the term in the year 1959 first having deep involvement and exploring the study and construction of algorithms; and gradually it has come to today's shape. Artificial intelligence is a system, procedure and mechanism regarding the designing and developing intelligent products, services which are able in human-like performance, activities, mimic their action, etc. It lies on problem-solving and decision-making process. Here rationalization and actions are possible based on case and context. Due to wider applicability and importance of artificial intelligence in different areas, different sectors and industries various educational institutes, research

organizations are putting their importance in research and practices in artificial intelligence. Artificial intelligence may be tested and used in the areas like healthcare and medicine, governance and administration, business houses politics and bureaucrat, transport and tourism, education and research, etc. [9, 22]. There are rises of AI-based systems and tools such as automated machines, intelligent devices and products, self-driving cars. In the industries like finance, banking also artificial intelligence applications have increased in recent past and drastically. The latest features of the artificial intelligence include:

- Artificial intelligence lies on simulation and also human intelligence systems which are required in devices and machines.
- AI is dependent on learning, reasoning in order to fulfill the aim and objective.
- Weak artificial intelligence and strong artificial intelligence are two different types of AI which are able to manage and effect more complex and human-like [23–25].

There are different type of approaches of AI, viz., supervised, unsupervised and reinforcement as depicted in Fig. 1.6. Furthermore, artificial intelligence is two types and here weak artificial intelligence is important in designing of the simple system that can do a particular job, whereas strong artificial intelligence is more advanced which acts like human and able to perform jobs with complex and complicated system. Many artificial intelligence jobs can be done or possible to solve problem without having a person. Here machine learning is a part or subset of the artificial intelligence within Computer Science. Some of the statistical techniques and mathematics are important in cutting-edge AI practice with the abilities in computation, decision-making, proper data management [21, 26, 27]. Some of the approaches of machine learning are depicted as under.

- Decision tree learning (DTL)
- Association tree learning (ATL)
- Artificial neural networks (ANN)
- Deep learning (DL)
- Inductive logic programming (IPL)

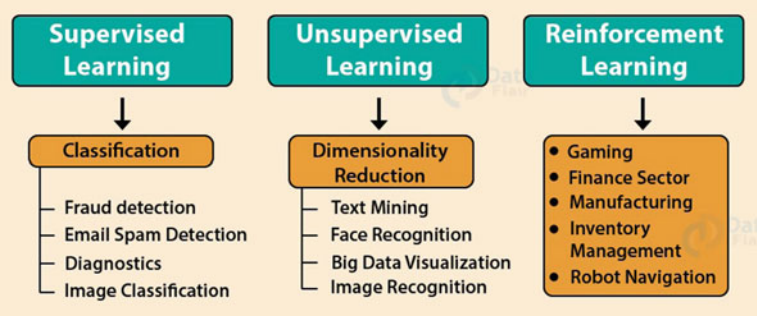

Fig. 1.6 Various approaches in AI with some other facets

- Clustering.

Healthy precision medicine treatment regarding individual patients becomes possible with AI and wearable sensor-dependent mechanism systems. Better and healthy integration of these systems is dedicated and brings better, efficient and healthy patient data management systems [8, 28]. Such sensors are able to collect fast and reliable data and help in health, fitness and allied aspects.

Allied and some other emerging technologies like Internet of Things (IoT), big data, AI-based biosensors are offering new opportunities, and at the same time it occurs few challenges too. Artificial intelligence is being used in analyzing the raw signal form a biosensor in by different means such as:

1.6.1 In Categorization

Artificial intelligence applications are able to increase the specificity of the sensor, and here the appropriate algorithms are useful in sorting the signal into different categories, and thus directly and indirectly it helps in healthy and sophisticated biosensor practices; it includes the wearable sensors.

1.6.2 In Detecting and Finding Anomalies

Sometime there are chances in error in signaling in the sensors, and here artificial intelligence can be effectively used in detecting anomalies. It can be directly expressed that a particular sensor is right or wrong. The advantages of artificial intelligence in biosensors help in healthy and effective biosensor practices.

1.6.3 In Reducing Noise on the Sensor Systems

Noise of the sensor is common; however artificial intelligence is perfectly suitable in reducing noise on the sensor while transferring data. Biosensor therefore required the support of the AI including other elements such as ML, DL and emerging robotics [9, 19].

1.6.4 In Identification of the Patterns

Identification of the patterns of different types on sensor data can be identified and split with the help of AI-based systems. AI can be trained to do pattern recognition.

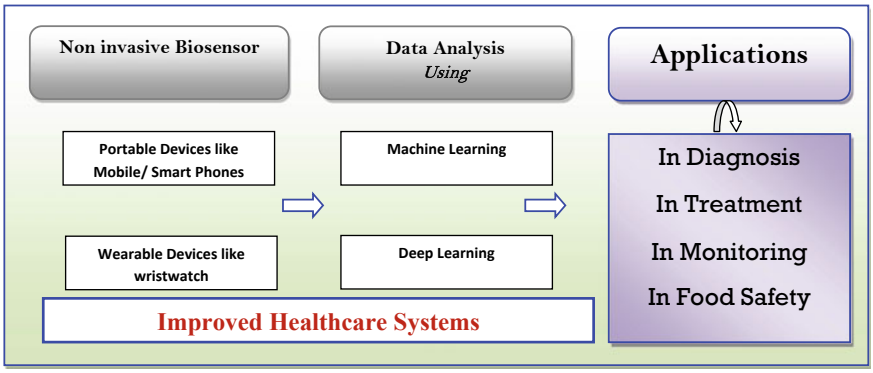

Fig. 1.7 AI and its subfields influences in biosensors in improving healthcare systems

The healthcare organizations, AI organizations are enhancing AI-based systems, and this scenario is rising [29, 30].

Therefore the AI and computational intelligence are being used in biosensor. Among different kinds of biomedical devices biosensors are widely increasing due to its benefit in healthcare monitoring and data collections. Here additionally Internet of Things, machine learning algorithms, cloud computing are being used in making of efficient and functional devices. For example in cardiac biosensor data are basically gathered based on heart symptoms, and it is useful in cardiovascular diseases. Cardian biosensors are able to gather different status and features of the cardiovascular diseases by analyzing status of the myoglobin, cardiac troponin, interleukins and interferons. With the biosensor irregular electrocardiogram patterns, rates of the respiratory, blood pressure, saturation of the blood oxygen, temperature of the body can be analyzed using AI-dependent biosensors. Additionally machine learning allows novel modeling and predictive methods in some of the following abilities, viz.:

- In identification of finding particular response
- In ability regarding finding delicate changes
- In proper and structural stability including functional stability
- In monitoring various health issues and systems
- In proper and sophisticated wireless transmission of the current and real-time data (Fig. 1.7).

1.7 Big Data Analytics, Allied Technologies and Biosensing

In the year 1990 the term big data was first coined and due to its role and importance gradually becomes an established name. The massive growth of data in various areas and domains leads more progress in data management using big data management tools and techniques [9, 31, 32]. The low-cost internet service providers created the craze in internet uses, and this results in huge data generation. However it is

important to note that before coined the term 'big data' in the year 1984 Tera Data Corporation first initiated and gives the concepts of big data or massive data with the 'parallel processing DBC 1012'. Soon after this various types of data, i.e., structured and unstructured management concepts and techniques were gradually improved and come to today's shape and periphery. In the year 2001 Seisent IMC also performed a perfect role for the development of the big data management systems. Google, Apache, MapReduce techniques (by Google) and Hadoop (by Apache) are important name in initial development of the big data management. Various other big data techniques and companies such as Oracle, EMC, Dell, IBM also take part in the proper big data management movement. Initially the concept lies on mining of the data with a particular timeframe and here database and data warehouse also allied and related. Here for managing and reducing the complexity of data various kind of tools and technique are used. Data become important in almost all the places and sectors, and as a result many other nomenclatures are being developed, viz., analytics, business analytics, data analytics, data science, etc. Various applications in other sectors lead other subjects and domains.

- Therefore, big data is concentrated on data that is huge in size. It is used to describe a collection of data huge in size and complex.
- Stock exchange, social media, jet engines, etc. are prime example in big data management.
- Big data therefore can be structured, unstructured and semi-structured in nature.
- Big data comes with the features of volume, variety, velocity and variability, and with this it offers the efficient customer service, operational efficiency, etc.

There are many applications of the big data and analytics in the fields of biosensors. As biosensor is dedicated in collecting various data throughout its operation, therefore the growth of the data and analytics can be seen all over. The AI algorithm-integrated data analytical system is important in collecting and providing accurate data and therefore important in managing different abnormalities in healthcare. AI-based data analytical tools are responsible to extract various information from various database and may be suitable in offering various data as per requirement such as data related to the heartbeats. As an example we can note long-term ST database which is able to collect data through the ECGs.

In collecting appropriate and proper data various methods of the artificial intelligence are being used such as supervised learning, unsupervised learning, reinforced learning. The data prediction in many ways depends on such methods [33, 34]. Since there are variety of the data already learned about among them wearable biosensor considered as important one which is very much sophisticated in regard to the monitoring health via proper analysis of the biological data like biofluids (e.g., saliva, sweat and blood). And such prediction is worthy to the physician and heath expert regarding further decision in patient monitoring and management. With big data-enabled biosensors following become easy and efficient such as:

- In managing proper medical imaging and DSS development.
- In proper and efficient real-time alerts.

- In healthy monitoring and optimization of healthcare systems.
- In better and preventative medicine analysis.

It is important to note that big data and analytics technologies required in knowledge discovery, problem-solving, data processing, analytical modeling which are worthy and important in healthcare, patient management and treatment. The big data analytical framework in connection with other technologies helps in remote monitoring of healthcare including physical daily activities of healthy as well as unhealthy population.

However, there are different technologies from the IT and computing also dedicated and required in biosensor and allied technologies and among them important are Internet of Things or IoT. ***Internet of Things*** lies on various objects, and it is able to collect required and timely data. The entrepreneur Kevin Asthton coined the term in the year 1990 and gradually improved various concepts, thoughts, techniques and strategies, etc. Different kinds of built-in sensor are integrated in the objects or system in order to collect various data. Some of the latest features are powered by the auto-adjustment like heating, lighting, etc. for building smarter life and technology enhanced society. Internet of Things plays an important role. The integration of the AI and ML in IoT devices is also valuable in order to do intelligent services and feedback systems. Here at Internet of Things IP address, wireless internet, embedded sensors are applicable to enhance the service and enhance professional and personal activities [34, 35].

The growth of the Internet of Things in other sector and areas leads other branches of IoT, viz., Industrial Internet of Things (IIoT), Agricultural Internet of Things (AIoT), Internet of Medical Things (IoMT), etc. as far as Internet of Medical Things or its medical applications are concerned; it is a worthy tool in collecting important health-related data. In some of the wearable devices used in healthcare Internet of Things is important for collecting various data in different formats in order to patient management.

These days developed societies are being connected with the advanced and intelligent healthcare network and here biosensor plays a critical role. Here in processing of advanced features of biosensor it lies on Internet of Things, big data analytics-integrated systems and obviously backed by the artificial intelligence. In recent past healthcare units and organizations are facing lot of challenges in data collection and generation and IoT, and some allied technologies come with the new hope of wellness. The growing healthcare data help in improvement in Electronic Heathcare Records, Mobile Healthcare, Telemedicines, etc. Remote monitoring systems using IoT are an important step in advancing Healthcare Informatics powered by the intelligent algorithms, tools and techniques with faster analysis and expert intervention for better treatment recommendations. The IoT-based sensor is responsible in collecting continuous data using proper signaling systems. Here signals are stored and thereafter properly stored in analytical techniques and systems using machine learning algorithms. Another important technology is cloud computing which helps in proving healthcare and medical systems like:

- Improving and advancing telemedicine systems powered by clouds

- Sorting and easy accessibility in medical imaging systems
- Proper and remote public healthcare systems
- Advanced patient self-management
- General healthcare operations and hospital management
- In allied healthcare and therapy management, etc.

Therefore artificial intelligence, robotics, Internet of Things, cloud computing and some other technologies are important in enhancing healthcare systems with due implications in the biosensors such as proper and efficient patient monitoring and patient management, cell-based biosensor activities, biosensor analysis and also imaging which are antibody based. As far as detecting a symptoms also this is considered as important. In cardiovascular areas too cloud big data-based systems are worthy similar to transduction technology. Biosensors are applicable in diabetes applications, and here directly and indirectly cloud computing, artificial intelligence and similar technological usages are important and valuable.

1.8 Biosensor, Futuristic Healthcare: Some Important Perspective

The advancement of the Information Technology leads various newer services in healthcare systems. As far as biosensor is concerned big data and allied technologies even also able in some other operations and dimensions such as X-rays, MRIs, ultrasounds and such generated various data through the images and to identification of such abnormalities here big data analytics including AI are important. Here cloud can be considered as important in storing and records management for current and future utilizations [9, 34]. Here machine learning can be considered as important in predicting various diseases. Analyses of the vast and large datasets are powered by the medical imagers using data analytics systems. In pharmaceutical industry also biosensors are useful, and here some of the analytical techniques are applicable and employed. It is a fact that machine can be used based on need and requirement; however it could not be considered as alternative. Though in pharma and healthcare industry biosensor can be useful in increasing the diagnostic speed and also helps in healthcare systems in enhancing patient care and communication.

Due to the advancement of the technology it is now creating new potentiality and possibility in advancing automated drug delivery, data and sensor-driven applications. The mobile-based applications are rising day by day for collecting data and patient management. Here business analytics are useful in analyzing and collecting data related to the patients. The biosensor is able to predict drug requirement based on AI-dependent prediction systems here body-worm sensor. Diet-tracking applications are also able to develop real-time data collections. Smart diagnostic and therapeutic devices cutting-edge engineering in the biosensors can lead more sophisticated healthcare systems. Here healthcare organizations can also be involved in advancing

the systems of healthcare with advancing biosensors [22]. Some of the interconnected advanced systems and technologies can improve biosensors uses, and these are:

- Cloud computing and big data
- Artificial intelligence with machine learning
- Deep learning and robotics
- HCI with usability systems, etc.

This technology is important in developing effective and low-cost biosensors and processing systems as well. In prediction analysis robotics and AI technologies, etc. are important in connection with the data analytics systems. Gradually the healthcare organization becomes able to offer cheaper health services, and in many context it is due to biosensors. Internationally biosensor industries have been increased, it was 25 billion USD market, and it is expected to grow 7.4 percentage in 2027. Demand for biosensors is increasing owing to wide use in clinical care and advancement in the areas such as diagnosis, patient health monitoring, detection of disease and human health and patient management with proper robust growth opportunities in the future. According to the Global Market Insights the biosensor market grows with following:

- Non-wearable biosensor segment market share is expected to rise at 37%
- Electrochemical biosensor segment is expected to grow 7.5%
- Home healthcare diagnostics is expected to grow 23%.

Furthermore as on 2020 North America market value is 10 BN USD, whereas European market is 8 BN USD and expected to grow in different categories and advances in biosensors [27, 35].

1.9 Conclusion

Biosensor including thermal, electrochemical, piezoelectric, optical—all types are increasing all over due to wide potentiality in different segments. Electrochemical biosensors are rising gradually due to wide scope and market. The needs of embedded systems have risen in recent past, and therefore healthcare organizations are also putting importance on biosensor. The advancement of Information Science and Technology leads to various kinds of benefits on society and human being, and healthcare is one of the latest. Lives become easy, effective and advanced with health informatics practice, and here emerging technologies are important in developing human lifestyle and comfort. Due to the implications of the biosensor in environmental monitoring, biological defense and food and nutrition, etc. the allied technologies of IT and computing play a leading role. Here artificial intelligence, machine learning, deep learning play a great role in modernization of the biosensors and allied activities. As biosensor is required in overall healthcare improvement therefore organizations, institutions and educational, research centers need proper step in developing manpower in the field of healthcare informatics.

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Chapter 2
On Few Electronic Properties of Nanowires of Heavily Doped Biosensing Materials

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Abstract **In this chapter, we study the** effective electron mass (EEM), the **Einstein relation for the diffusivity–mobility ratio (ER)**, **the Einstein's photoemission (EP)**, the field emission (FE) **and** the thermo-electric power (TP) in **heavily doped nanowires (HDNWs) of** different **biosensing materials** together with the relative comparison of the said transport features with that of the HDNW compounds. The EEM is an important transport quantity which is used in the analysis of different devices of low-dimensional electronics. The ER is useful in the characterizations of various types of hetero-structures and occupies a central position in the field of materials science. The EP is a physical phenomenon which finds extensive application in modern opto-electronics, and the FE is a quantum mechanical process. Besides, with the advent of quantum Hall effect, there has been considerable interest in studying the TP for various low-dimensional compounds. Although biosensing materials find wide applications and many physical properties have already been studied, nevertheless the investigations of the said electronic quantities for nanowires (NWs) of heavily doped (HD) biosensing materials are becoming increasingly important. Keeping this in mind in this chapter, an attempt is made to study the aforesaid quantities, **talking HDNWs of** various **biosensing material**s. We observe that the EEM is quantum number dependent. T**he ER oscillates with** the electron statistics (n_0) and t**he magnitude and nature of oscillations are totally different as compared with the ER in**

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HDNWs of other materials talking HDNW of InSb as an example. The Einstein's photo current from HDNWs of different biosensing materials **also oscillates with n_0 in radically different fashion as found from HDNWs of other materials. The field emitted current oscillates with increase in electric field due to van Hove singularities and the TP increases with increasing n_0 in oscillatory ways. The most important realization is that the quantum signatures in all the cases are not only totally different, but also the variations of the said electronic quantities as compared with that of HDNWs different compounds excluding biomaterials are also different**.

2.1 Introduction

The EEM [1–4], ER [5–8], EP [9–12], FE [13–16] and TP [17–20] have extensively been investigated in the recent literature, and they have important contributions in controlling control the transport phenomena in biosensing materials. Although biosensing materials find wide applications and many physical properties have already been studied [21–37], nevertheless it appears from the literature that the study of the said electronic properties has yet to be made. In this chapter, they are being investigated in HDNWs of biosensing materials. It may be noted that HDNWs are also being studied by various workers [38–40]. The theoretical background is described in Sects. 2.2, and 2.3 contains the results and discussion in this context.

2.2 Theoretical Background

The $E - k_x$ relation assumes the form [37]

$$k_x^2 = A_{11}(E, \eta_g, n_y) \tag{2.1}$$

where

$$A_{11}(E, \eta_g, n_y) = \left[\frac{2}{\sqrt{3}}\cos^{-1}\left[\left[[f\gamma(E, \eta_g) + g]^2 - 3 - D - 2\cos\left(\frac{n_y\pi}{d_y}\right)\right]\Big/\left(4\cos\left(\frac{3\pi n_y}{2d_y}\right)\right)\right]\right]^2$$

and the other notations are defined in [37]

The use of (2.1) leads to the expression of EEM as

$$m^*(E_F, \eta_g, n_y) = \frac{\hbar^2}{2}A'_{11}(E_F, \eta_g, n_y) \tag{2.2}$$

where the notations have their usual significances.

The n_0 can be written as

$$n_y = \frac{2g_v}{\pi}\sum_{n_y=1}^{n_{y\max}}\left[\sqrt{A_{11}(E_F,\eta_g,n_y)} + \sum_{r=1}^{r=n} 2(1-2^{1-2r})\xi(2r)\frac{\partial^{2r}}{\partial E_F^{2r}}\left[\sqrt{A_{11}(E_F,\eta_g,n_y)}\right]\right] \tag{2.3}$$

where the notations have their usual significances.

The ER can be expressed as

$$\frac{D}{\mu} = \left(\frac{n_0}{e}\right)\left[\frac{\partial n_0}{\partial(E_F - Z)}\right]^{-1} \tag{2.4}$$

where Z is given by

$$A_{11}(Z,\eta_g,n_y) = 0 \tag{2.5}$$

Thus by using (2.2)–(2.4), we can study the DMR numerically.

Incidentally, the photo current I can be written as

$$I = \frac{\alpha_0 e g_v k_B T}{\pi\hbar}\sum_{n_y=1}^{n_{y\max}} \ln(1+\exp[(E_F-(Z+W-h\upsilon))(k_B T)^{-1}] \tag{2.6}$$

where α_0 is the probability of photoemission

The field emitted current (i_f) assumes the form

$$I = \frac{2e g_v k_B T}{h}\sum_{n_y=1}^{n_{y\max}} [\ln(1+\exp[(E_F-Z)(k_B T)^{-1}]\exp(-Q)] \tag{2.7}$$

where

$$Q = \frac{4[A_{11}(V_0,\eta_g,n_y)]^{3/2}}{3eF_x\left[A'_{11}(V_0,\eta_g,n_y)\right]},\quad V_0 = E_F + \phi_w$$

2.3 Results and Discussion

The plot of the normalized EEM in HDNWs of MOS_2 versus n_0 for three sub-bands is given in Fig. 2.1. The plots of normalized DMR ($\overline{D}$) in HDNWs of MOS_2 versus

n_0 are given in Figs. 2.2, and 2.3 shows the same for HDNWs of InSb for the purpose of relative comparison. Figures 2.4 and 2.5 explore the normalized photo current ($\overline{I}$) from HDNWs of MOS_2 versus n_0 and the same for HDNWs of InSb respectively. Figures 2.6 and 2.7 exhibit the plots of the normalized FE and TP for different HDNW biomaterials versus n_0 respectively.

The salient features are given below:

1. In Fig. 2.1, the EEM increases with increasing n_0 where the value of EEM for $n_y = 1$ is the greatest.
2. In Fig. 2.2, the $\overline{D}$ in HDNWs of MOS_2 oscillates with enhanced n_0, and the magnitude and nature of oscillations are totally different as compared with the $\overline{D}$

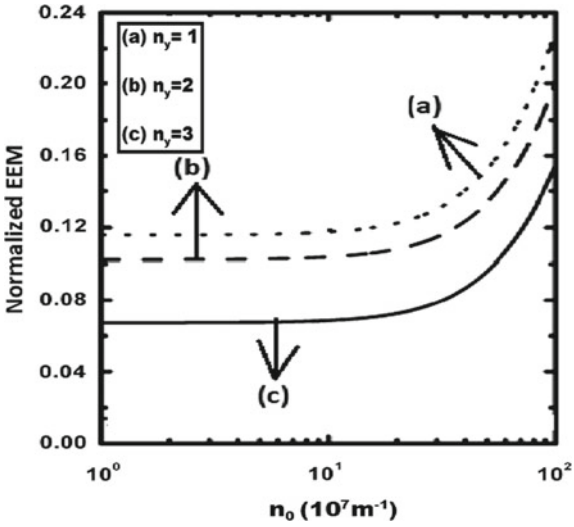

Fig. 2.1 Plot of the normalized EEM versus n_0 for three different values of n_y in HDNW MOS_2 where $d_y = 20$ nm

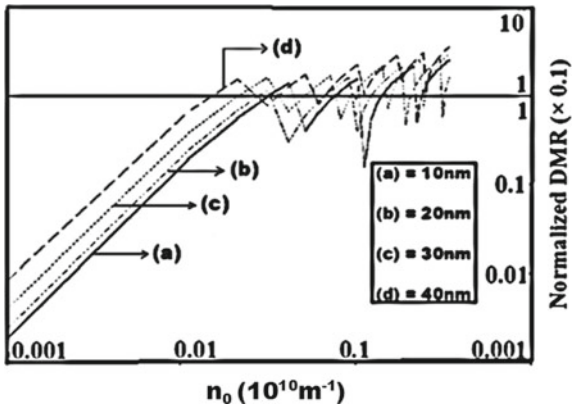

Fig. 2.2 Plot of the $\overline{D}$ in HDNWs of MOS_2 versus n_0 for four different values d_y as shown in the figure

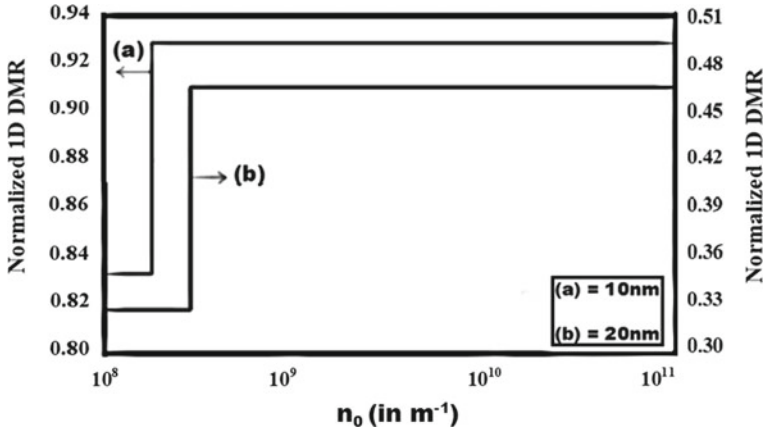

Fig. 2.3 Plot of the $\overline{D}$ versus n_0 for the NWs of $n-$InSb with two different values of d_y

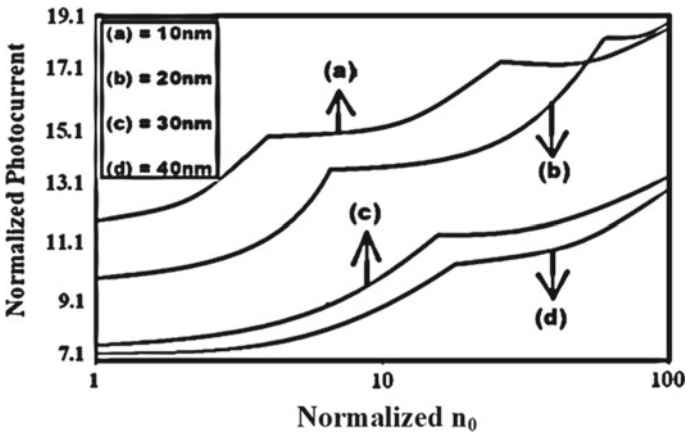

Fig. 2.4 Plot of the $\overline{I}$ from NWs of MOS_2 versus n_0 for four different values of film thickness

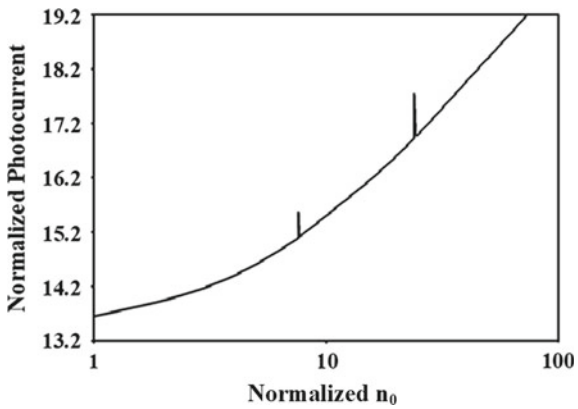

Fig. 2.5 Plot of the $\overline{I}$ versus n_0 for the NWs of $n-$InSb

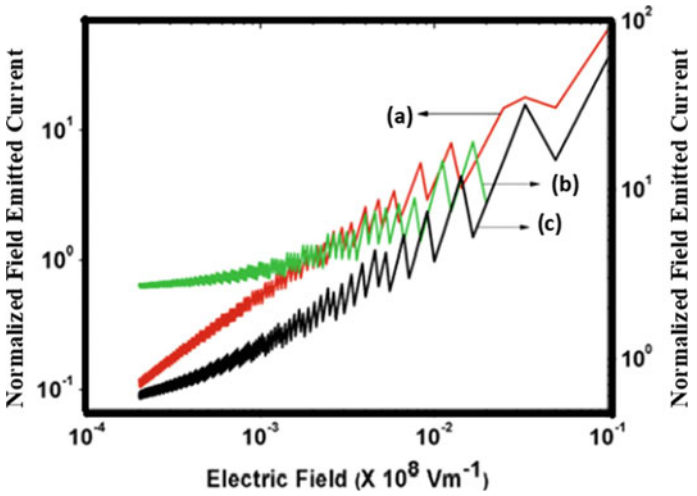

Fig. 2.6 Plot of the normalized field emitted current versus electric field for three different HDNWs of biomaterials

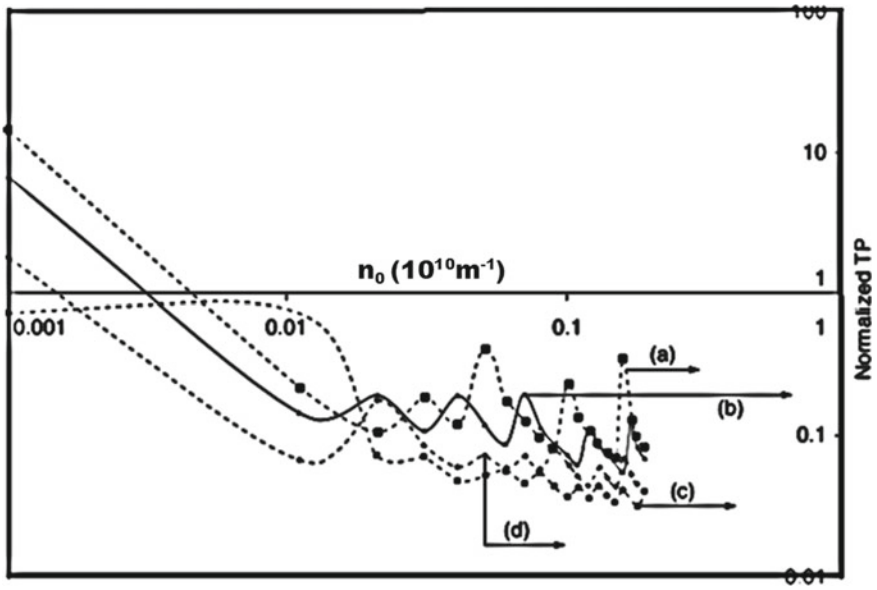

Fig. 2.7 Plot of the normalized TP versus n_0 for four different HDNWs of biomaterials as shown by a, b, c and d, respectively

in HDNWs of other material as given in Fig. 2.3. The quantum signatures of two different types of 1D motion can be assessed by comparing Figs. 2.2 and 2.3.

3. From Figs. 2.4 and 2.5, it appears that the $\bar{I}$ HDNWs of MOS_2 oscillates with n_0 in radically different manner as compared with that from HDNWs of other materials.
4. From Fig. 2.6, we note that the field emitted current oscillates with increase in electric field due to Van Hove singularities
5. From Fig. 2.7, we note that the TP increases with increasing n_0 in oscillatory ways.

Most important to realize is that the quantum signatures in all the cases are not only totally different, but also the variations of the said electronic quantities as compared with that of HDNWs different materials excluding biocompounds are also different.

2.4 Conclusion

In this chapter, we study the EEM, **ER, EP**, FE **and** the TP in **heavily doped nanowires (HDNWs) of** different **biosensing materials** together with the relative comparison of the said transport features with that of the HDNW compounds. We observe that the EEM is quantum number dependent. T**he ER oscillates with** the electron statistics (n_0), and t**he magnitude and nature of oscillations are totally different as compared with the ER in HDNWs of other materials talking HDNW of InSb as an example. The Einstein's photo current from HDNWs of** different biosensing materials **also oscillates with n_0 in radically different fashion as found from HDNWs of other materials.** The field emitted current oscillates with increase in electric field due to Van Hove singularities, and the TP increases with increasing n_0 in oscillatory ways. **The most important realization is that the quantum signatures in all the cases are not only totally different, but also the variations of the said electronic quantities as compared with that of HDNWs different compounds excluding biomaterials are also different.**

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Chapter 3
Overview of Biosensors and Its Application in Health Care

Sandip Bag and Dibyendu Mandal

Abstract In recent years, biosensor is referred to as most impressive and ingenious analytical tool consisting biosensing element with wide range of medical utilizations like detection of diseases, diagnosis, treatment, patient well-being monitoring and individual fitness management. In addition, these sensing devices have been emerged as the prospective apparatus of cardiac segment. In modern medicinal avenue, biomedical analysis of diagnostic report is of thriving interest because biosensors have excellent potential, easy to use, ascendable and impressive in construction procedures of medical devices. Additionally, improved qualities of biosensing technology grant the competence to recognize disease and track the body's reaction to utmost concern. Scientists and doctors always prefer secure and lucrative modes of operating their exploration, assuring people health & security and freight patient specific fitness choice. As a result, present and future trends of medical science have shifted into implementation of low-cost biosensors to examine foodstuff & water pollutants, monitoring human physiological systems, appraise explicit health analysis and so on.

3.1 Introduction

By definition, biosensor is a measuring device or system which incorporates a probe united with either biologically sensitive element as perception material or bioreceptor as physicochemical identifying element and a transducer for the recognition of a chemical reagent that blends a biological component with a physicochemical detector [9, 14]. So, this is an interpretive tool combining an entrapped biological component like antibody, enzyme, DNA/RNA, hormone, whole cell, etc. that precisely bind with an analyte to develop physical, chemical or electrical signal as an output which is comparable to amount of analyte in the specimen solution (e.g. glucose, urea, drug, pesticide) in the reaction process whose concentration has to be measured [2, 36].

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Various sensitive biological elements like nucleic acids, enzymes, antibodies, tissue, microorganisms, organelles, cell receptors, etc., which is either a biologically synthesized material or biomimetic substances that can easily reacts or binds with, or recognizes the measurand of the testing sample [48]. The transducer is considered as detector element to converts one form of signal into another using physicochemical approach, i.e. optical, piezoelectric, electrochemical, etc., after combining of marked analyte with the bioelement, for easy measurement and also compute the measurand [35].

Biosensor produces some detectable physical changes after combines with measured analytes and transformed into a measurable electrical output with the help of a transducer. Output signal is then intensified, analysed and displayed as analyte concentration in the sample [48, 51].

The mechanism of action between the analyte and biorecognition material of biosensors is of two categories:

(a) Catalytic biosensors in which the measurand may be transformed into a synthetic product by enzymes and
(b) In affinity biosensors, the analyte may directly react with the biological element embedded on the biosensor (e.g. to antibodies, nucleic acid).

3.2 Components of a Biosensor

A biosensor typically composed with a bioreceptor in the form of aptamer, nucleic acid, enzyme, antibody, cell, etc., a transducer made of either semi-conducting material or nanomaterial, and also an electronic system includes signal amplifier, processor & display unit [22].

So, a biosensor is developed with two distinct components mentioned below:

1. Biological element such as enzyme, antibody, etc.
2. Physical instruments like transducer, amplifier, processor, display, etc.

The following components are essential for a typical biosensing device which is displayed in Fig. 3.1.

- Analyte—It is a chemical component or substance or that is of interest in a biosensing procedure which requires identification. For sugar level measurement in bloodstream, biosensor is designed to identify the analyte glucose.
- Bioreceptor—A biological molecule (e.g. enzyme, antibody) is considered as bioreceptor that is sensitive to recognize the specific chemical substance. Enzymes, proteins, nucleic acid and antibodies are common examples of bioreceptor. Biorecognition is the step of signal generation as heat, light, pH, charge or mass change, etc. when interaction takes place between bioreceptor and the analyte.
- Transducer—Devices which mould one form of energy into other have played vital role in biosensing system that transforms biological interaction into a measurable

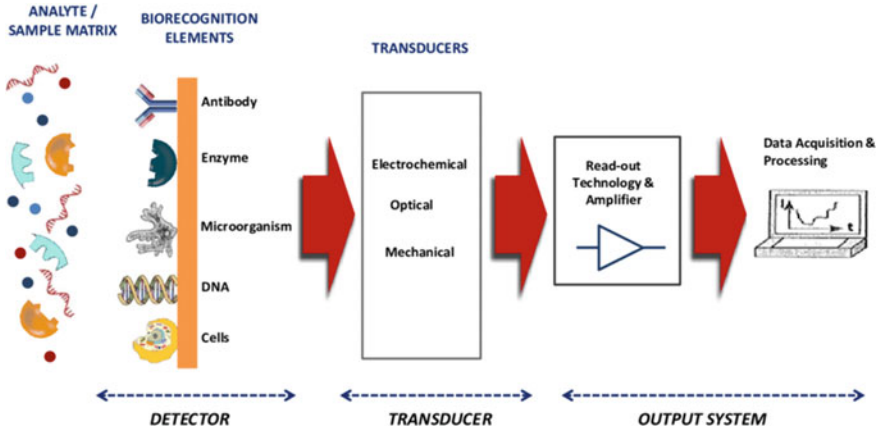

Fig. 3.1 Basic components of a biosensor

signal. Most biosensors produce either optical or electrical signal as an output after interaction with substrates, and the magnitude of such outcome is corresponding to the amount of analyte–bioreceptor reactions.

- Electronics: Signal produced after transduction is generally refined and keep ready for exhibit by electronic components of the biosensor. It composed of complex electronic circuit that operates as signal processing system to amplify the signal as well as change it from analogue to digital phase. After that the display unit of biosensor will quantify the processed signals.
- Display: This section composed of a translation system such as liquid crystal display of a computer or direct printer as a numbers or graphical representation or image according to the demands of the final user which is easily recognizable by the customer. The outcome component usually composed of amalgamation of hardware with software that customizes output of the biosensor in a user-friendly manner.

Three basic segments, namely detector, transducer and output system, are shown in the block schematic diagram of the biosensor. In the initial part, the sensing element acts as a responsive biological factor to identify analyte, whereas the later section is considered as the identification portion that modifies output data from the interaction with analyte and provide result as a display form by an accessible way. The concluding part encompasses with a signal conditioning unit, a demonstrating segment & a processor to amplify and display the signal in a suitable manner [35]. The biorecognition element first admits and binds with target analyte to generate signal resulting from substantial change that can be identified by transducer present in biosensor. In general, bioreceptor is suitably embedded on transducer, and thus, so-called biosensors are regularly used number of times for a long span [29].

3.3 General Features and Characteristics of Biosensors

(a) Features of Biosensor

Biosensors are utilized in number of medical applications like detection of disease, drug revelation, identification of contaminants, illness causing pathogens, markers, etc. are signals of disorders in blood, urine, saliva, sweat, etc.

Successfully designed biosensors must have certain features as follows:

- It should be specific and selective towards analyte.
- Biosensor must have sufficient sensitivity and resolution.
- It should be highly accurate and repeatable.
- It must have satisfactory speed of response and acceptable dynamic range.
- The mechanism of interaction should be free from external factors such as pH of sample solution, temperature, exhilarating speed, etc.
- Chemical reaction should be linear for a specific length of analyte concentrations.
- Output signal must be relevant to measurement environment.
- Device should be highly biocompatible and miniaturized so that it can be easily implemented within the body.
- This system must be cost effective, portable, user friendly and suitable for repeated use.

(b) Characteristics of Biosensor

Every biosensing element possesses certain static and dynamic attributes that are essentials requirements for any biosensor. The performance of each biosensor depends on the optimization of these properties. Biosensors are characterized by eight essential static and dynamic parameters, which includes-

Selectivity—Perhaps the most decisive quality of any biosensor is selectivity which indicates the strength of a bioreceptor to identify and respond only to a destiny analyte within a sample combined with other combinations. The simplest way of selectivity is explained by the coupled of an antigen with marked antibody. Generally, antibodies act as bioreceptors and are embedded on face of the transducer to detect specific analyte, i.e., antigen. A solution containing desired pathogen is then exposed to the transducer where antibodies combine only with such pollens. So, during design and development of a biosensor, selectivity is one the primary attributes of attention while selecting bioreceptors.

Sensitivity—It defines as the ratio of biosensor response and change in analyte concentration in the sample. In number of monitoring applications in healthcare sector, a biosensor is mandatory to expose analyte concentration in the range of ng/ml to confirm the presence of minute quantity of antigen in solution. For example, a prostate-specific antigen (PSA) concentration of 4 ng/ml in blood indicates prostate cancer for which doctors prescribe biopsy tests. Therefore, this character treated as one the essential features for biosensor fabrication.

Reproducibility—It means capability of a biosensor to produce similar reply for an identical condition and procedure. It is expressed by two parameters of the transducer named precision and accuracy with which the sensor's output depends because precision of sensor means exhibiting analogous results always when same sample measured repeatedly and accuracy expresses sensing ability of transducer to obtain a mean value close to the actual value when a specimen is measured more than once. The reliability of the reproducible signals is very high, and it provides compactness to the assumption made on the basis of biosensor.

Stability—This factor characterizes the shifting of its baseline or sensitivity over a specific time duration. By definition, it is the degree of sensitivity against surrounding interruption on biosensing environment that create flutter in output signals of a biosensor. As a result, it can create a mistake in measurand concentration and affect the precision and accuracy characters of biosensor. So, stability is the most influential factor in monitoring applications where a biosensor requires long incubation steps. The response of a biosensor depends on two factors such as (a) influence of temperature and (b) affinity of the bioreceptors. Deterioration of bioreceptor over a period of time is added factor that alters the stability of any instrument.

Linearity—This property exhibits the accuracy of the measured response of any biosensor in a straight line, and it is closely related with the verdict of biosensor and territory of analyte concentrations under inspection. Linearity of any biosensor should be steep for the detection of high analyte concentration. Based on operation, good resolution is appropriate because most biosensing applications need not only analyte detection but also measure the concentrations of chemical constituent over a large range of operation.

Operating Range—Operation range in biosensor is termed as concentration range over which response of biosensing elements changes linearly with the concentration, i.e. the sensitivity of such sensor is good. Occasionally, this character is also known as dynamic range.

Response Time—In other word, it is called as reaction time and can be expressed as time required to respond from no load to a step change in load to illustrate more than 60% of its final response for step input change in analyte concentration.

Life Time—Life time of a biosensor is specific time frame on which sensor can perform without severe degradation in functional characteristics.

3.4 Basic Principle and Working Mechanism of Biosensor

Usually, a specific enzyme is considered as desired biorecognition element which is deactivated by some natural process known as electroenzymatic approach and immobilized bioelement is in touch with the transducer. The analyte coupled with biological substance to shape a clear analyte which converts enzymes into corresponding electronic reaction with the help of a transducer that can be calculated. Oxidation of enzyme is most common biological response in biosensor where oxidation catalyses the reaction and alters the pH of the biological material. Alterations in pH will

automatically influence the current carrying ability of the enzyme that has direct relation with the enzyme being measured. In some instances, analyte is altered when it connected to a device that is associated with release of gas, heat, electron ions or hydrogen ions. Outcome of the transducer in form of current is a direct rendering of the enzyme that being measured. The current is usually transformed into voltage for proper analysis and depiction.

Biosensors are operated on signal transduction principle for recognition of biological element. The transducer measures analyte–bioreceptor interaction and provides an output signal. Power of output signal is related to the amount of the analyte. After that output is amplified and processed by the associated electronic circuit. The flow diagram of biosensing principle is shown in Fig. 3.2.

A number of bioreceptors is used as sensing element in this biosensing device, but the most suitable biomolecule is usually a specially deactivated enzyme which immobilized at the tip of the transducer. The target analyte connects to the specific biological material embedded on transducer (bioreceptor) by conventional methods and inducing an alteration in biochemical nature of enzyme which results an electronic response through electroenzymatic approach. Now, the electrical signal comes from transducer is a direct depiction of biological substance that can be calculated. The block diagram of biosensors principle is shown in Fig. 3.3.

In some instance, antigen is transformed into a product that may connected with the liberation of heat, oxygen, electrons or hydrogen ions. The consolidation of sensitive organic molecule and transducer is liable to disciple the biological material to a corresponding electrical signal which can be amplified and measured. Based on nature of enzyme, the output of the transducer is different, i.e. either current or voltage. If output is current instead of potential, a converter needed to change into an equivalent voltage before proceeding further. Working mechanism of biosensor is depicted in Fig. 3.4.

The output voltage signal obtained from chemical reaction is sufficiently low in intensity and overlaid on a high-repetition commotion signal. Electrical impulse is then intensified and moved through a low-pass RC Filter. A signal conditioning or processing entity of the electronic circuit is responsible for amplification and filtration

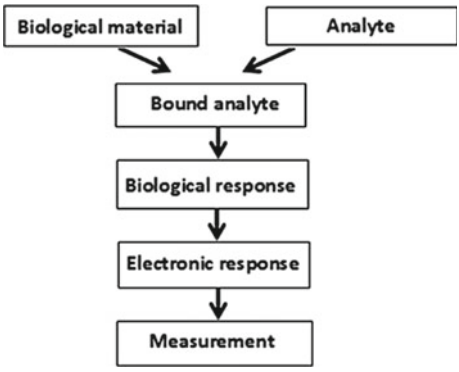

Fig. 3.2 Flow diagram of biosensing principle

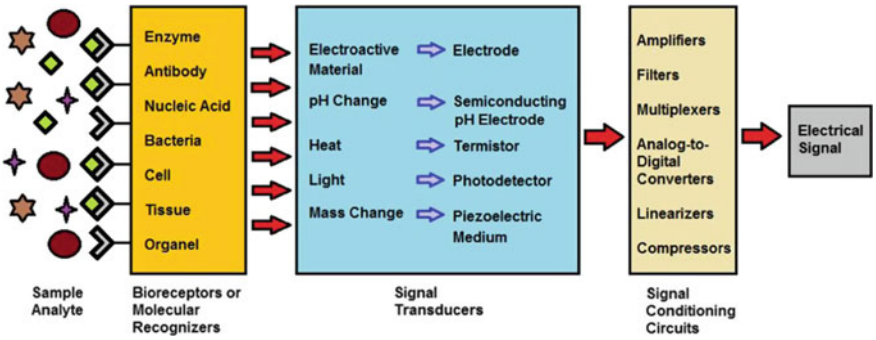

Fig. 3.3 Operating principle of biosensor

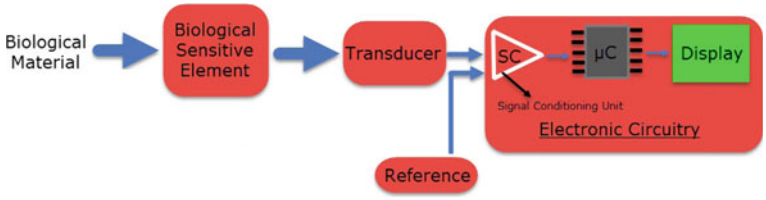

Fig. 3.4 Working mechanism of biosensor

of the signal. An output signal obtained from conditioning unit is proportional to the organic material which is being measured. After that the filtrate signal is fed straight to the LCD for presentation, but normally, this analog signal is transmitted to a microcontroller unit, where this output is transformed into digitized mode that is easier to analyze, process or storage.

Therefore, three elements are mainly involved in the basic working mechanism of any biosensor:

(i) At first, highly specific biorecognition element interacts with the analytes present in the sample.
(ii) Then, transducer detects and transforms signal into electrical one from biological target receptor molecule after electrochemical reaction.
(iii) Finally, transduction output is converted from biological to electrical signal where its elaboration takes place and processed values are displayed in monitor.

3.5 Evolution of Biosensor

Biosensor's development becomes the broadly studied research discipline due to simple, faster, cost-effective, highly sensitive and selective biosensor contribution leading to advances in imminent medicines such as ultrasensitive pin point detection of markers for diseases and health inspection. Aptamer biosensors can identify a

vast spectrum of target analytes of small molecules such as ions, vitamins, large molecules like proteins, whole cells, etc.

In the year 1950, an American Biochemist named LL Clark invented the first biosensor for use. This device is applied to measure the O_2 content in blood, and later this electrode is known as Clark electrode or O_2 measuring electrode. After that, the enzyme glucose oxidase incorporated gel was coated on the O_2 electrode to determine the glucose level. Similarly, enzyme urease was also applied with an electrode for NH4++ ion measurement useful for calculation urea concentration in blood and urine.

Three geneses of biosensors accessible in the retail according to the degree of incorporation of biological components like the technique of adherence of the biorecognition molecule towards element of the base transducer.

For first generation, biorecognition element is physically enticed within the environs of the sensor backside of a semipermeable sheath such as dialysis membrane. In the successive evolutions, the entrapment of bioreceptor can be accomplished by covalent bonds on an appropriately customized transducer junction otherwise inclusion within a polymer matrix of transaction surface. In this stage of biosensors, the return of the product dissipates to sensor and causes the electrical reaction, and subsequently, electrons are transferred to O_2 molecule and the analysis is made when reduction in O_2 concentration and/or increase in H_2O_2 production.

In second generation, the individual components remain noticeable or stay separate like control electronics, electrode and biomolecule. Second-phase biosensors use synthetic, partially toxic mediators or nanomaterials to transit the electrons towards electrode in a better way.

In third generation, biorecognition elements are indispensable part of base sensing material, whereas these explanations were possibly expected for enzyme electrode systems, akin gradations are pertinent to biosensors usually can be made. It is basically in between second and third generation families that the major developmental attraction which currently observed. In this generation of biosensors, no mediator is directly associated to initiate the reaction. As a result, the electrons are directly moved to electrode from enzyme without any intermediate stages or use of nanoparticles.

3.6 Types of Biosensors

Different classes of biosensors are available depending on the sensing devices and type of biochemical substances used [32]. The different types of biosensors are described below.

1. Electrochemical Biosensor

These are the simplest biosensing devices according to measurement of electric current, ionic or conductance changes imposed by electrochemical transducer. They can easily identify the biological materials such as enzymes, whole cells, specific

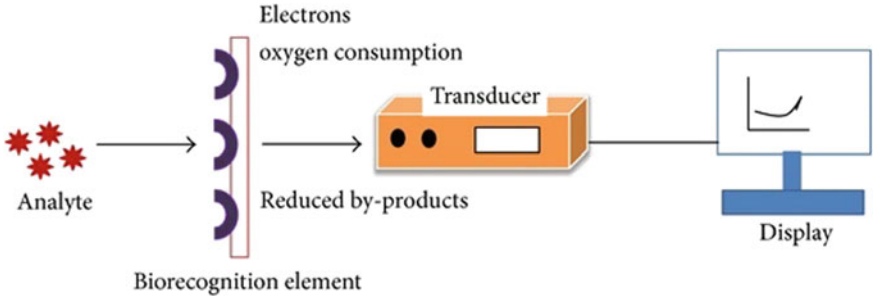

Fig. 3.5 Schematic diagram of electrochemical biosensor

ligands and tissues, along with non-biological matrixes [43]. Generally, the principle of electrochemical biosensor depends on enzymatic catalysis that evolves or consumes electrons [15]. This biosensor usually composed of three electrodes such as measuring, reference and counterelectrode. Block schematic diagram is represented here as Fig. 3.5.

In this sensor, target substance is committed towards response that takes place on the base of measuring electrode which is the origin for electron transmission through the dual-layer potential. Electrode potential or current can be determined at definite voltage [17].

A number of electrochemical biosensors available in the market depend on their working mechanism to secure organic selectivity or their signal transduction procedure or both [54]. The bioreceptors are specific for particular biocatalytic event such as a reaction that is catalysed by enzymes, or for a selective bioaffinity, such as the interaction between an analyte and a biorecognition element irrespective of their biological environment. Electrochemical biosensors are generally categorized into four types [50] such as follows.

(a) Amperometric Biosensor

These kinds of biosensors are self-supporting unified devices based on the developing current from redox reaction of an electroactive biological element offering exact quantitative analytical information. The current developed in amperometric biosensors is corresponding to the concentration of the target substances [5]. In comparison with potentiometric biosensors, it shows less response times, maximum energy spectrum and higher sensitivities. Clark oxygen electrode is the simplest amperometric biosensor which is frequently used.

The functioning principle of such biosensors depends on the movement of electrons as a result of enzyme-catalysed interaction and oxidation/reduction reactions [8]. Normally, a pre-set bias voltage applied through the electrodes being measured. In an enzyme-based reaction, the substrate or product can easily transport an electron-to-electrode surface which are either oxidized or reduced (Fig. 3.6).

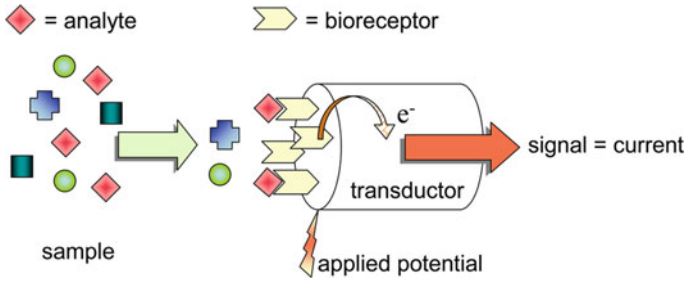

Fig. 3.6 Working principle of amperometric biosensor

When a voltage difference is created between two electrodes, a current is produced which is equivalent to substrate concentration. These are the first-generation biosensors. A typical example of such sensor is glucose sensor where we can measure the blood glucose level by redox reactions using glucose oxidase enzyme [20]. The considerable drawback is their affinity to dissolved O_2 content in analyte solution. This problem can be solved using mediators that relocate the electrons generated by the interaction directly to the electrode other than slashing dissolved O_2 in analyte solution. That's why these are referred as second-generation biosensors. Modern electrodes directly expel the electrons from the reduced enzymes without any help of mediators and are coated with power-conducting organic salts [19].

(b) Potentiometric Biosensor

It is a class of chemical sensor where biosensing element is attached with an electrochemical potential transducer applied to find the analytical concentration of some components of the gaseous substrate or solution [52]. These sensors usually measure the electrode potential without applying voltage as it relies on a biochemical reaction leading to a simpler chemical species and its subsequent electrochemical detection of NH_4OH, CO_2, pH, H_2O_2, etc. Potentiometric biosensors utilize ion selective electrodes to transduce the biological reaction into an analytical electrical output. For example, in pH measuring device a specific enzyme immobilized on membrane which surrounds the probe.

The electrical current is calculated as voltage difference between the active & reference electrode and is critically rely on the analyte concentration in gas or solution phase.

Potentiometric biosensors have been categorized under three distinct groups.

(i) Type I sensors contains an ionic solution having free ions inside chemical component of gas phase which is to determine. The example of commercially available type I sensor is YSZ oxygen sensor.

(ii) Although type II sensors having no free ions in the chemical compound that to be assessed, an ion pertinent to target gas can diffuse in the solid conductor to maintain equilibration with the surroundings. Thus, both type I and II sensors have the similar design with gas electrodes united with metallic strip

and an electrolyte where oxidized or reduced ions are in equilibrium through the electrochemical cell.

(iii) Type III sensors consist an auxiliary phase to the electrodes for promoting the selectivity and stability of the potentiometric biosensors. In the designing of a solid-state sensor, the ancillary phase acts as of part of the electrode but due to less electrical conducting nature of the auxiliary phase materials, it is not considered as an electrode. Irrespective of this distraction, these sensor designs attempt more usefulness in designing of various sensors with different accessory materials and electrolytes.

The main classes of potentiometric biosensors include membrane-based ion-selective electrodes, ion-selective field effect transistors (ISFETs), solid-state devices, screen-printed electrodes & electrodes modified through chemical reaction like metal oxides otherwise electrodeposited conducting polymers like sensitive layers. The cost-effective ISFET devices can be easily practised for miniaturization of potentiometric biosensors [26].

In potentiometric biosensors, alterations in ionic concentrations are usually calculated through ion-selective electrodes (Fig. 3.7). pH glass electrode is the most frequently used ion-selective electrode as lots of enzymatic reactions involve in the formation of hydrogen ions. Other important example includes ammonia-selective electrode and CO_2 selective electrodes.

The power variation obtained among the measuring and the reference electrode can be determined which is proportional to the concentration of the substrate. The main drawback of potentiometric biosensor is the sensitivity of enzymes towards H + and NH4 + concentrations.

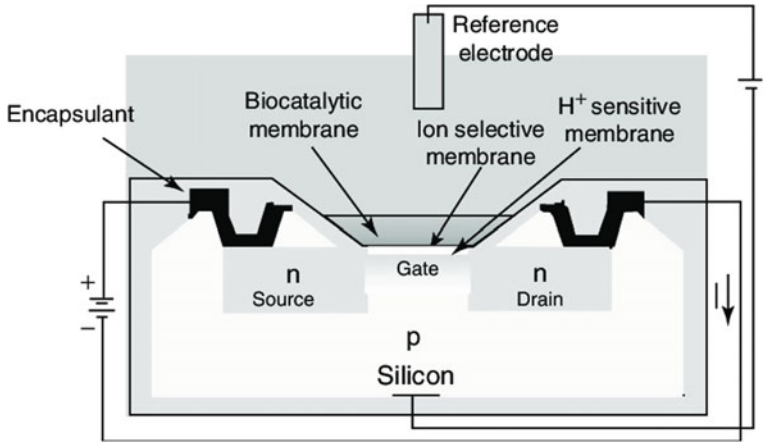

Fig. 3.7 Components and principle of potentiometric biosensor

(c) Impedimetric Biosensor

Impedimetric biosensors generally measure the conductance and capacitance changes at sensor surface due to selective binding of the target analytes (Fig. 3.8) In impedance spectroscopy, the hindrance of electrochemical cell surface is inspected which is dependent and proportional with the concentration of analyte [21]. Electrochemical impedance spectroscopy (EIS) is applied for a wide range of physical and chemical properties measurement. At present, there is ascending tendency towards the inflation of impedimetric biosensors in medical field. Impedimetric technique has been implemented to distinguish the invention of the biosensors and examine the catalysed reactions of various biological element such as lectin enzyme, nucleic acids, receptors, whole cells and antibodies [47].

(d) Voltammetric Biosensor

This biosensor was developed with a carbon glue electrode customized with Hb (haemoglobin), which includes four prostatic groups of the hem (Fe). This type of electrode shows a reversible oxidation or reduction procedure of Hb (Fe) [28]. This device detect analyte by calculating the change in current as a function of applied voltage. The peak current value is used for identification, while the peak current density is corresponding to the concentration of the analogous species. The advantages of this type of electrochemical biosensor are highly sensitive measurements, and multiple analytes can detect simultaneously.

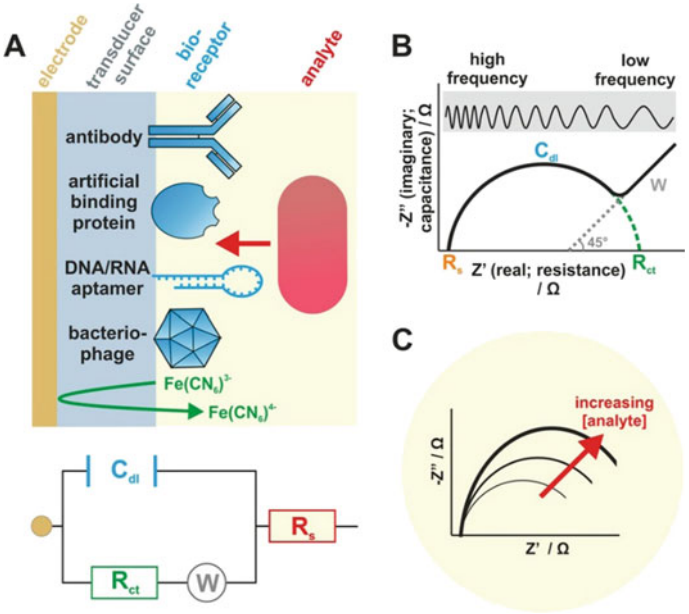

Fig. 3.8 Structure and function of impedimetric biosensor

2. Piezoelectric Biosensors

Piezoelectric biosensor, class of analytical tools, works on the principle of "affinity interaction recording", i.e. by measuring the shift in frequency when the antigen interacts with the antibody receptor [39]. The platform of a piezoelectric biosensor made of sensing element that works on the vibrations transform law due to collection jump on the surface of a piezoelectric crystal. In this measurement, biosensors having their modified surface with specific antigen or antibody, a molecularly stamped polymer, and genetic information [40]. The piezoelectric platform appears to be ideal for the construction of biosensors. It can simply record affinity interactions without the necessity to apply any specific reagents.

Piezoelectric biosensors are also known as acoustic biosensors as they work on the principle of acoustics (sound vibrations) (Fig. 3.9). In this sensor, the crystals having positive and negative charges oscillate with attribute frequencies [55]. Consumption of some molecules on crystal surface amends the resonance frequencies which can be recorded by electronic apparatus. Enzymes with gaseous substances or hindrances can also attached to these crystals.

These biosensors utilize ion-selective electrodes to disciple the biological return into electronic signal. The electrodes employed are most commonly glass pH electrodes coated with a specific gas selective membrane (for CO_2, NH or H_2S) or solid-state electrodes [13].

Many reactions produce H+ ion which is identified and measured by gas sensing electrode where very weak buffered solutions are used. An example of such ion selective electrode is based on urease which catalyses the following reactions:

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO^{-3}$$

The above-mentioned reaction can be measured by either a pH or ammonium ion or NH_3 or CO_2-sensitive electrode. Biosensors can now be prepared by placing enzyme-coated membranes on the ion-selective gates of ISFET which are extremely small.

Piezoelectric biosensors are readily available and reliable devices suitable for the determination of analytes by interactions without application of any reagents [41]. When it is compared with surface plasmon resonance methods having the same character, piezoelectric biosensors can be performed same function with low costs and simple analysis devices. In modern bioanalysis, diagnosis based on the determination of macromolecules can attain popularity for piezoelectric biosensors.

3. Thermometric Biosensors

These are also termed as thermal or calorimetric biosensors. Numerous organic reactions are responsible for the liberation of heat, and this form of calorie generation is the basis of thermometric biosensors [42]. A simple diagram of a thermal biosensor is shown in the following Fig. 3.10. It composed of a heat insulated box along with aluminium cylinder as heat exchanger. In thermometric biosensors, the reaction occurs in a small enzyme-packed bed reactor. When substrate penetrates the

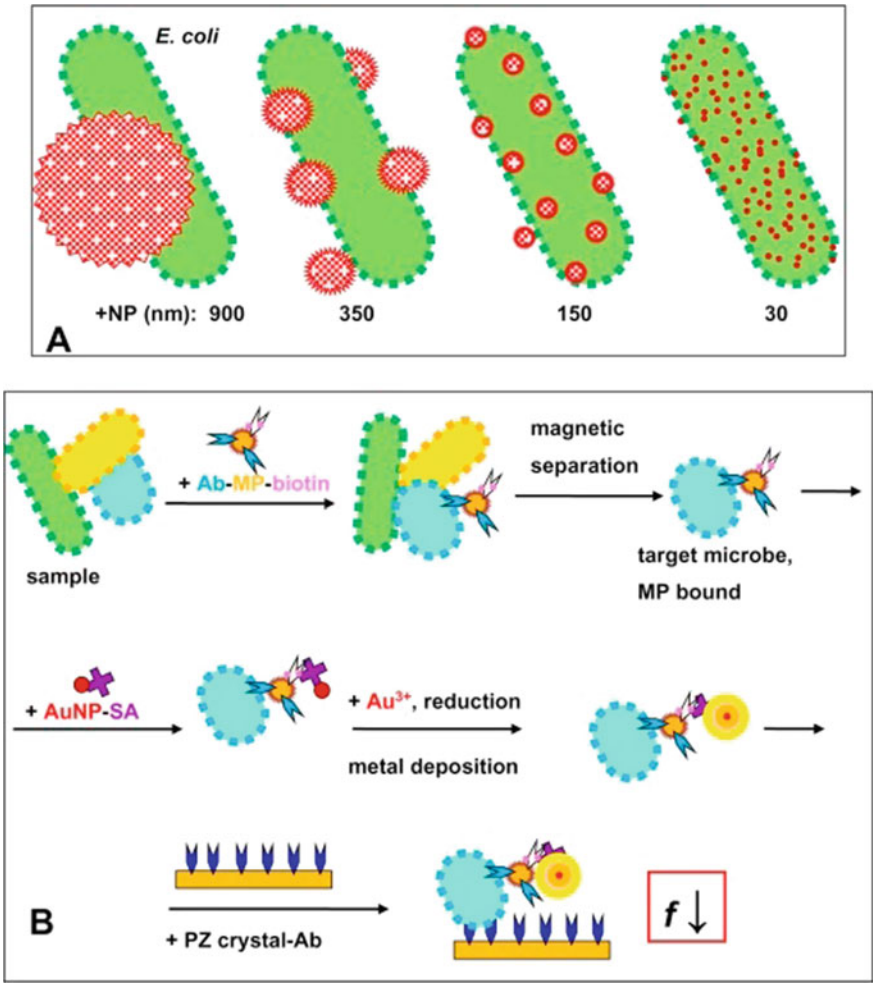

Fig. 3.9 Working principle of piezoelectric biosensor

bed, it transformed to a product and generated heat. The temperature difference between substrate and product is measured by thermistors because a small variation in temperature can easily be detected by thermal biosensors.

Thermometric biosensors are used to measure or estimate the serum cholesterol. When cholesterol gets oxidized by the enzyme cholesterol oxidase, the heat will produce which can be calculated. Similarly, assessments of glucose (enzyme-glucose oxidase), urea (enzyme-urease), uric acid (enzyme-uricase) and penicillin G (enzyme-P lactamase) can be done by these biosensors. In general, their utility is, however, limited. Thermometric biosensors can be used as a part of enzyme-linked immunoassay (ELISA), and the new technique is referred to as thermometric ELISA or TELISA.

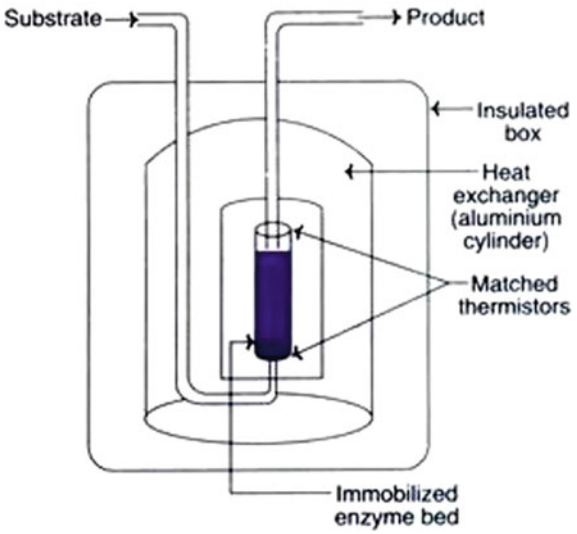

Fig. 3.10 Components and structure of thermometric biosensor

4. Optical Biosensors

Another type of analytical tools implemented using fibre optics and optoelectronic transducers and utilizes the principle optical measurement, i.e. absorbance, fluorescence, chemiluminescence, etc. [11] (Fig. 3.11). Antibodies and enzymes are considered as two main transduction molecules in optical biosensors [3]. The main advantages of optical biosensors include it allows a safe non-electrical remote sensing materials and usually do not require any reference sensors as the correlative signal can be generated using a similar light source like sampling sensor.

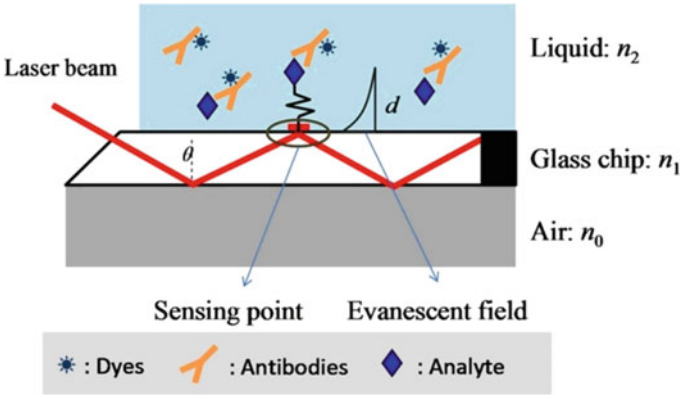

Fig. 3.11 Working mechanism of optical biosensor

Both catalytic and affinity reactions are measured with these biosensors. Optical biosensors measure the fluorescence deviation or absorbance caused by the developed products after every catalytic reaction. Alternately, they determine the alterations induced in the inherent optical properties of biosensor exterior due to loading of dielectric molecules like protein in affinity-type reactions. A most encouraging luminescence biosensor uses firefly enzyme luciferase for identification of bacteria in food or biological samples [10]. The bacteria are specially degraded to release ATP, which is used by luciferase in presence of O_2 to generate light that is measured by such biosensor.

Optical biosensors allow secure non-electrical inaccessible sensing of equipment. The optical biosensors are categorized as non-labelled and labelled optical detection biosensors.

5. Immunosensors

It is a class of biosensor that incorporates a biological recognition mechanism with a transducer which produces a measurable output in reply to alterations in the concentration of given biomolecule [27]. Immunosensors are compact device that reveal specific immunoreaction between antibody and antigen, i.e. creation of a stable immunocomplex is detected by using a transducer and an electrical signal is measured [30]. This distinct immunoreactions between these specific molecules makes immunosensor very interesting and attractive in recent years as a tool for several applications in different fields such as kits for clinical diagnosis & health monitoring, food management, industrial analyses and environment monitoring. These biosensors use antibodies as bioreceptor and a transducer which changes the antibody–antigen interaction event to a measurable outcome. Some examples of immunosensors are optical, evanescent wave, surface-plasmon resonance, fluorescence and chemiluminescence that used for clinical and environmental monitoring. Continuous and selective detection of analyte, yielding a response in real time, are the major advantages of these sensors.

Based on working principle, immunosensors can be classified into two types named nonlabelled and labelled immunosensors. First group of immunosensors are designed in such a way that the antigen–antibody immunocomplex is directly determined by measuring the physical changes that induced by the formation of this compound. On the other hand, a sensitive label is incorporated into second category for detection is referred as labelled immunosensor. As a result, the immunocomplex is sensitively determined through the label identification.

Label-free immunosensors are suitable for point-of-care analysis as devices work very fast and use simple technique [33].

Labelled immunosensors are designed to identify the immunochemical complexation that takes place on the exterior of the sensor matrix. There are number of variations in the procedure to form an immunocompound on this matrix.

3.7 General Working Principle of Immunosensors

Immunosensors are solid-state biosensing devices, where immunochemical reaction is coupled with a suitable & pertinent transducer and considered as most interesting categories of affection-based biosensors depends on the specific recognition of antigens by antibodies to form a stable immunocomplex. Based on the type of transducer used, there are three types of immunosensor available as electrochemical, optical and piezoelectric (Fig. 3.12). All these types can operate as either set of non-labelled or labelled immunosensors.

In recent years, most commonly used bioelements for the development of electrochemical immunosensors are antibodies (Ab), followed by aptamers (Apt) and microRNA (miRNA). Here, the highly specific reaction occurs between variable regions of an antibody and the epitopes of an antigen involving different types of bonding/interactions such as hydrophobic & electrostatic interactions, van der Waals force and hydrogen bonding. The immuno-reaction is reversible due to the relative weakness of the forces that holds the antibody and antigen together; the formed complex would dissociate easily based on reaction environment, i.e. pH and ion strength. The binding strength of immunocompound could be characterized by its affinity constant (K), ranges between 5×10^4 and 1×10^{12} L mol^{-1}. The high affinity and specificity of this antigen–antibody binding reaction confirms the unique immunosensor characteristics.

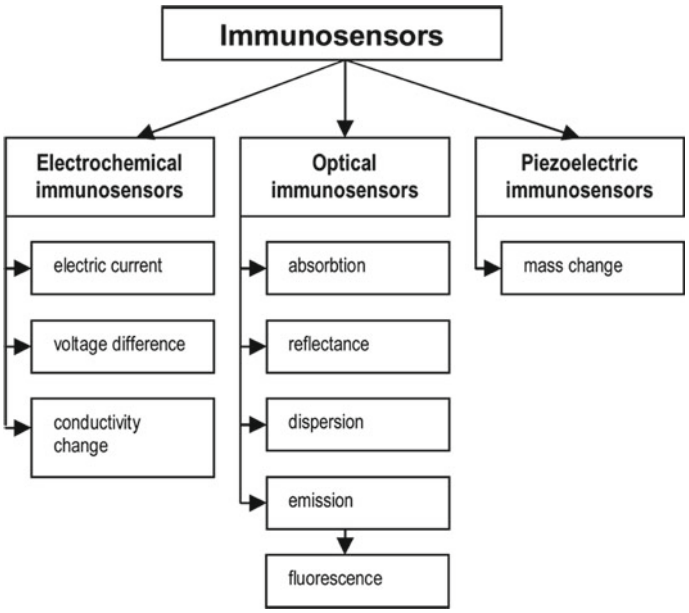

Fig. 3.12 Types of immunosensor

The recent developments made in the immunosensor field include incorporation of nanomaterials for increased sensitivity, multiplexing or microfluidic-based devices, may have potential for promising use in industry and clinical analysis. Some examples of assays for several commercially available biomarkers will be presented. The main application fields, beside biomedical analysis, are drug abuse control, food analysis and environmental analysis.

Immunosensors are often used to identify or quantify the disease-related substances in clinical diagnosis, providing their increased attraction towards antibody–antigen complex with greater selectivity. For example, a novel strategy for AFB1 detection based on DNA tetrahedron-structured probe (DTP) and horseradish peroxidase (HRP) triggered polyaniline (PANI) deposition was developed. In this process, the carboxylic group was associated with the AFB1 monoclonal antibody (mAb) to fabricate DTP.

3.8 Wearable Biosensor

Wearable biosensor is a digital electronic device that can mainly attached on the human limb to monitor and transmit physiological information continuously and non-invasively [24]. Various wearable devices in the form of smart clothing, smart watch, ring, smart shoes, belt, arm strip or band, etc. allow the person to monitor vital signs and detect crucial biological anomalies such as glucose level in blood, blood pressure, heart rhythm, body temperature and other physiological variables that is necessary to calculate regularly and invariably (Fig. 3.13) [31]. Advanced biosensors are able to track targeted biomarkers and help to understand the disease in better way.

Presently, we observe that wearable sensors are providing an indication of development for better universe. Suitable application of such user-friendly sensors permits an authentic degree of knowledge into a patient's real-time well-being stature [6]. This real-time data convenience will furnish preferable clinical choices and will affect embellished fitness results and added proficient use of health systems [7].

For human subjects, wearable sensors may support in incomplete realization of health actions as well as avoid the chance of hospitalization [46]. The major advantages of such sensors are curtailed hospital stays and readmissions that will obviously create positive awareness in upcoming days. Furthermore, investigated report reveals that these sensors will undeniably provide low-cost wearable health apparatus to the world [1].

Fig. 3.13 Wearable biosensors

3.9 Types of Wearable Biosensors and Their Applications

3.9.1 Smart Socks

A pressure sensor is infused within comfortable, textile shocks that can restrict GAIT pattern by which feet-ground interactions and foot-landing technique in various activities and positions like walking, running, standing or sitting position (Fig. 3.14). It is really a wonderful supportive tool to guidance the elders having difficulty in walking and training kit for children who trying to learn walk, inhibit potential injuries during walking, etc. Athletes can also apply such socks to modify training arrangement. User's mobile can store the data recorded by pressure sensors and transmitted wirelessly to the cell phone for satisfactory analysis through a proprietary program, and necessary alarming indicator is set for the subject if necessary. They inform us in real time when we are striking with the heel on the ground.

Fig. 3.14 Smart socks

3.9.2 Ring Sensors

Ring sensor is basically a pulse sensor that allows us to monitor heart rate and oxygen saturation in real time. Light-emitting diode (red & infrared) and photodiode are entrapped into the ring-shaped device and can be worn for long period of time for constant monitoring (Fig. 3.15). When heart muscles contract in each beat, blood ejects from the ventricles with pressure pulse that transferred through the circulatory system. During transmission, pressure pulse travels through the blood vessels as a result vessel wall displacement takes place which can be measured at various points to detect pulse or blood volume changes by the optical method. Generally, photo conductors are applied, but sometimes photo resistors are used to amplify the signals. The entire system is designed and guarded by a special processor. The transmitted waves are conveyed through a digital wireless transmission network to home computer and analysed after receiving.

3.9.3 Smart Shirt

This unique shirt is designed & developed at Georgia tech incorporating special sensors and optical bears to detect wounds and interconnected with the monitor to measure the vital signals of our body (Fig. 3.16). Smart shirt caters a structure for monitoring, instruction processing systems and sensing of the shocks. The sensors can be fixed on the right positions of the shirt for all the users, and it can be washed without damage.

Fig. 3.15 Smart ring sensor

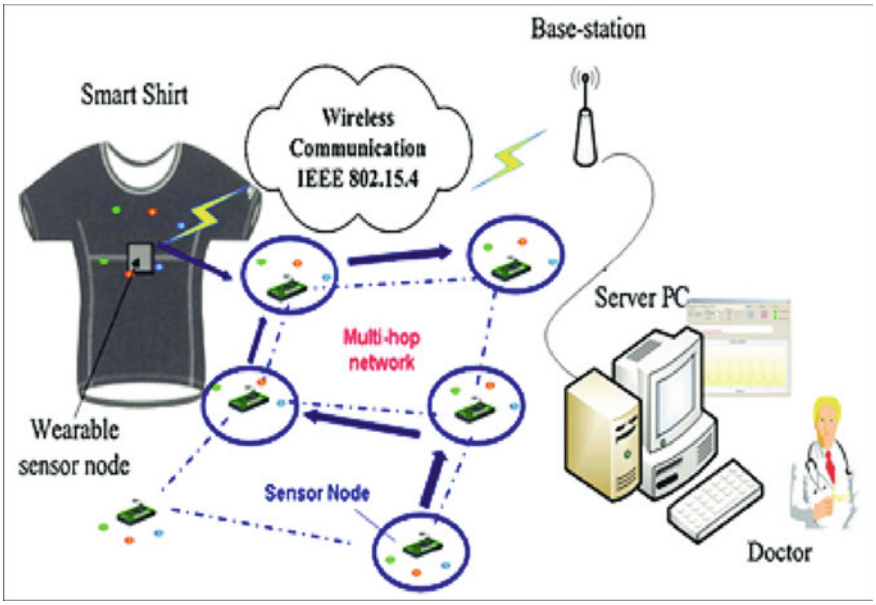

Fig. 3.16 Smart shirt as wearable biosensor

Primary advantages of wearable smart shirt are to help to monitor the physiological variables like heart rhythm, respiration rate, physique temperature, etc. Integrated sensors and conductive fibre grid are accustomed in smart shirt having band connectors that recognize important parameters and transmit signals to wireless server system that carries out future analysis for medical care.

3.9.4 Smart Clothing for Premature Babies

Over 15 million premature babies are born annually in this world, out of which more than one million of this populations dies or suffers physical and psychological complications due to the loss of body water. Researchers from Poland have successfully designed an intelligent clothing for premature babies to prevent the unwanted fluid loss. This clothing is composed of two different layers in which first layer is made of ordinary fabric and second layer is basically a membrane which counters excessive sweating in the baby.

3.9.5 Digital Clothing for Examining Mental Status

Infused miniature sensors in digital clothing can monitor the physiological indicator for determining the psychological state of the people, i.e. heart rhythm, body temperature, as well as skin conductance very efficiently [44]. After that, data are fed to a database through a cell phone, where an appropriate reply is sent considering the present situation and general interest of people. The display curtain in the clothing is equipped with LED lamps for displaying cheerful symptoms when people are in regret or scare.

3.9.6 Benefits of Wearable Biosensors

The biosensors with wearable technology offered most crucial role in managing fitness by sending useful information about performed activities and physical status of the body. Medical sensors in wearable form are the revolution in many application avenues ranging from cardiovascular monitoring to battlefield personnel monitoring and sports mechanics including medicines. Recent advancements of such tiny devices have favoured a sensational gain of attraction in wearable technology.

The usage of wearable biosensors facilitates consistent investigation of physiological signals crucial for upgrading both diagnosis and treatment of diseases. Further, this portable device will help to restrict the use of expensive technologies in patient monitoring for days, weeks or months.

3.9.7 Enzyme-Based Biosensors

An enzymatic biosensor is one kind of analytical device used where enzyme is merged with transducer for recognition and then reacts with the destiny analyte to

form chemical signal. The produced output is comparable with the amount % of target analyte which can be amplified, stored, processed for future analysis.

Over the past several decades, development of enzymatic biosensing devices has been extensive for overall investigation of variety target substrates on a vast range of applications [34]. The noticeable benefits that enzyme-depended biosensors provide are high sensitivity & specificity, portability, low cost and the possibilities of miniaturized form and point-of-care diagnostic testing which makes them one of the most extensive and attractive areas for clinical assays, maintained food safety or disease monitoring.

The principle behind the sensing mechanism of the enzymatic biosensor is to identify the existence of some substrates by measuring alterations like H+ ions number, release or consumptions of gases such as CO_2, NH_3, O_2, etc., emission or absorption of light, heat generation and so on, which substrate is consumed or formed a bi-product due to an enzymatic reaction [23]. After that, the transducer transfers those transformation into measurable signals as electrical, thermal or optical that are utilized to find the analytes of concern. Enzyme-dependent biosensors are classified into various groups based on transduction categories such as electrochemical, optical, thermal and piezoelectric biosensors. The working principle of enzyme-based biosensors is shown as block schematic in Fig. 3.17.

In these biosensors, enzyme particles are entrapped over solid support or matrix transforming substrate into the desired product. Thus, confinement of enzyme is considered as protection of catalyst on base. To make it more workable, the enzyme has to heed within the matrix of ascertaining action. Biosensors are anticipated for high stimulant storing in such a way that a sufficient amount of biocatalyst gets accustomed to the surface to assure the biocatalyst receives the appropriate environment to continue their enzymatic activities.

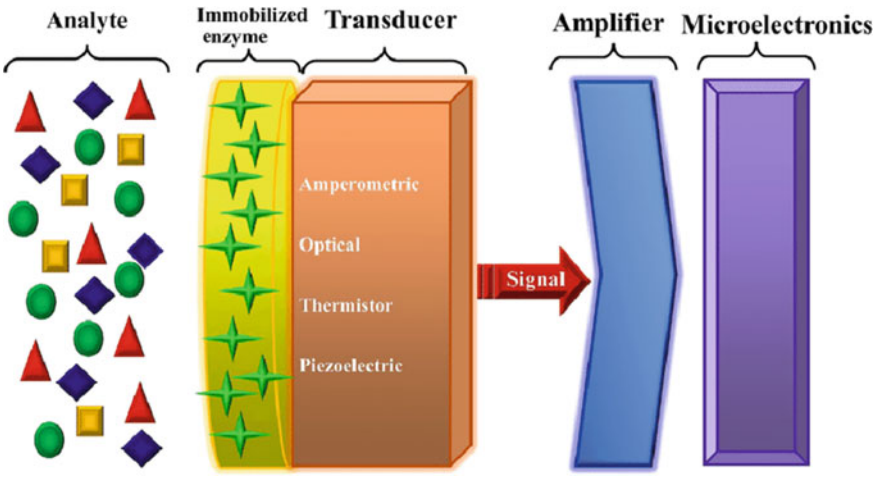

Fig. 3.17 Schematic diagram of working of an enzyme-based biosensor

3.9.8 DNA Biosensor

Based on nucleic acid realization technique, DNA biosensors are being fabricated for the rapid analysis, easiest and cost-effective examination of genetic and contagious diseases. Nucleic acid recognition layers can be easily produced & refurbished for numerous applications that is not possible for enzymes or antibodies [53].

DNA-based sensing devices are considered as application biosensors and play decisive role in disparate domain such as environmental surveillance, foodstuff control, drug exploration, forensic and biomedical research.

Nowadays, application of nucleic acids has become the utmost modern tools for identification and monitoring lots of relevant complexes. As a result, tremendous requirement of detection systems is not only for specific DNA fragments determination but also calculate the total amount of nucleic acid present in sample without error [37]. Over a decade, DNA biosensor technologies have gained immense interest with immense pledge for rapid and inexpensive detection of definite DNA arrangements in human, bacterial and viral nucleic acids [12].

The analysis of nucleic acids has accomplished vast acknowledgement in various domains including diagnostic test, pharmacological findings and various other areas such as animal farming & determination of transgenes. The increasing number of DNA-based tests has accelerated requirement for mechanized, and low-cost testing kit provides miniaturized inspection platform and exquisitely the associated instrumentation.

The functioning of electrochemical DNA biosensor is based on principle of biological synergy between biomolecules & marked analytes that can either generate or consume electrons, which alters the electric current, potential or any other electrical properties of the solution being tested.

The working mechanism of these widely used biosensors depends on hybridization technique via unplanned H bonding between the specific DNA and its reciprocal strand (Fig. 3.18). This principle is usually consummate by entrapping the single-stranded DNA onto a suited platform. Amalgamation episode usually invented by two distinct process: (i) identification of certain electroactive indicator (labeling) and (ii) detection of signal produced by the maximum electroactive base of DNA as adorned in Fig. 3.19.

3.9.9 Biosensors Applications in Medical Field

Healthcare or clinical service sectors are the considerable dominant area where biosensors found their best possible applications. Figure 3.20 illustrates a number of noticeable applications of biosensors that fit under one umbrella of healthcare services and allied areas. Major application area of biosensors includes detection of disease, prosthetic eye, divergent imaging during MRIs, cardiovascular diagnosis, medical mycology, health monitoring, etc. These potential abilities further exhilarate

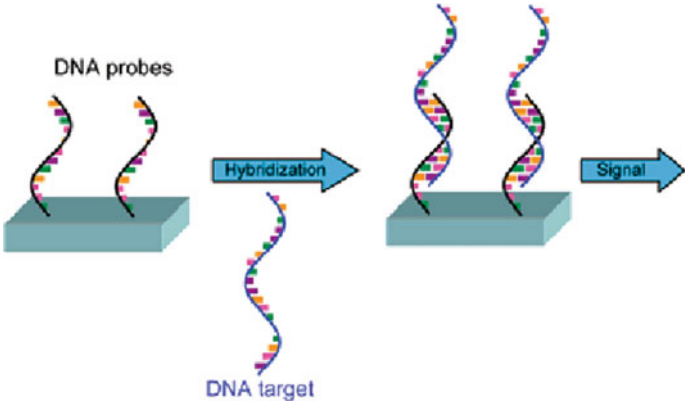

Fig. 3.18 DNA biosensor

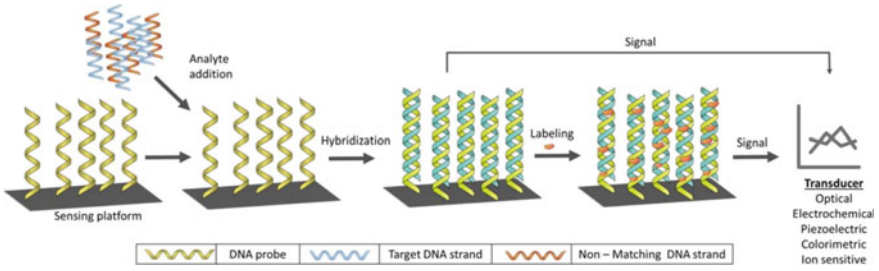

Fig. 3.19 Design and working principle of DNA biosensor

health care to a new pinnacle with admirable social services [25, 49]. The virulent diseases such as avian influenza, SARS, Hendra, Nipah, etc. have gained significant interest in recent years along with latest COVID-19 disease which is highly infectious and is caused by a newly discovered coronavirus that has impacted the world. Thus, biosensors have mammoth power & promise to identify the outbreak caused by virus and/or any disease.

Presently, a number of biosensing devices are used in the medical field not only for regular healthcare monitoring, clinical analysis, diagnosis of disease, etc. but also useful for treatment purposes [18]. These apparatuses are useful to expose a molecule in sensitive bioelements utilizing microorganisms, cell receptors, enzymes, antibodies and DNA/RNA [16]. Biosensors' design and fabrication have been especially symbolic and contributed towards exceptional developments in healthcare sector, leading to exploring new horizon of decisive and potential analytical sensors [38]. In present condition, nanomaterial-immobilized biosensors are used for the COVID-19 detection. Some of the significant applications of biosensors in health care are as:

(i) Tracking of Biological Flaws—Biosensors can solve various medical problems as it measures the indispensable symptoms of a subject and identifies the

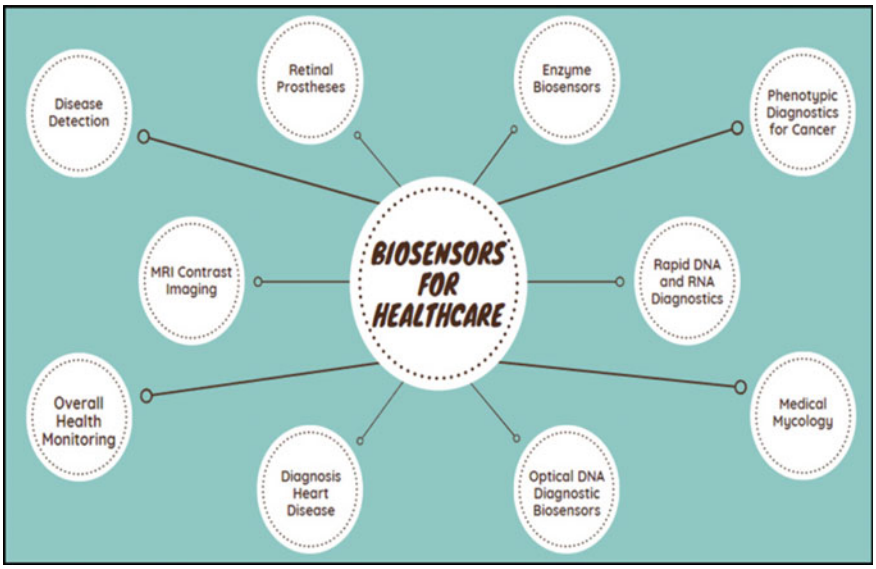

Fig. 3.20 Biosensors in healthcare services

biological abnormalities & irregularities in an efficient manner. Early intervention drive for detection and supervision of danger factors are very essential to reduce the medical costs and prevention of flaws as well. Effective time execution on diagnosis is promising as patient will recover their disorders by faster and effective treatment. With help of medical biosensor, physiological changes can be identified quickly which leads to proactive, earlier treatment. Recent technological advancements in monitoring systems using biosensors can be expanded at lesser cost than hospital remedy.

(ii) Heart Rate Monitoring—A sensor-based watch or band can repeatedly track the heart rhythm and other physiological information. Modern biosensors can identify the targeted biomarkers quite effectively and help the medical personnel to understand the disease clinically. A bioreceptor senses a biomarker's activity and generates associated electrochemical and optical signals. A transducer transforms this crude data to an electric signal which can relay biological data. These technologies are being utilized as an evolutionary algorithm to perform various essential assignments regarding healthcare applications.

(iii) Biochemistry Tracking—Implantable biosensor confined beneath the skin would allow tracking a patient body's chemistry uninterruptedly. The applications of such investigation technology can easily carry out for blood sugar tracking and exercise habits. With this revolution, medical professional is able to monitor the health of the patient from home.

(iv) Diet Inspection—Currently, public can examine the foodstuff with diet sensor, which can also monitor the supplied calorie [45]. Biosensors are being developed for expeditious detection of food pathogens, carcinogens, toxins and pesticides like contaminants [4]. Apart from their quick response, selectivity, easy function, and low cost make biosensors a potential tool for diet monitoring. Health care uses modern nanosensor to test an acetone molecule inpatient breath.

(v) Air Quality Inquiry—Implementing most recent sensor technologies, people can easily monitor air quality. Biosensing technology aids to track everyday atmosphere and measures the human body's core temperature to notify users and healthcare professionals about disease diagnosis. Wireless biosensors meticulously monitor internal body temperature, sleep pattern, sports physiology, clinical testing and hospital uses applications.

(vi) Sugar Level Measurement—For diabetic patient, glucose monitoring is performed by applying electrochemical test strips. Prior to design and development of biosensor in miniature form, blood collected from fingertip was used to determine glucose levels, but nowadays researchers are looking for biosensor-based wearables devices that will monitor blood glucose level continuously. Therefore, biosensors are suitable for some common monitoring applications such as glucose monitoring and diagnostics purpose like maternity and fertility testing. They are also convenient for lifestyle devices like cholesterol monitoring. In recent years, biosensors are also incorporated as medical instruments for cancer and genetic tests.

(vii) Authentic Results and Decision Authoring—These devices provide original information as well as instant and accurate data. Multimodal testing using biosensing instruments can be valuable assets in several medical applications as it has been investigated for decades in decision-making. These devices are used to measure, early diagnosis and treatment of diagnosed disease and future study in allied areas of health care. It facilitates the possible advantages of a multimodal approach to medical applications.

(viii) Heart Rate Tracking for Cardiac Patient—The potential applications of biosensor have increased extensively after screen-printed electrodes make the wearable biosensing devices portable and furnish maximum customized data. By this portable biosensor, heart rate of cardiac patients can be tracked rapidly. It also records the degree of tension levels for service people, legislation enforcement bureau, engineman and many more. Therefore, to continue persistent prosperity and advanced outcomes, these mechanisms can explore to check athletes' strength before and after physical exercise.

(ix) Patient Condition in Medical Unit—Biosensors embedded smart watch or arm band can support children, athletes, older people, and many others. In large context, the healthcare sector is responsible for the exponential development of biosensor technology that biosensors have been used for patient status surveillance in hospitals. These tools easily track the biological processes and provide insightful analytical data for both doctors and patients. Biosensors also have the advantage of making biological activity

such as the levels of blood oxygen minimally invasive. The requirement for biosensing process increases many folds due to various conditions including sleep apnoea, peripheral artery disorders and pulmonary obstruction.

(x) Disease Management—Healthcare unit relies on the identification and management of effective diseases through the applications of biosensors. Innumerable clinical disorders such as infection and cancer can be detected via biosensor technology. Biosensors are gain immense opportunity for medical applications with higher sensitivity and accuracy at a lower cost. Therefore, there is increasing requisition for better sensors. As a result, biosensors gain leading role in medical equipment, as well as very common in smart cities, IoT-based devices and wearable equipment. Wearable biosensor can analyse individuals' health features including heart rate, sleep habits, recognition of personality and many more.

(xi) Monitoring of Physiological Parameters—Medical patch on patient's body can investigate physiological variables like skin temperature and respiration rate. In recent years, home monitoring kits for biosensor producers have flourished all over world. These kits allow patients to track their health status every day without facing difficulties. Many critical illnesses such as cancer may be monitored at home. Patients would potentially make the device accessible in a high percentage of medical facilities when biosensors are easily available in hospitals. It can greatly enhance the patient experience because they continue to play a pivotal role in personalizing health care.

(xii) Tracking Cell Protein—The biosensor is highly effective to measure live cell proteins without labels in real time. A microfluidic system consists of a cell module and a biosensor module structures. It is positioned middle on a single zigzag channel where the architectural module of microfluidic cell provides the secreted proteins with a straight-forward tube network to nearby biosensor module. The dynamics of protein secretion can be pursued by invariably transcript the spectral shifting. The proposed microfluidic stage has explicit capacities in small footprint for multiplex and label-free disclosure that pledge to be miniature and integrated into lab-on-chip instruments.

(xiii) Symptom Detection in Patient—For quick identification of symptoms in patient, medical practitioners use biosensors rather than standard procedure. Biosensor manufacturers have given a special attention on enhancing it application in health sector to improve the quality of life. Hospitals and healthcare centres routinely used this to control blood pressure & sugar and cardiac rhythm in many distinct ways. These devices facilitate the physicians to execute patient diagnostic analysis in real time. A number of biosensors in miniature form can be tattered to collect patient data meticulously which makes it simple for medical set-up to grasp smart wearable technology without delay.

(xiv) Biomolecule Detection and Evaluation—Biosensors are fundamental tools of analytical process for biomolecular recognition and analysis. It allows the researcher to observe the levels of O_2 continuously in chips in actual time. This grants our body systems to reproduce the function of essential systems

more precisely. The main objective of sensor research and design of new drugs with small-scale biomolecules mimics the specific organ functions, including O_2 exchange into bloodstream from the air.

(xv) Postsurgical Treatment Monitoring—Biosensing devices also contribute the opportunity to strengthen follow-up treatment for postoperative surgery by using dissolved pressure sensors for brain and sensors which control implants infection & inflammation. Working principle and process for getting more systematic and logical processing, storing and exchanging this information with appropriate stakeholders are decisive to make this data a significant influence on patients' treatment. Successful application of biosensors in medical care has very imperative for growing public confidence during handling of patient information.

3.10 Conclusion

Detection and diagnosis of several human diseases at early phases of their growth grant successful treatment of the patients at the initial stage and are most essential. Therefore, it is indispensable to design and establish a cost-effective, elementary, highly responsive, user-friendly and competent diagnostic tools like biosensors that efficiently detect the diseases. Biosensors are analytical devices that are engaged in various medical operations such as disease monitoring, drug exploration, pollutant disclosure, identification of disease-creating microbes and disease-indicating markers in body fluids mainly in blood, urine, saliva, sweat, etc. Biosensing device is considered as highly decisive tool which grants people to regulate the target glucose levels in bloodstream and resolve green feedback to the disease. Due to its rapid diagnosis of diseases as well as accurate inspection of patient health, biosensors can help medical practitioners and patients in many ways like control sickness, clinical care, precautionary treatment, patient health information and disease scrutiny more accurately. Recently, wearable biosensors have gained considerable enthusiasm as they are highly capable of providing uninterrupted, real-time physiological information via dynamic, non-invasive measurements of biochemical markers in in sweat, tears, saliva and interstitial fluid. Present and future evolution have been focused on amalgamation of electrochemical and optical biosensors along with the advancement in the monitoring of biomarkers including metabolites, microbes, enzymes, DNA, hormones, etc. Thus, fusion of multiplexed biosensing, microfluidic sampling and transport systems must be interspersed, shortened and combined with flexible materials for improved wearability, better performance and simple operation process.

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Chapter 4
Graphene and Carbon Nanotubes (CNTs)-Based Biosensor for Life Sciences Applications

Apurba Das and Adil Wazeer

Abstract Growing interests to meet the demand for precise and trustworthy detection of disease have laid the research path for biosensors. Biosensors may be employed as analytical instruments to detect and quantify a variety of analytes, and they have a number of benefits over other methods. Sensors and biosensors, in contrast to standard lab tests, which can take hours or days to offer exact results to healthcare experts, can produce correct results in seconds, making them fascinating analytical instruments for medical applications. For surface modification or biorecognition element of the transducer, several materials are employed. CNTs and graphene are recently used as the appropriate materials for electrochemical, electronic, and optical sensors due to its excellent chemical, electrical, mechanical, optical, and structural characteristics. Because of the different function and combinations that may be utilised in various biosensor designs to allow for detection with high sensitivity and stability, two-dimensional graphene sheets have captivated the interest and creativity of physicists and chemists. Many research institutions across the world are working on graphene biosensors and biosensing approaches that are adapted to specific applications. CNT and graphene-based electrochemical biosensors especially optical biosensors and field-effect transistor biosensors have made substantial progress. Despite the fact that CNTs and graphene have made significant advancements, obstacles still persist, and the area field of biosensors remains a subject for further study in multidisciplinary platform combining biology, materials engineering, and electrical sciences.

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4.1 Introduction

Biosensor is very broad field with different form of sensing method that basically depends on the method of transduction or the type of bio-receptor used. A biosensor is a microbiological device that leads to biological activity via bio-receptor to electrical module, resulting in production of a computable signal with increased sensitivity for identifying particular chemical. A biosensor is described as sensing instrument which turns biological action into signal that can be measured. A specific biological component plus transducer which turns the biological data into calculable signal are often included in biosensor [1]. Leland C and C Lyon were the first to work on biosensors in 1962. Biosensors can detect antibodies, nucleic acids, enzymes, viruses, and bacteria, among other biological substances [2]. Food, environmental, and human samples are all tested with biosensors [3]. Specific biological components are frequently attached upon surface of higher bioactive transducers for aiming. Entrapment, adsorption, covalent binding, encapsulation, and cross-linking are some of the attachment mechanisms [4] which are used in biosensors. Following that, the relationship between both the detection component and objective is examined and further transformed into measurable current. Subject to the communication prototypes, several categories of transducers could be employed for transfiguring distinguishing actions into digital signal comparative to the occurrence and volume of objective. Optical, piezoelectric, magnetic, and electrochemical transducers are the most commonly used. Electrochemical, electrical, and optical approaches are among the most preferred because of their quick responses and versatility in using recognition elements [5]. Biosensors with superior selectiveness and sensitivity have seen lot of advancement in latest years. Rapid expansion of nanomaterials had significant impact on biosensor development. All technological apparatuses for biosensors, from identification parts up to signal processors, have been given nanomaterials' treatment. When the size of a material is lowered to the nanoscale, surface effect and quantum effect alter in chemical and physical characteristics. Nanomaterials' ratio of surface to volume improves considerably when associated to its mass form, allowing them to develop biosensor sensitivity by expanding interface for detection element distribution. Electrical and optical characteristics of nanomaterials are influenced by their size and form as band-gap energy increases with decreasing size. Dimension, contour, and chemical configuration of nanomaterial can be tailored for particular biosensor applications. Nanomaterials comprise a broad variety of materials with different qualities such as nature, size, shape, content, chemistry, and so on. CNTs in addition to graphene, for example, are extensively employed for biosensors and are at the top of the list [6, 7]. In the enormous family of nanomaterials, these two are much frequently utilised [8]. Owing to distinctive chemical and physical characteristics of CNTs as well as graphene, they are extensively being explored for biosensor applications. This chapter provides a complete review to researchers highlighting the summary of current breakthroughs in CNT- and graphene-based biosensors. The article also includes a brief summary of obstacles that such biosensors experience in terms of practical implementation.

4.2 Graphene-Based Biosensors

When aligned correctly into a honeycomb pattern, graphene, which is defined as a sp^2 hybridised carbon, generates a graphitic 2D monolayer structure akin to polycyclic aromatic hydrocarbons with quasi-limitless dimension [9]. Despite the fact that graphene had been vaguely acknowledged for several decades, Geim and Novoselov were the first to create graphene by continuously peeling graphite utilising regular scotch tape in the year 2004 [10]. Mechanical exfoliation approach produces pure monolayer to few-layer graphene (Fig. 4.1). This mechanically exfoliated graphene was used to demonstrate the remarkable properties of graphene [10]. Although this process produces superior-class graphene, it includes drawbacks comprising high-scale production and inadequate transference procedures that limit its application in cost-effective device manufacture.

Another type of process, prevalent in nineteenth century, is called chemical exfoliation. This method is built upon graphite exfoliation utilising strong acids plus oxidisers. Some examples of the chemical exfoliation techniques are Brodie, Hummer, and Staudenmaier methods [14]. Hummer's technique is regarded as among utmost frequently employed approaches. Such technique does not yield pristine graphene, but produces sheets of graphene oxide (GO). These oxygenated functional groups produce imperfections in planes of sp^2 graphene, making it less electrically conductive. However, the restoration of conductivity could be done to larger level by following reduction techniques (thermal, electrochemical, or chemical) to generate reduced graphene oxide (RGO). Economical, simple processing, aqueous solubility, and existence of reactive edges because of oxygen functional group richness mark GO a standard material for development of electrochemical biosensors.

Variety of routes are present regarding the production of graphene as well as its derivatives, namely rGO, GO, GNRs, and porous-reduced graphene oxide (prGO). Single or few-layer nanosheets of graphene can be produced from nickel or copper through chemical vapour deposition (CVD) technique. These graphene sheets could be delivered to any transducer interface on a regular basis using mostly polydimethylsiloxane-supported transfer methods [15]. These electrodes are useful for G-FETs and plasmonic biosensing applications due to their enhanced CVD graphene quality and opportunity for producing mono- as well as bilayer improved electrical interfaces. The most extensively utilised synthetic technology for the creation of G-biosensors is chemically manufactured GO as well as rGO nanosheets, which are synthesised using graphite precursors by solution-based exfoliation targeted to minimise van der Waals contacts amid sheets of graphene. It is a reasonably low-cost technology for mass scale generation of GO/rGO with porosity and shape variations in nanosheets. Additionally, doping along non-metallic elements like sulphur and boron modifies the electronic structure of such materials, resulting in improved electro-catalytic and electrical properties. Improved dispersible structures of size approximately 3–20 nm with less than five layers result from the GO-flake size reduction [16]. Graphene quantum dots, which have a larger surface area, are the

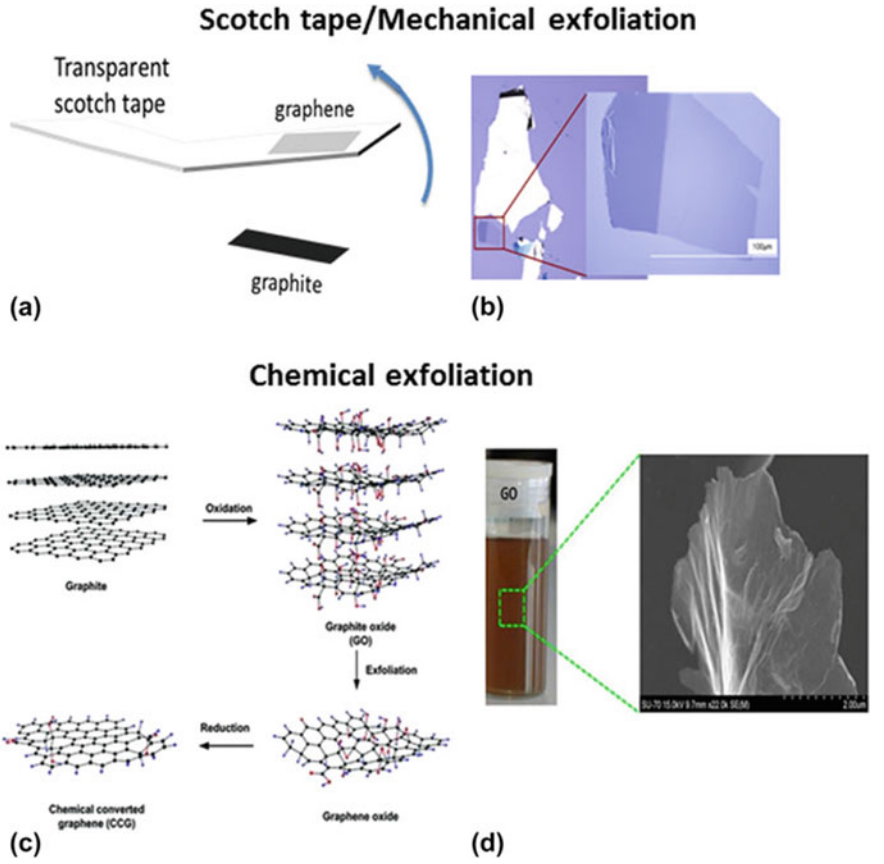

Fig. 4.1 Graphene by mechanical exfoliation **a** scotch tape method and **b** optical image of SiO_2/Si monolayer graphene [11]. Graphene by chemical exfoliation method **c** Hummer's method of graphene exfoliation from graphite [12] and **d** SEM image of graphene oxide [13]

name for these structures (GQDs). Some of the methods for coating inert and electrical surfaces having chemically obtained graphene elements include spin-coating, drop-casting, and electrostatic contact among positively charged interfaces as well as negatively charged nanosheets of GO/rGO, electrophoretic deposition (EPD), and electrochemical reduction of GO.

4.2.1 DNA Biosensors Based on Graphene

DNA adsorption via detecting DNA hybridisation practices [17, 18] plus fluorescence-quenching features of graphene in addition to GO arose as unique stage for producing DNA-based biosensors. Electrochemical G-based DNA sensors

propose higher sensitivity, higher selectivity, and quick and economical study for sensing biomolecules that are substantial in clinical treatment and diagnosis. The majority of DNA detection research has engrossed upon sequence-specific identification as well as mutation of single-stranded DNA (ssDNA) [19, 20]. Double-stranded DNA (ds-DNA) is critical considering both direct imaging of genetic evidence for living cells and progress of cell-based expertise [21]. There exist two varieties of electrochemical DNA biosensors, namely label-free (grounded upon nucleic acid target's intrinsic electrochemical characteristics) and labelled (using redox-active species involving ds-DNA) [22]. Due to the characteristics of graphene, several studies have discovered that G-based DNA biosensors have greater selectivity and sensitivity (limits of detection of 8 nM, 10 fM, and 1 pM, respectively) [23–25].

Tian et al. [26] used methylene blue and functionalised graphene (FG) to develop a technology for sequence-specific DNA sensing (MB). When FG was introduced for aqueous analytes and MB is utilised as electrochemically active DNA intercalator, they observed that DNA may be identified having 48.15% sensitivity that is significantly higher from the sensitivity obtained without FG. The findings show that FG was significant in boosting DNA recognition sensitivity via mingling MB solution close to electrodes. Layer-by-layer construction of DNA biosensor combining graphene having polyaniline nanowires (PANIw) for increased DNA sensing sensitivity was disclosed by Bo et al. [27]. During analytical operation of the DNA sensor, GN/PANIw displayed effective differential pulse voltammetry (DPV) current reactions for appropriate DNA sequences utilising immobilised probe to hybridise along varying quantities of target DNA. Kakatkar et al. [28] used CVD Graphene FET biosensors to create another DNA sensing application. In the light of this statement, DNA-immobilised GO electrode is improved as an electrochemical biosensor in order to measure hydroquinone. Tian et al. [26] used functionalised graphene (FG) and methylene blue to create an approach regarding sequence-specific DNA detection (MB). It was observed that addition of FG to aqueous analytes and using MB as electrochemically active DNA intercalator allowed them to detect DNA with a sensitivity of 48.15%, which was much greater than the sensitivity achieved without FG. The experiments show that by mixing MB solution with electrodes in close proximity, FG significantly increased the sensitivity of DNA detection. The layer-by-layer construction of DNA biosensor combining graphene with polyaniline nanowires (PANIw) for increased DNA sensing sensitivity was disclosed by Bo et al. [27]. Throughout analytical operation of DNA sensor, GN/PANIw displayed effective differential pulse voltammetry (DPV) current responses for matching DNA sequences employing the immobilised probe for hybridising with varying quantities of target DNA. Kakatkar et al. [28] used CVD Graphene FET biosensors to create another DNA sensing application.

4.2.2 Graphene-Based Bacteria Detection Biosensors

Nanostructured materials have previously offered a plethora of biosensing possibilities due to their exceptional physical, optical, and electrical properties. Nanowires [29, 30], carbon nanotubes [31, 32], and most recently graphene [33] have all been used to create distinct nanoelectronic biosensors. Recently, field-effect transistors (FETs) were constructed from single-walled carbon nanotubes (SWCNT) [34] for detecting occurrence of bacteria. SWCNT-based FET network having sensitivity of 100 cfu/mL was developed by Vallamizar et al. [35] for detecting S. Infantis.

Authors verify graphene biosensor for electrically detecting *E. coli* bacteria having improved specificity and sensitivity in research [36]. The large-scale graphene sheet was created using the chemical vapour deposition approach and functionalised with Anti-*E. coli* antibodies and a passive layer. When exposed to *E. coli* bacteria at modest concentration of 10 cfu/mL, a substantial increase in conductance is observed, despite the fact that greater concentrations of another bacterial strain showed no significant effect. This biosensor was also utilised to monitor the attached *E. coli* bacteria's glucose-induced metabolic activity in real time. Theoretically, these kind of simple, rapid, label-free, and sensitive nanoelectronic biosensor may be used to detecting any harmful bacteria, as well as for functional research and antibacterial drug screening.

4.2.3 Graphene-Based Glucose Biosensors

Glucose is a stimulating and important chemical to keep track of. Hyperglycaemia, or diabetes, causes early death owing to microvascular and macrovascular issues; therefore, increment in levels of glucose is detrimental to human health. Close monitoring of blood glucose levels might fundamentally assist in diabetes management. There has been a lot of work put into developing dependable and efficient glucose sensing methods. Sensors made from graphene-based glucose oxidase (GO_x) where glucose is used on surface of graphene, similar to graphene–FET, were projected by Huang and team [37].

Shan and co-researchers [38] used mainly graphene-based glucose biosensor having liquid nanocomposites where glucose response (2–14 mM, $R = 0.994$) was higher. It also provided a good reproducibility with standard deviation 3.2% and greater stability. Kang and team [39] examined ability of chitosan to disperse graphene plus develop glucose biosensors showing appropriate sensitivity. Chitosan appeared to help generate well-dispersed graphene dispersion and immobilise biomolecules, resulting in a biosensor with higher sensitivity (37.93 A mM1 cm^2) and longer stability for measuring glucose. Qiu [40] developed Pt/PANI/G-based glucose and H_2O_2 biosensor (hydrogen peroxide). H_2O_2 revealing limit of the Pt/PANI/G-based sensor was about 50 nM. Pt/PANI/G improved electrode stated glucose recognition limit of roughly 0.18 M after GO immobilisation. Although GO_x allows for more specific glucose detection, non-enzymatic glucose sensors built on inclusion

of electro-catalytic sites for glucose in nanoparticle form were developed by many researchers [41, 42]. While such sensors are less sensitive to glucose and must be used in an alkaline medium for the most part, they have exhibited a number of advantages, including improved stability, detection limits, and range compared to GO_x-based sensors.

4.2.4 Graphene-Based Cholesterol Biosensors

Increased cholesterol levels of arteries might lead towards severe health troubles like cerebral thrombosis, coronary heart disease, and atherosclerosis [43]. Consequently, it is clinically important for computing the cholesterol levels in arteries. Cao et al. [44] used high sensitive platinum–palladium–CS–graphene hybrid (PtPd-CS-G) nanocomposites functionalised GCE to study an electrochemical biosensor for detecting cholesterol. PtPd-CS-G nanocomposite not only induced direct electron transfer to electrode surface via a redox enzyme, but it also increased the amount of cholesterol oxidase immobilised (ChOx). This biosensor demonstrated broad linear responses to cholesterol in concentration arrays of roughly 2.2 106–5.2 104 M/L in optimal conditions. While 0.75 M/L (S/N = 3) was calculated as the detection limit. The reaction time was below 7 s, and Michaelis–Menten constant was 0.11 mM/L. Furthermore, the biosensor developed displayed great levels of efficiency, stability, and repeatability.

Dey and Raj [45] created complex amperometric biosensor built upon hybrid material consisting of Pt nanoparticles and graphene to detect H_2O_2. To make the cholesterol biosensor, cholesterol oxidase and cholesterol esterase were immobilised on GR/PtNP hybrid material surface. The sensitivity and limit of detection were enhanced owing to synergistic effect of PtNP as well as graphene (Fig. 4.2).

4.2.5 Graphene-Based Haemoglobin Biosensors

Haemoglobin is the main component of blood and also accountable for conveying oxygen through the body. Changes in Hb concentration levels in the blood might lead to a variety of illnesses and even death. As a result, pathologically, exact assessment of Hb level is critical. Hb levels in men range from 13.0 to 18.0 g/dL, while in female range from 12.0 to 16.0 g/dL [46], and Hb total beneath these ranges results in anaemia. Noting that anaemia affects over 2 billion humans worldwide, the majority of whom are children and women. As a result, a clinically relevant element is the computerised assessment of Hb component in the blood [47].

For haemoglobin electroanalysis, the Xuand group [48] developed a chitosan-graphene (CS-GN)-modified electrode. On cyclic voltammogram (CV), Hb displayed a clear redox peak at the CS-GN/GCE, which was comparable to the CS-GCE. The Hb current reaction improved linearly from 30 to 150 mV/s at the

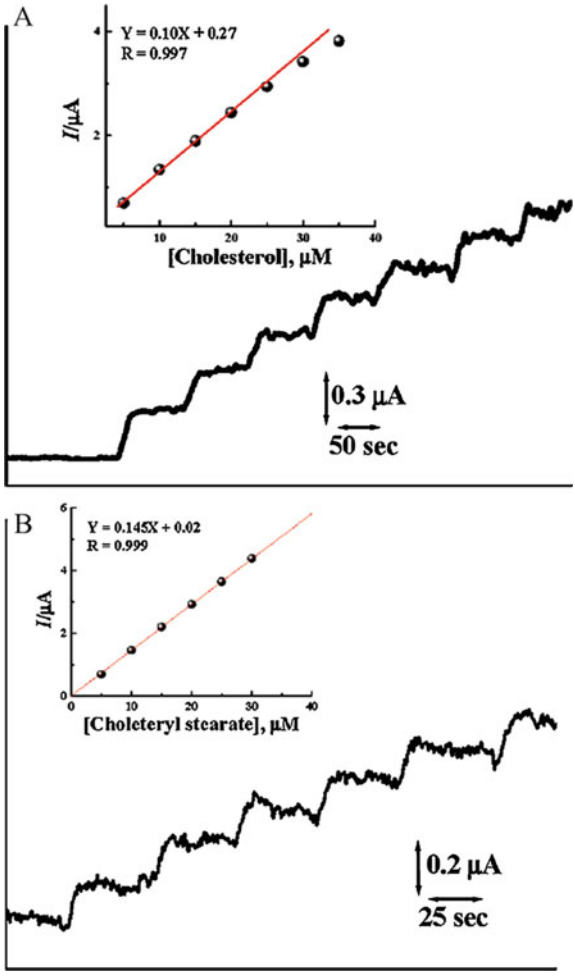

Fig. 4.2 Biosensors for cholesterol (**a**) and cholesterol ester (**b**) amperometric responses [45]

CS-GN/GCE, suggesting a surface-controlled electrochemical pathway. Xu and team [49] employed a CS–GR-modified electrode regarding Hb electroanalysis. In comparison with CS/GC electrode, cyclic Hb voltammogram at CS–GR/GC electrode showed a distinct redox peak. The Hb current response at CS–GR/GC electrode rose linearly from 30 to 150 mV/s, showing a surface-controlled electrochemical route as depicted in Fig. 4.3.

Sun and colleagues [50] developed a new electrochemical biosensor that uses 3D graphene as substrate electrode and Hb immobilisation on electrode surface using chitosan layer. Such electrochemical approach revealed the appearance of a unique redox peak pair on CV, demonstrating detection of Hb direct electron transfer. Grounded on its increased conductivity as well as larger surface area, electron-transfer coefficient and apparently heterogeneous electron-transfer rate constant

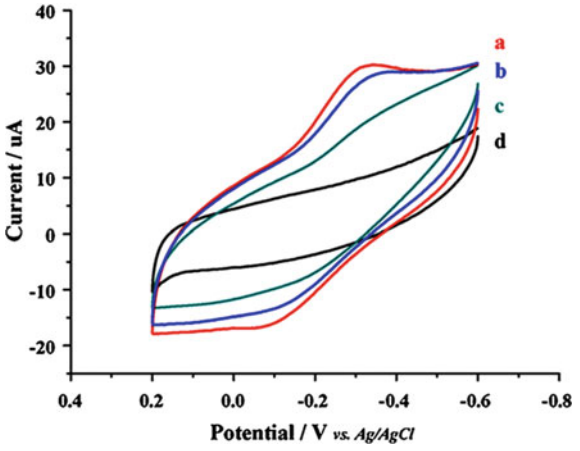

Fig. 4.3 Cyclic voltammograms of CS–GR-Hb/GC (**a**), GR-Hb/GC (**b**), CS-Hb/GC (**c**), and CS–GR/GC (**d**) in PBS (pH 7.0). Scan rate: 100 mV s^{-1} [49]

(ks) of 3D graphene were determined around 0.426 and 1.864 s1, correspondingly. Customised electrode demonstrated effective electro-catalytic activity for reducing trichloroacetic acid (TCA). Simultaneously, a linear response to TCA concentrations ranging from 0.4 to 26.0 mM/L was detected having limit of 0.133 mM/L (3σ).

4.2.6 Graphene-Based Biosensors for Protein Biomarkers

Protein biomarkers are explicit molecules found in tissues/blood that may be used to diagnose, monitor, and forecast cancer and other diseases based on their amount or determination. The capacity to compute femto- to picomolar concentrations is required for biomarkers to be clinically helpful for identifying illness, and it is also necessary to distinguish biological activities and seek for novel protein biomarkers. The LODs of the most common analytical processes for biomarker identification (e.g. enzyme-linked immunosorbent assays) fall short of the necessities for clinical use and study. The sensitivity of graphene-based immunoassay systems, in which definite antibodies are immobilised on graphene for specifically retaining biomarker analyte, has been demonstrated [51]. Kim et al. [52] quantified fM PSA levels having dynamic extent of nearly six orders of magnitude using a rGO-based FET customised having prostate-specific antigena1-antichymotrypsin (PSA-ACT). Upon graphene-coated Au chips coated involving pyrenenitrilotriacetic acid (NTA) having LOD of 5 pg/ml, Singh and colleagues [53] employed an SPR-based read-out to detect cholera toxin.

4.3 CNTs-Based Biosensors

CNTs are seamless hollow tubes made by rolling sheets of graphite. Conditional to quantity of graphite layers, mainly two kinds of CNTs were used, namely single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [54, 55]. SWCNT is one-molecule nanomaterial composed from a single layer that folds a single graphite sheet called graphene. It has a diameter distribution of 0.75–3 nm plus length distribution of l–50 m, respectively. Although the MWCNT is built up of many layers of coiled graphite sheets having widths variable from 2–30 nm up to a few hundred nm [56], the interlayer gap is just 0.42 nm.

Due to its better strength and important physical/chemical features, and exclusive tubular nanostructure with considerably higher length to diameter ratio (132 million:1) [57], CNTs are usually used for biosensor applications. CNTs also have superior conductivity, sensitivity, biocompatibility, and chemical stability than other materials [58]. Furthermore, CNT sidewalls and ends might be enhanced by connecting practically any chemical species. Because of their high sensitivity, carbon nanotubes might be outstanding transducers in nanoscale sensors. CNTs have the potential to boost the electrochemical reactivity of significant biomolecules and accelerate electron-transfer kinetics involving extensive variety of electroactive class [59].

CNTs are an attractive nanomaterial regarding manufacturing electrochemical biosensors fluctuating between amperometric enzyme biosensors to pathogenic microbe detectors due to their superior electrochemical reactivity and facile detection of biomolecules. The combination of these properties, as well as the use of the appropriate cross-linking agents for receptor enzymes and antibodies on electrodes, results in biosensors for analytes that are fast, sensitive, dependable, cost-effective, and reusable. Another notable feature of carbon nanostructures is its distinct photothermal activity. Photothermal treatment is employed for trimming down dimension of tumours and removing them. SWCNTs used near-infrared (NIR) laser irradiation for producing heat, which was then used to treat cancer cells all around the world [60, 61].

The use of carbon nanotubes in biosensors is gaining, and research activity related to this is increasing. As a result, the need for carbon nanotubes in industry has become increasingly significant. CNTs have been produced using a variety of techniques, including laser irradiation, arc discharge method, and chemical vapour deposition (CVD) [62].

4.3.1 Immobilisation of Enzymes for Biosensors

Enzyme immobilisation is procedure for confining or localising the enzyme in specific spot while retaining its catalytic activity, allowing it to be used again and consistently [63]. A good biosensor requires enough enzyme loading and optimal

activity. It is important to handle enzymes delicately since they can lose their function if they are subjected to harsh chemical treatments or exposed to high temperatures. The immobilisation of enzymes upon surface of carbon nanotubes (CNTs) offers framework for the creation of biosensors that leverage the unique optical and electrical features of CNTs for signal transmission.

The most excellent method for producing an enzyme biosensor is to design an arrangement that allows electrons to be transported directly from the enzyme redox centre to the fundamental electrode. Use of covalent immobilisation or physical adsorption on the surface of immobilised CNTs might indicate a well-organised method for proving direct electrical connectivity amid electrodes and active site of redox-active enzymes. CNTs' electro-catalytic properties are predicted to be used in the progress of enzyme biosensors that do not require mediators in the future.

The biological activity of an enzyme immobilised on carbon nanotubes may be enhanced, as well as the durability and stability of the enzyme. As a result, immobilisation of enzyme is critical step in development of CNT-based enzyme biosensors. Physical activity adsorption, cross-linking, embedding, and covalence are only a few of the immobilised approaches [64]. Immobilisation methods are frequently distributed into three groups: (i) physical approaches, which employ weak contacts; (ii) chemical methods, which use stronger connections; (ii) chemical techniques, in which enzymes and support matrix form covalent connections; and (iii) entrapment, in which enzyme molecules were imprisoned [65]. The following are several ways for enzyme immobilisation on CNT platforms [66].

4.3.1.1 Physical Adsorption

Adsorption is the process of attaching a molecular probe to a CNT platform via van der Waals, hydrogen bonds, hydrophilic, hydrophobic, or ionic contacts. The simplest, cheapest, and least time-consuming method for producing biocatalytic systems is immobilisation by adsorption. Furthermore, enzyme activity is not harmed since adsorption immobilisation uses weak links between supports and enzymes, and the enzyme is not chemically impacted. This approach has a number of advantages, but it also has a number of drawbacks. When compared to other techniques, the danger of enzyme leakage through the support is higher since enzyme-support exchanges are comparably weaker. CNT-modified electrode is made via casting/evaporating CNT dispersion over GCE (glassy carbon electrode) and further releasing a Nafion-involving solution of selected enzyme on top of the electrode and then allowing it to vaporise. A tyrosinase-based amperometric sensor to detect phenolic chemicals has been developed in this way [67]. It is feasible to limit enzyme leaching and increase biosensor durability by repeatedly placing a Nafion layer over the electrode [68]. The layer-by-layer approach [69], which includes putting alternative layers of opposite charges polyelectrolyte and enzyme upon electrode, is another way for enzyme adsorption.

4.3.1.2 Covalent Bonding

While noncovalent ways to building CNT-based electrochemical biosensors contain certain advantages, such as easier manufacturing and lower costs, poorer stability and enzyme leaching can often have an important influence on performance of biosensors. Countless efforts were made to covalently bind enzymes to the surface of electrodes using a variety of methods and chemicals to overcome this limitation. The most difficult sort of connection between enzyme and carrier is formed when enzyme is linked to a backing through chemical process or upon activated or monomer-grafted surfaces. A bond forms among amino acid residues in enzyme backbone and functional group that was initially existing in support matrix or moulded through change in such procedure. Most frequent reactive groups upon side chains of amino acids in enzymes are amines of lysine or arginine, carboxyl of aspartic or glutamic acid or threonine, hydroxyl of serine, sulfydryl, and terminal amino and carboxyl groups of polypeptide chains. Because covalent bonding is often supported by chemicals and at various pH levels, the process is likely to experience a decline in enzyme activity. Figure 4.4 shows glucose oxidase covalently attached to CNT nanoelectrodes using carbodiimide chemistry to form amide connections among its amine residues and the carboxylic acid clusters on CNT tips [70]. In a few glucose biosensors, covalent bonding of Gox to a SWCNT enhanced Pt electrode and Au substrate has been observed [71, 72].

4.3.1.3 Entrapment

In the entrapment technique, enzymes were combined along monomer solution and further polymerised for producing a gel. Enzyme molecules were trapped in gel pores throughout the operation. Meanwhile, no chemicals are used to produce the trapping, and enzyme function is not disrupted. It is an easy approach showing the potential to increase protein loading. Furthermore, due of the increased surface area and enhanced electrical connection among redox centres of hydrogel or sol–gel derived matrix and electrode, these sensors have improved sensor response. Polyacrylamide, nylon, starch gels, silastic gels, and conducting polymers are among the most often used gels. To create sensitive biosensors, a wide range of enzymes were efficiently immobilised on CNT-incorporated redox hydrogels [73]. In contrast, a glucose sensor was created by capturing GOx plus MWCNTs in appropriate amounts in Nafion matrix [74].

4.3.1.4 Electropolymerisation

Enzyme is coupled along monomer that is electropolymerised at GCE or metal electrode and then implanted into the polymer matrix. Electrostatic interactions commonly drive enzyme incorporation into the matrix, as evidenced by multiple occurrences [75, 76]. The ease of one-step arrangement, removal of electroactive and surface-active interferences, monitoring of film wideness, and localisation of

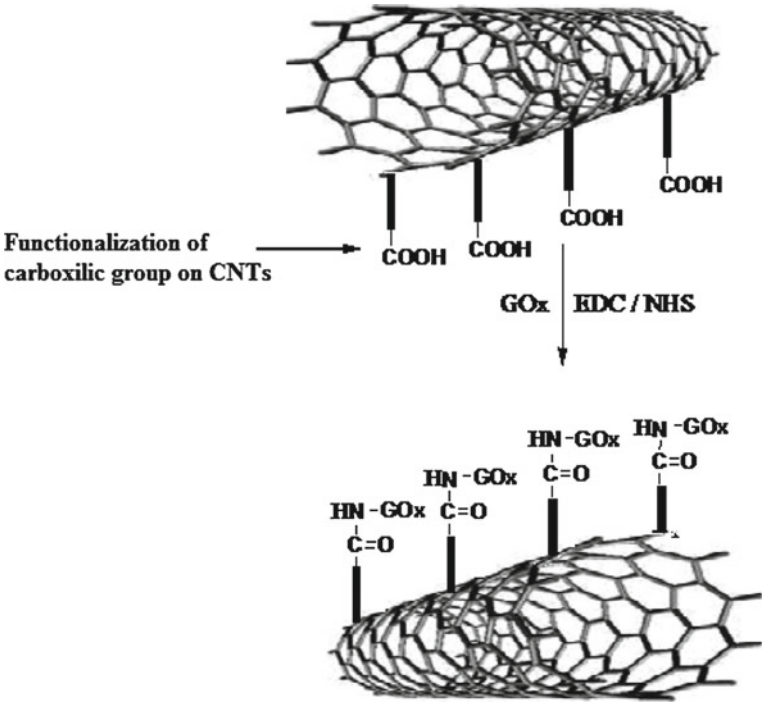

Fig. 4.4 Carbodiimide covalently immobilised glucose oxidase on CNT nanoelectrodes [70]

biocatalysts on minute electrode surfaces are all important advantages of this immobilisation technology. In several studies, conductive polypyrrole (PPy) was employed as polymer matrix. The preference arises that pyrrole could be electropolymerised in aqueous solutions at neutral pH with reduced oxidation potentials, making it appropriate for extensive spectrum of biological molecules. Polypyrrole had been recognised to be beneficial in linking enzymes and carbon nanotubes as a primary electrode.

4.3.1.5 Cross-Linking

Typically, bio-material is chemically attached for solid support to a substance such as a cross-linking agent in this cross-linking procedure to significantly increase the attachment. It is a good idea to keep the adsorbed biomaterials stable. It usually comprises residues that are not involved in catalysis, such as the amino group of lysine residues [77]. Glutaraldehyde, adipoyldichloride, and epichlorohydrin are the cross-linking chemicals used to achieve the goal. Despite this, glutaraldehyde can respond to a variety of functional protein groups, including amine, phenol, imidazole, and thiol [78]. Enzyme cross-linking is rapid and simple and has a wide range of

applications. Because a lesser fraction of enzyme molecules occasionally acts as a support, there is a potential that the enzyme's activity will be reduced. The drugs may also inhibit enzyme action, specifically at superior dosages.

4.3.2 Practical Concerns of CNT-Based Biosensor

While CNT-based biosensors are extensively employed owing to its excellent functioning, they have a number of practical problems. The size and helicity of CNTs are often required for biosensor fabrication [79]. However, choosing the helicity and size of CNTs is a difficult task since controlling the size of CNTs throughout the growth process is difficult. Furthermore, with current technology, creating a profitable mass manufacturing of CNTs and achieving better purity is difficult. Purity is critical for CNT-based biosensors; just a few experiments are required to achieve high purity (99.99%), which is challenging to achieve. As a result, the existing market price of CNTs for some real-time marketable usage is quite costly.

Considering a CNT-based biosensor, enzyme is continually trying to immobilise itself on the surface of the CNTs. Immobilisation, on the other hand, will almost certainly damage their biological activity, structural stability, and biocompatibility. As a result, its prospective influence upon sensitivity plus selectivity of CNT-based biosensors has to be deliberated. Simultaneously, their cytotoxicity [80] must be investigated, leading to biological species for CNT incorporation in biological tissues and cells. As a result, regularisation [81] of organisational and surface properties of CNTs, as well as typical parameter for cytotoxicity calculation, is critical. Currently, the manufacturing of sensors and applications is still in research phase; nevertheless, if CNT-based biosensors are promoted, the aforementioned challenges must be overcome.

4.4 Challenges and Future Perspectives

Various methods for fabricating innovative biosensors are followed where graphene and CNTs are used as sensing materials have been identified. However, research activity in this field is not completed and there are plenty of scopes available for improvement. CVD-synthesised graphene with a higher surface area might be employed for perceiving small sized biomolecules (DNA), gaseous components, and heavy metal ions. Novel biosensors might be developed using heteroatom-doped graphene. The electrochemical function of IL in conjunction with graphene might potentially be investigated. Graphene/conducting polymer nanocomposites and graphene/carbon paste electrodes have received less attention than CNTs.

CNTs are considered as most attractive areas of current materials' research, and their growth in biomolecule sensing is critical for biomedical and bioengineering

applications. The increased cost of CNT materials and CNT-based enzymatic biosensors had produced serious challenges that have severely limited their use. Dropping these prices is a difficult challenge that would nearly positively necessitate use of material investigation and nano-engineering. A non-enzymatic sensor based upon CNTs is viable solution that had piqued the interest of a few research organisations. Soft lithography and nanoimprint lithography are two new nanotechnologies that are valuable.

The description of such innovative materials at molecular state is vital and fundamental scientific test as new nanomaterials including CNTs continues to advance. In addition to research, molecular design must be created as instrument for calculating functioning of novel materials regarding certain biomolecule. These computer approaches would allow for rapid examination of novel materials. Finally, thorough knowledge of structure–function relations would be useful in guiding as well as monitoring exploratory attempts in the direction of the utmost auspicious biosensing configurations.

4.5 Conclusions

Graphene has a significantly shorter history than carbon nanotubes. With the recent advancement of graphene in biomedical and biological fields, the question of which is better for making biosensors has arisen. CNTs and graphene possess a number of mutual traits, comprising exceptional mechanical, thermal, and electrical characteristics. Graphene could be mass-generated at lower price and will not include the metallic contamination that is common in carbon nanotubes. Graphene is far more suitable for large-scale designs than one-dimensional CNTs with a larger aspect ratio. Because of its defect-rich nature, graphene may chemically be functionalised for forming functional groups in 2D plane with ease. Such characteristics provide graphene a lot of potential in therapeutic and biological imaging applications, as well as a lot of sensitivity. Graphene absorbs light from the UV to the NIR ranges and has good fluorescence-quenching properties. Lot of focuses are being given on carbon nanomaterials like graphene and carbon nanotubes for biosensor applications. Researchers have confirmed that graphene and carbon nanotubes might provide fresh detection techniques while also increasing the detection limit over present protocols. CNT- or graphene-based sensors have a fast reaction time, are inexpensive, and are compact in size. Graphene and carbon nanotube (CNT) biosensors, however, have several hurdles.

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Chapter 5
An Overview of Integrated Miniaturized/Microfluidic Electrochemical Biosensor Platforms for Health Care Applications

Khairunnisa Amreen, Koushik Guha, and Sanket Goel

Abstract The increasing demand of biosensing in the health care sector is meticulously associated with the need of fabricating diagnostic tools with an instant, point-of-care (POC) approach. These devices are expected to have high sensitivity, portability and selectivity. Off lately, an extensive focus on designing biosensors with an imperative technique has been accomplished to ensemble the materials, equipment and methodologies to improve their performance. Basically, biosensor is an analytical sensor which has a biological moiety, like enzymes, antibodies, live cells, etc., as an electron transfer mediator to detect analytes via a suitable detection mechanism including electro-catalytic activity. These sensors tend to produce electrical current signals whose intensity is dependent on the concentration of the selective analyte. Since, 1999, when IUPAC designated biosensor as a sovereign tool for selective qualitative and quantitative analyte detection, several chemically modified biosensors have been reported. However, most of these are bulk electrodes that are laboratory-based, use large sample volumes, lack POC approach and therefore cannot be employed for real time field sensing. To overcome this, significant research has been carried out to miniaturize and integrate microfluidic concept with these devices. The advent of microfluidics not only makes these biosensors suitable for real-time practical application but also makes them cost-effective, portable and more sensitive. During the last few years, several research groups globally have successfully developed miniaturized/microfluidic biosensor-integrated electrochemical platforms for health care applications. The present chapter briefly describes the fabrication, characterization, materials used and types of these biosensor devices and summarizes some of the recent advance applications in health management. The future prospects and present limitations are also highlighted.

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5.1 Introduction

Over the last few years, researchers are copiously working on the design and development of biosensors. Diverse important technical advances have been accomplished so far to ensemble the materials, equipment and methodologies to fabricate biosensors with improved performance [1]. Since 1999 by the IUPAC, biosensor has been regarded as a tool for selective, sensitive qualitative and quantitative estimation of analytes [2]. Primarily, biosensor is an analytical sensor wherein a biological component or moiety is used as a redox mediator (immobilized over the matrix or electrode substrate) that can detect biological, biochemical and chemical analytes by a suitable detection scheme [3]. During electroanalytical detection, these devices generate electrical signals which are directly proportional to the concentration of the chosen analyte. These biosensors have transducers incorporated in them which converts the biological signal obtained due to electro-catalytic reaction mechanism into a current throughput. Clark et al., in 1962, prepared an oxygen electrode for monitoring oxygen during cardiovascular surgery [4]. Since then, massive progress in this regard, especially for enhancing the sensitivity and selectivity, has been made. Today, biosensors portray significant role in applications in the diversified fields of biochemical, medical, environmental, pharmaceutical, clinical and industrial research. A typical biosensor is comprised of three main components: (a) a biological component or bioreceptor, (b) transducer and (c) signal processor (Fig. 5.1a). Figure 5.1b shows the schematic of the working mechanism of a biosensor.

Biological components: Electroactive bio-components/like enzymes, antibodies, live cells, protein, DNA, etc., that can shuttle electrons between the electrode/electrolyte interfaces are used as redox mediators. These bio-mediators are immobilized over matrices of choice, like carbon nanomaterials, nanorods, nanosheets, polymers, composites, etc., either via trapping onto the matrix or integration inside the matrix [5].

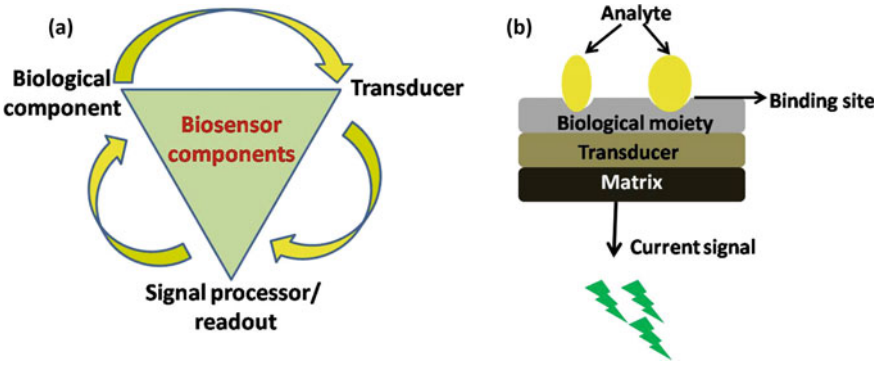

Fig. 5.1 **a** Schematic for components of an ideal biosensor. **b** Schematic for working mechanism of a biosensor

Transducer: When the biological redox mediators interact with analytes, an electrochemical reaction (oxidation or reduction) occurs along with alterations in the oxidation states of analytes. Further, changes in the parameters, like pH, heat or temperature, at the electrode/electrolyte interface take place leading to chemical changes. The transducer traps these reaction signals and converts them to a measurable electrical response. Since the bio-mediators are highly selective, these reactions with analytes occur only at the specific binding sites. There are three ways of attachment of bioreceptor and transducer. (a) First generation: biological entity is trapped or attached onto the membrane fixed to transducer, (b) second generation: biological entity attached via covalent bond to the transducer and (c) third generation: biological entity attached directly with the transducer [5].

Signal processor: The signal processor amplifies and converts the received current signals into a quantifiable digital display that can be understood in the readout device [5].

5.1.1 Types of Biosensors

Based upon the type of biological redox mediator and transducer, biosensors are of different types. Figure 5.2 is the diagrammatic representation of various types of biosensors.

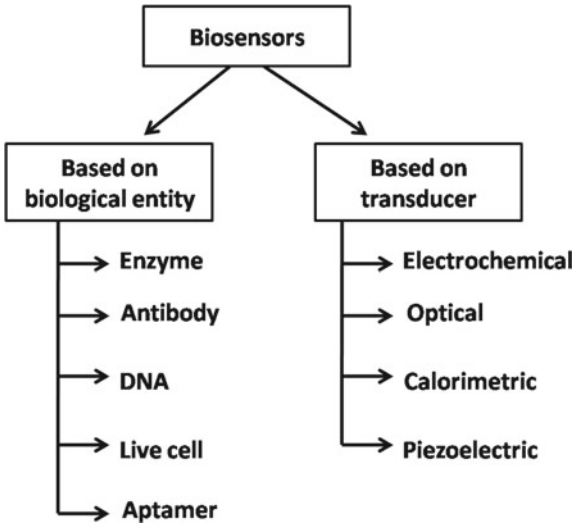

Fig. 5.2 Diagrammatic representation of various types of biosensors

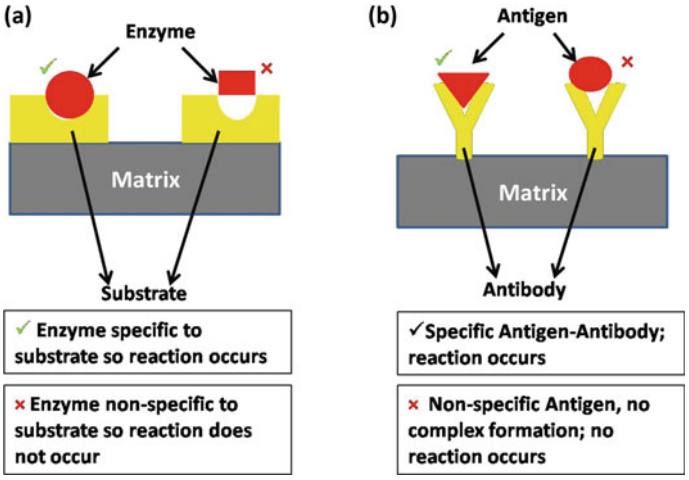

Fig. 5.3 Schematic representation of **a** enzyme-based biosensor and **b** antibody-based biosensor

5.1.1.1 Classification Based on Biological Component

Enzyme-Based Biosensor

Herein, the biosensor has an enzyme as a bio-component over the matrix that can detect the specific analyte substrate compatible with the enzyme. This obeys the lock and key model wherein only specified analyte can bind with the active site of enzyme and the electrochemical reaction can take place. Figure 5.3a shows the schematic representation of the mechanism of enzyme-based biosensor [6]. These reactions typically are dependent on various parameters, like substrate concentration, pH of the electrolyte, temperature, etc., as enzymes loses their activities if these are not controlled. Several enzyme-based biosensors, for detection of disease biomarkers as an indication of ailment, have been developed both in bulk and microfluidic way.

Immunosensor or Antibody-Based Biosensor

These types of biosensors are excellent candidates for pathogen and infection detection. Herein, a specified, usually monoclonal antibody (Immunoglobin-Ig), which is target antigen specific is immobilized over the underlying matrix as a bioreceptor. The Ig has two heavy and two light chains with a variable portion that selectively binds with the antigen to give antigen–antibody complex which gives electron transfer-based detection. Figure 5.3b gives the schematic representation of an immunosensor [7].

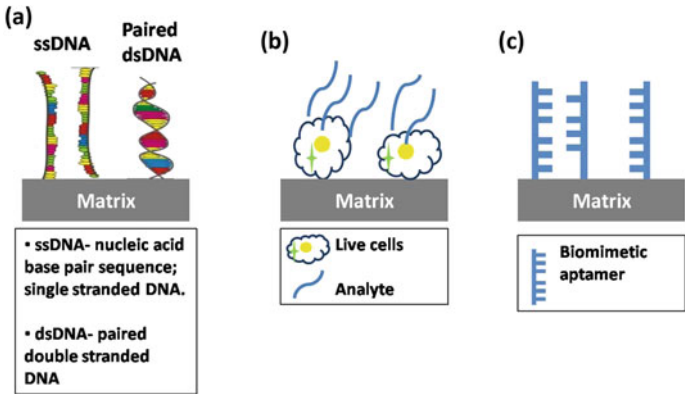

Fig. 5.4 Schematic representation of **a** DNA-based, **b** live cells-based and **c** aptamer-based biosensors

DNA Probe-Based Biosensor

In these types of biosensors, a nucleic acid sequence capture DNA (ssDNA) is immobilized over the matrix as a bioreceptor. Each ssDNA can bind with the complimentary strand forming a double-stranded DNA (dsDNA). The target analyte DNA is denatured chemically and added to the electrolyte. The complimentary sequences adhered to the matrix, and upon exposure to these target ssDNA, they can capture and form hydrogen bond resulting in dsDNA, and hence target DNA can be detected. These biosensors are of great importance in clinical diagnosis of pathogen DNA presence in the biological samples [8]. Figure 5.4a is the schematic representation of this type of biosensor.

Live Cell-Based Biosensor

In these biosensors, live cells of microorganisms like fungi or bacteria are immobilized over matrix as a bioreceptor. The proteins present in these cells can be used as bioreceptor for detection of précised target analytes. Nevertheless, these sensors have limited stability, low detection limit. The live cells might lose activity due to environmental parameters like pH, temperature, etc. But in terms of sensitivity, these biosensors are advantageous [9]. Figure 5.4b is the schematic representation of this type of biosensor.

Aptamer-Based Biomimetic Biosensor

In these biosensors, a bioreceptor, imitating natural system, like aptamers, is immobilized over the matrix. Aptamers are synthetic nucleic acid sequences that biomimic

nucleic acid base pairs. These are useful for selective detection of amino acids and proteins. These are more advantageous over live cell and antibody-based sensors, as they are more stable, not effected with environmental parameters and safe to handle [10]. Figure 5.4c is the schematic representation of the aptamer-based biosensor.

5.1.1.2 Classification Based on Transducer

Electrochemical Transducer

In these, chemically modified or non-modified electrode acts as a transducer. Different types of techniques like impedance, potentiometry, amperometry, conductometry are used in this.

Optical

Herein, optics-based devices are used as transducers. Techniques like absorption, Raman spectroscopy, fluorescence, phosphorescence, etc. are utilized [11].

Calorimetric

Mostly, enzyme-based biosensors use this transducer. The heat generated during reaction is measured, and alterations in the temperature are recorded.

Piezoelectric

This type of biosensor has transducer made up of piezoelectric substance like quartz that resonates at a set frequency when exposed to target analyte. The bioreceptor is also coated using a piezoelectric material. When the reaction occurs, the frequency is changed which is measured as signals.

5.1.2 Matrices for Biosensors

Figure 5.5 is a general schematic representation of various types of matrices generally used for entrapping the bioreceptor as well for fabrication of microdevices. Generally, carbon nanomaterial matrices are used owing to their excellent electron shuttling capabilities. The schematic shows some of the commonly used materials. Likewise, metallic nanoparticles have also proven to be useful in entrapping the bio-component and giving high electron transfer. Some of the generally used ones are stated in the schematic. Likewise, for fabrication of microdevices integrated

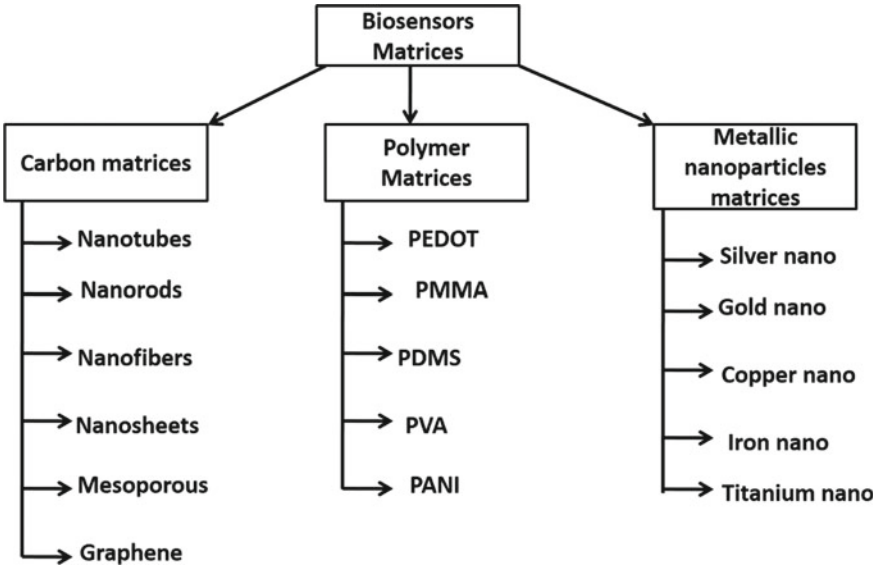

Fig. 5.5 Schematic for various types of matrices used in biosensors

with biosensors, as well as for immobilization of bio-components, polymer and poly-nanomaterials composites are also employed. Commonly used polymers are mentioned in the schematic [12, 13].

5.1.3 Physico-chemical Characterization Techniques for Biosensors

Since the biosensor has multiple layers of modification, strategized physico-chemical characterization of these layers is needed. The interaction and the electron transfer mechanism between the matrix and the bioreceptor, analyte and the redox mediator, can be studied via these approaches [14]. In further, the morphological and surface characteristics can also be studied. The commonly applied techniques are shown in Fig. 5.6.

5.1.4 Microfluidic and Miniaturized Devices

Microfluidic and miniaturized devices are portable, fully integrated, wherein minimal sample volume and reagent volume are used. The major advantage is that these can be used as point-of-care testing devices (POCT). Clinical diagnosis of ailment

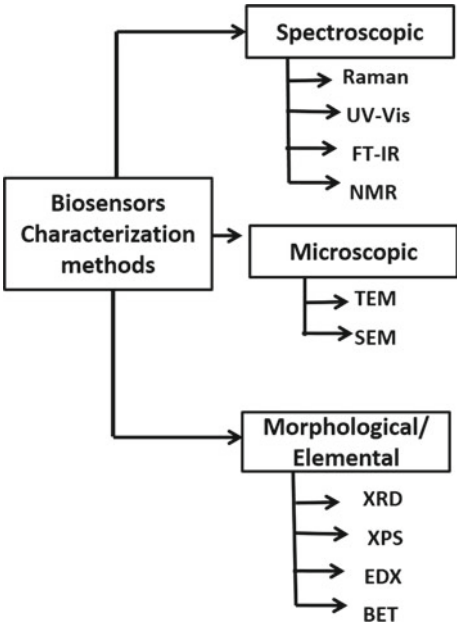

Fig. 5.6 Schematic summarizing various physico-chemical characterization techniques for biosensors

biomarker, pathogens and other health parameters can be accomplished without laboratory-based procedures; hence, they can be employed in health monitoring. Ideal POCT devices must have the following features.

1. **Portability**—compact and handheld device.
2. **Instant results**—less waiting time.
3. **Easy to use**—user-friendly, no skilled training required.
4. **Less volume**—for sample and reagent.
5. **No preparation of sample**—whole sample analysis.
6. **High sensitivity and selectivity**—interference mitigated.
7. **Robust**—unaffected with the environmental parameters.
8. **Lesser cost**—affordable.
9. **Inbuilt calibration**—calibration integrated.

The microfluidic biosensor-based electrochemical platforms can be generally categorized into two types: (1) single use and (2) multiple use. These can be either quantitative or qualitative estimations. Dip sticks, strip-based, digital output-based, multiple use cassette device with more than one analyses, benchtop devices are some of the commercially available ones.

5.1.4.1 Fabrication Methods

Quite a few approaches for fabricating these microfluidic electrochemical platforms have been reported so far. Several underlying substrates, electrodes, matrix materials for biosensor integrated microfluidic device development have been explored in recent years. For instance, paper, polyimide, glass, conductive filaments like vinyl alcohol (PVA), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET), polylactic acid (PLA), wood fiber (cellulose + PLA), polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA) are some of them used commonly. Based on these types of materials, there are various methods of fabricating. Figure 5.7 gives various types of microfluidic/miniaturized electrochemical platforms.

1. **3D printing**: Also referred as additive manufacturing where suitable filaments are used as material and are deposited layer-by-layer forming a 3D structure as per the device design. In this method, the device is drawn in a computer-aided design (CAD) software. This file is changed to a Standard Triangle Language (STL) file which is compatible to 3D printer. Finally, a G-code file is made based on which the printer deposits material into a 3D structure. Figure 5.8 summarized different types of commercially available 3D printers.
2. **Molding**: Molten liquefied polymers are modeled and solidified as device form. There are two approaches of this. (a) Inject molding: master mold with electrode cavities is made with lithography, and molten polymer is injected and solidified

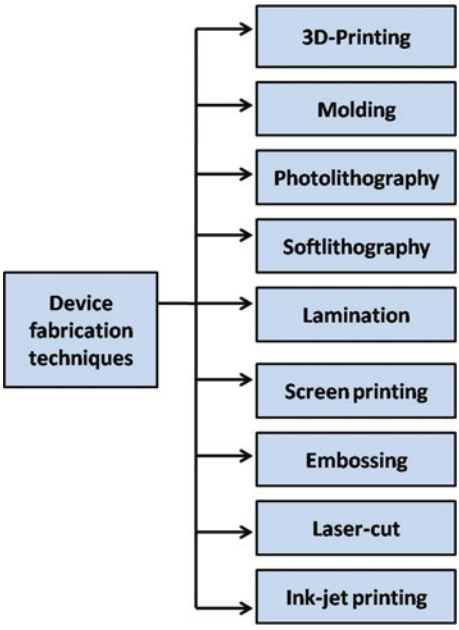

Fig. 5.7 Schematic giving various microfluidic electrochemical platforms fabrication techniques. Brief description of all these methods is presented here [15–19]

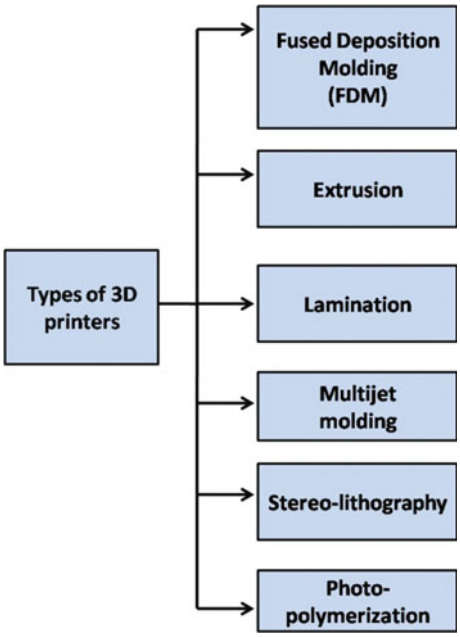

Fig. 5.8 Schematic giving various types of 3D printers available commercially

(Fig. 5.9). (b) Replica molding: master mold of silicon is made, over which liquid polymer is poured, cooled and solidified.

3. **Photolithography**: Herein, optical beam is used to draw desired electrode patterns on the substrate. Beams like UV, X-ray, electron beam and ion beam are employed. Figure 5.10a is a schematic of this approach.
4. **Soft lithography**: liquid polymers like polyimide, PDMS are poured over a master mold made up of elastomers. Solidification is done to obtain the designed microfluidic platform. Figure 5.10b is the schematic of this approach.

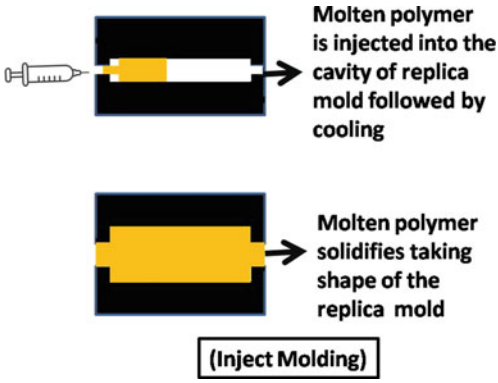

Fig. 5.9 Schematic for inject molding method

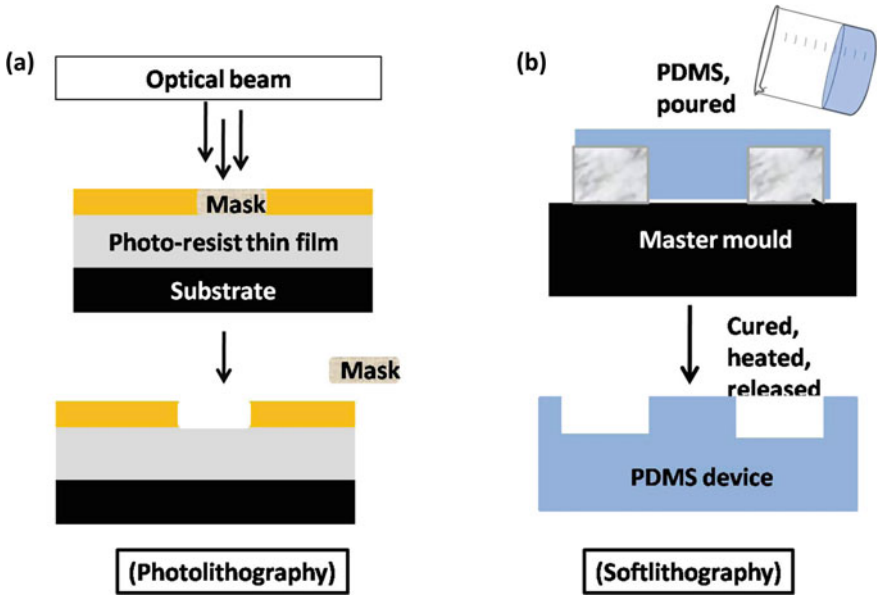

Fig. 5.10 Schematic representation of **a** photolithography and **b** soft lithography method

5. **Lamination**: Herein, independently cut layers are stacked on top of each other and bonded together. Generally, three layers are bonded, bottom, intermediate where microchannels are patterned and top layer. Glass slides, PMMA, PLA, etc. are used as materials. Laser or knife plotter is used for designing patterns. Adhesives like double-sided tape, thermal bonding are used for firmly bonding these devices.
6. **Screen Printing**: Herein, paper based or any other flexible substrate is used to design electrode patterns. Frame is used to fix the paper over frame, mask of electrode pattern is placed, and conductive ink is poured over it. The obtained pattern is dried in over. Figure 5.11 gives the schematic representation of this method.
7. **Embossing**: this technique uses a mold with desired cavities for microchannels. A thermoplastic polymer sheet is placed, and temperature and pressure are applied. Sheet melts and takes the shape of cavity. Upon cooling, it solidifies and gives microchannel based device. Figure 5.12 is the schematic representation of this method.
8. **Laser cut**: Herein, substrate materials like paper, glass, carbon, plastic, polymer sheets, etc., are exposed to laser ablation for obtaining laser-induced graphene electrodes and laser cut microchannels for sensors. Lasers like CO_2, UV, pulsed, diode, etc. are used.
9. **Inkjet printing**: Herein, inkjet printer is used where a conductive ink of desired viscosity is filled in the nozzle of the printer and printed over substrates like paper, glass, etc. The substrates are affixed over the printer to keep still. Post printing,

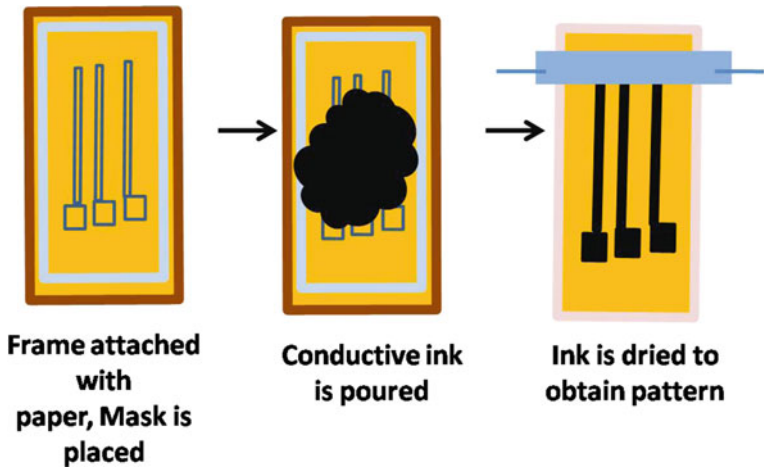

Fig. 5.11 Schematic representation of screen printing of electrochemical platforms

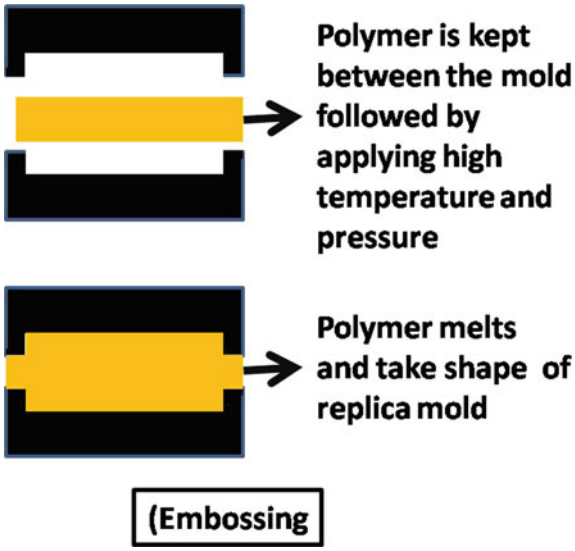

Fig. 5.12 Schematic representation of embossing method

the substrate is dried in oven. Figure 5.13 is the schematic representation of inkjet printing method.

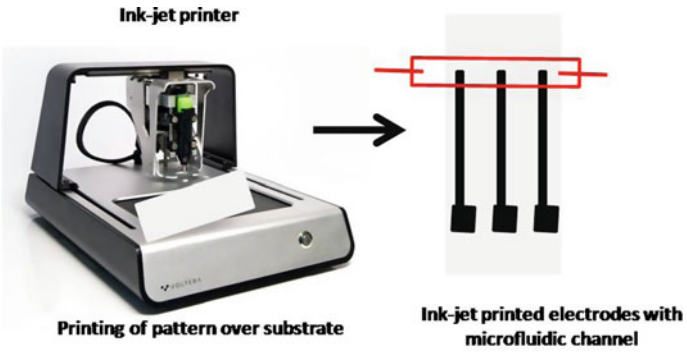

Fig. 5.13 Picture of inkjet printer with nozzle printing over the substrate

5.2 Microfluidic/Miniaturized Electrochemical Biosensors

While integrating biosensors into microfluidic system, either the bioreceptor component can be immobilized over electrodes and then introduced into the microchannel or the bioreceptors can be immobilized inside microchannel via microparticles or can also be introduced into the microchannel as a solution phase via syringe pumps. Figure 5.14 gives the schematic representation of these approaches. Although literature has substantial reports, herein a few of the recently reported microfluidic biosensing platforms with various biological components have been discussed.

5.2.1 Enzyme-Based Electrochemical Microfluidic Biosensor

Microfluidic enzyme-based electrochemical platforms have been extensively developed in the last few years. Campana et al. recently gave a detailed overview of various advances in microfluidic electrochemical biosensing platforms for an efficient detection of pharmaceutical pollutants in wastewater [20]. Likewise, Nadar et al. gave a critical review of paper-based microfluidic enzyme-integrated microPADs (paper-based analytical devices) [21]. Meanwhile, several research groups have also developed every effective methods to realize microfluidic biosensors. For instance, Mohammad et al. reported an enzyme-metal–organic framework (MOF-enzyme) composite-based biosensor. Herein, they used a polydopamine (PDA) and polyethyleneimine (PEI) coating to integrate MOF-enzyme into microchannel. Glucose oxidase and horseradish peroxidise (HRP) enzymes were used for detecting glucose. In further, polymer substrate of PDMS was used as base material. The device gave good sensitivity toward glucose in 8 μM–5 mM linear range and a limit of detection (LOD) as 8 μM. The similar approach can be used for various antibodies and aptamers as well. Figure 5.15 is the reprint of their schematics of fabrication [22].

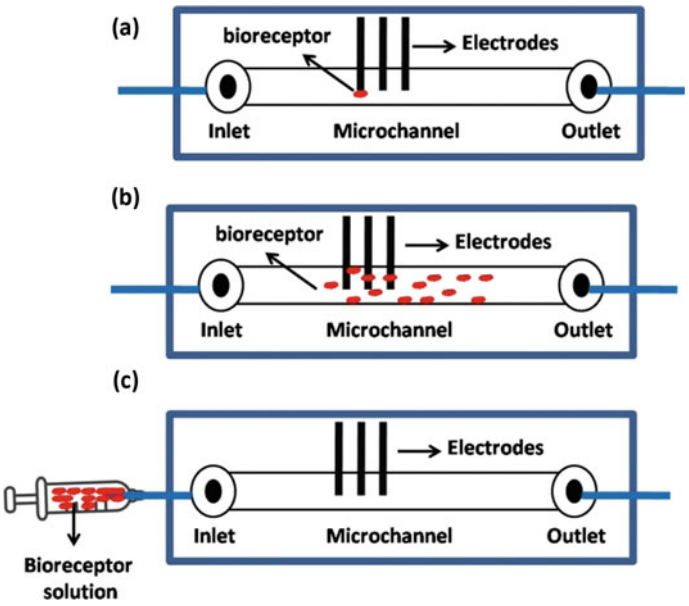

Fig. 5.14 Schematic representation of **a** electrode immobilized bioreceptor, **b** microchannel immobilized bioreceptor and **c** bioreceptor solution injected via syringe pumps

Similarly, in an impressive work, Cao et al. developed paper-based screen-printed microfluidic electrode. Herein, photolithography and screen printing were used. Working electrode of paper modified with redox mediator Prussian blue over reduced graphene oxide composite with tetraethylenepentamine was used. Glucose oxidase was immobilized for sensing hydrogen peroxide. There device gave 0.1–25 mM detection range with LOD as 25 μM. Further, real sample of human blood and sweat was analyzed. They validated the device with commercial glucose meter results [23]. Li et al. reported a one-step method for POCT microfluidic biochip fabrication. Herein, a gold nanoparticle-carbon nanotube composite was used as matrix for enzyme immobilization. Efficient detection of creatinine in biological samples was done [24]. Zhu et al. reported a unique microfluidic paper-based biosensor fabricated by wax printing method. A hybrid flower-like composite of two enzymes, glucose oxidase and horseradish peroxidase, with copper nanostructures was patterned. This microPAD showed a sensitive glucose detection from 0.1 to 10 mM with LOD as 25 μM. Further, they tested real sample of human blood [25]. In a very interesting approach, Shitanda et al. developed a screen-printed microfluidic biosensor where carbon modified with MgO-1,2-naphthoqui and lactate oxidase enzyme was used on a PDMS base substrate. The device gave lactate detection from 0.3–50 mM and LOD of 0.3 mM. This microfluidic biosensor was a wearable device and gave continuous monitoring of lactate in sweat [26]. Overall, several reports pertaining to use of enzymes as microfluidic biosensors in real-time monitoring have been reported with plenty scope of future. However, these devices have certain limitations with regard

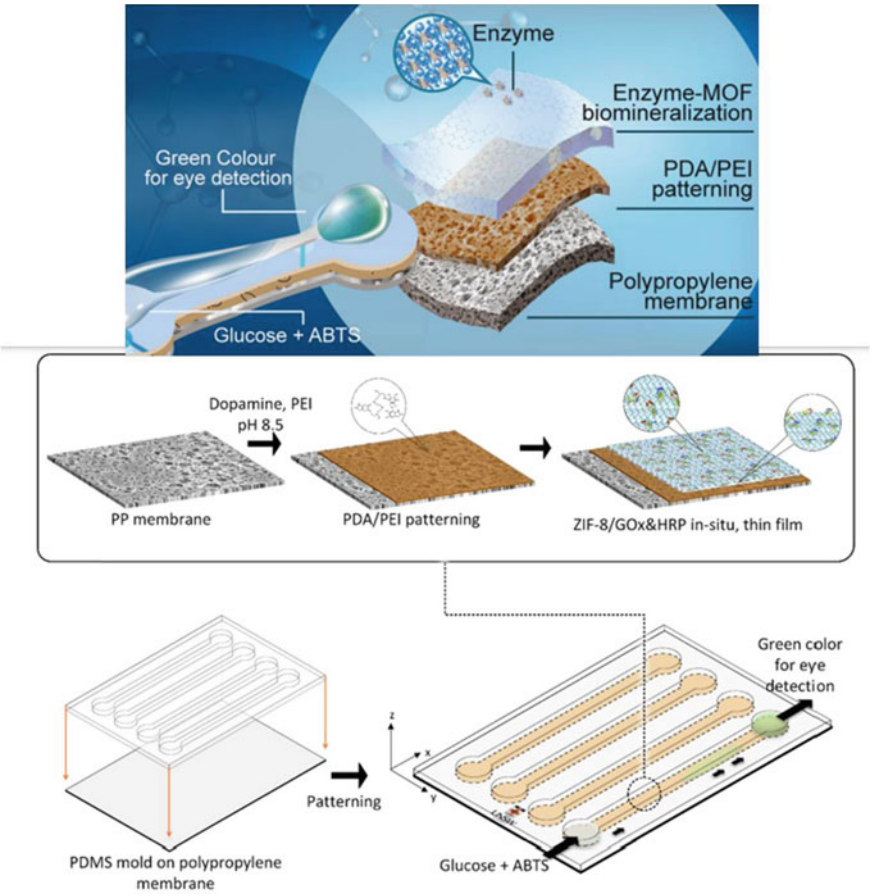

Fig. 5.15 Schematic of the microfluidic biosensor device reprint with Copyright © 2019, American Chemical Society from [22]

to stability and shelf life of enzyme. Enzymes often are prone to get denatured with external parameters like pH, temperature and therefore may lose their activity soon. To overcome this, suitable and compatible matrices with novel materials are being tested.

5.2.2 Antibody-Based Electrochemical Microfluidic Biosensor (Immunosensor)

Like enzyme, several antibody-based immunosensors in microfluidic electrochemical platform have been reported. Nasseri et al. gave a detailed review summarizing

various POC microfluidic biochips for detection of various pathogens focusing on viruses like HIV, HVB, ZIKV. Specific antibody-based immunosensors have been reported [27]. Similarly, Akçapınar et al. also gave a critical review on various reported biosensors devices focusing on apoptosis detection [28]. Several research groups have worked off lately on developing immunosensors focusing on specific pathogens. For example, Funari et al. recently reported a microfluidic chip for detection of antibodies against SARS-CoV-2 which has become a major concern of pandemic today. Herein, gold nanospikes were electrodeposited for detecting the antibodies of this virus in human plasma in less than 30 min. The gold nanospikes were functionalized with antigen. The entire device was made with PDMS substrate. The antibodies in the real sample, if present, forms antigen–antibody complex detecting LOD up to 0.08 ng/mL [29]. Zhang et al. reported a microfluidic biosensor for detecting interleukin-8 (IL-8), a cancer biomarker. The microfluidic device had two microchannels, wherein one microchannel was immobilized with IL-8 capture antibody and biotin-streptavidin linkage was established. The antibody was used in linear concentration of 7.5–120 pg/mL. They further utilized this device for real-time detection of IL-8 in tumor cells and validated their device with commercial assay method [30]. Nunna et al. reported fabrication of interdigited electrodes modified with gold nanoparticles for detection of cancer antigen CA-125. Specific antibody was immobilized over electrodes to capture the antigen via complex formation. Impedance analysis was done changing various concentrations [31]. Similarly, Evans et al. demonstrated preparation of POCT microfluidic device based on circuit board for cytokine detection. Gold working and counter electrodes with Ag/AgCl reference electrode were used [32]. Liu et al. prepared a Salmonella type B and type D detecting microfluidic impedance interdigitated biosensor. LOD up to 300 cell/mL for these pathogens were detected. Turkey samples were used as real samples. Antibodies were immobilized over electrodes which forms complex with Salmonella type B and D. The immunosensor was selective and gave no interference from other pathogens like *E. coli* strains. Further, the effect of antibody concentration, time duration, etc. were also examined [33]. Overall, microfluidic immunosensors have proven to be potential candidates for POCT detection in critical conditions. However, these devices have a limitation in terms of cost-effectiveness as specific antibodies are expensive. Approaches for economic antibody preparation should be adapted.

5.2.3 DNA-Based Electrochemical Microfluidic Biosensor

Microfluidic biosensor electrochemical platform wherein electrodes are modified with ssDNA as capture DNA, also or detecting the DNA, RNA in real samples have been reported. These approaches have also been employed for detection of biomarkers of diseases and syndromes. Adampourezare et al. reported a detailed review focusing on microfluidic bioassays of DNA methylation focusing on the recent advances for detection of this cancer biomarker [34]. Likewise, Wu et al. presented a review summarizing microfluidic electrochemical biosensing platforms

for detection of food pathogens [35]. In further, Bruch et al. designed a multiplexed microfluidic biosensing platform with electrochemical approach. Herein, the device has subsections over the chip and could detect 8 miRNAs. CRISPR was used as a bioreceptor here [36]. Caneira et al. reported a microfluidic platform for DNA hybridization whereby a 22-mer sequence of DNA which is a prototype of miRNA tumor biomarker was detected. Nanoporous beads, modified with immobilized probe DNA, were used as the bioreceptor [37]. In another work, Ghrera et al. developed an impedance-based microfluidic electrochemical biosensor. The biochip was fabricated with PDMS, consisting of electrode of mutliwalled carbon nanotube-indium-tin-oxide substrate. The MWCNT was modified with probe DNA for detection of DNA sequence of chronic leukemia [38]. Similarly, Pursey et al. developed an array microchip for detection of bladder cancer biomarker. Three types DNA biomarkers of cancer were simultaneously detected with LOD of 25 fM. Herein, porphyrin marker embedded was used to detect target DNA [39]. Alsabbagh et al. prepared impedance-based microfluidic electrochemical chip for detection of troponin I, a cardiac ailment biomarker. A glass slide substrate with PDMS microchannel was employed. Probe DNA sequence was the bioreceptor used for capturing the protein [40]. Overall, DNA probe-based microfluidic biosensors are being greatly explored especially in cancer detection and prevention studies. Drawback pertaining to cost is a concern to be worked out in the future.

5.2.4 Live Cells-Based Electrochemical Microfluidic Biosensor

Electrochemical microfluidic cell-based biosensors have witnessed remarkable advances in recent times. Various cells, including stem cells, and tissues have been utilized as bioreceptors for various applications in health monitoring like anti-microbial susceptibility, drug screening, etc. Gupta et al. gave a review about these cell-based biosensors, their emerging trends, drawbacks, challenges and future outlook [41]. Likewise, Zhai et al. gave a detail review of the microfluidic platforms for cell-based screening of drugs. This review summarized the toxicity and drug metabolism over isolated cells with integrated microfluidics [42]. Liu et al. also reported a comprehensive review on cell-based biosensors focusing on the biomedical applications [43]. Likewise, a concise review of microbial cell biosensors for varied applications has been reported by Su et al. [44]. Chen et al. reported a microfluidic cell-based biosensor for detection and monitoring of physiological components where a pulse generator was used to detect selective signals [45]. Brennan et al. reported a novel impedance microfluidic biochip wherein fish cells from rainbow trout fish, gill epithelial cells were used as biocomponent for analysis of drinking water quality against pesticides toxicity [46]. Overall, these cell-based microfluidic sensors although have limitations pertaining to stability, yet are an efficient electroanalytical sensor.

5.2.5 *Aptamer-Based Electrochemical Microfluidic Biosensor*

These are oligonucleotides or peptides chain of selective sequence which shows great affinities toward target biomolecules. Owing to their high stability, these aptamers have gained much attention than enzyme and antibody-based biosensors. As a result, advances in terms of microfluidic lab-on-chip platforms, POCT devices using aptamers as bioreceptors have grown significantly. Khan et al. gave a detailed review about these aptamer-based lab-on-chip microfluidic platforms for health monitoring and other applications [47]. Vandghanooni et al. also gave a detailed review about aptamer-based nano- and-microfluidic systems for early detection of biomarkers for ovarian cancer [48]. In an interesting work, Jiang et al. developed a microfluidic PDMS-based platform. Herein, a dendrimer aptamer was used as a bioreceptor for detection of *E.coli* [49]. Tanu et al. reported an impedance-based aptamer microfluidic biosensor for detection of Ranibizumab drug [50]. In another example, Wang et al. developed paper-based, microfluidic, aptamer-dependent sensor that was multiple cancer biomarker detection [50]. Overall, aptamers as bioreceptor have proven to be more specific, selective and stable over various other bioreceptors.

5.3 Conclusion and Future Outlook

Biosensors in health monitoring have played significant role for quite some time now. The integration of microfluidics, cloud computing, automation to realize a fully integrated systems, has made point-of-care amenable. Such platforms are extensively helpful for diagnosis in critical situation. The advent of these platforms has made clinical analysis laboratory-free, and with minimal sample and reagent requirements, these devices improve cost-affectivity. The user-friendly approaches, simple operating procedures and no bulkier hardware, make them portable and easy to handle. Off lately, the bulk biosensors utilizing various bioreceptors as redox mediators, like enzymes, antibodies, aptamers, DNA, cells, etc., are being converted to miniaturized or microfluidic and nanosystems. Since these biological components are less robust, they often lose their activity due to external environmental parameters like pH, temperature, etc. Hence, utmost care for protecting them within the microfluidic devices has to be taken care of. Several fabrication approaches, materials, matrices are being explored to overcome this limitation. In the future, tremendous growth in design and development of such lab-on-chip platforms is expected. The present chapter briefly summarizes the importance of biosensors for health management application. Various types of biosensors, classified based on their components, are also discussed here. The matrix materials used for fabrication, characterization techniques and overview of fabrication approaches have also been discussed in detail. Furthermore, some of the recent examples reported in the literature of each type of electrochemical microfluidic biosensor are also discussed. The

chapter aims to concisely present the recent progress in miniaturized/microfluidic biosensor-integrated electrochemical platforms for health care applications.

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Chapter 6
Application of Nanomaterial-Based Biosensors for Healthcare Diagnostics

Arpan Deyasi, Arighna Basak, and Angsuman Sarkar

Abstract Diagnoses of multifactorial diseases are always difficult owing to lack of proper sensors which can not only detect the low level concentration molecularly imprinted polymers and peptides, but also able to generate electrically measurable signal from biochemical interactions. Nanomaterial-based biosensors are the most accepted and trusted tools in this regard, which serves both the purpose with comparatively lower cost, reliable, and transportable. These sensors are mostly built on wide variety of nanomaterials, including quantum dot, carbon nanotube, grapheme, magnetic nanomaterials, nanofibres, optical nanoresonators and a few imprinted structures. These novel aspects of applications make a new insight of material science research directed towards biological tiny sensor elements, particularly detection of those elements which are not possible by any other physically realisable detecting elements. Applications of nanobiosensors hover around from environment protection by detecting pesticides, water contaminants, etc. to determination of drug residue in food and drinking water. In present day, they are utilised in forensic science, thanks to the possibility of making intra-cellular detection. Laser base sensors at submicron level are capable of detecting organism inside living cells. The final target is to obviously develop lab-on-a-chip, which will make revolution in present medical care facility, and should be affordable to all financial class of people.

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6.1 Introduction

Rapid identification of any infectious illness while it is still in its early stages is critical for public health and efficient clinical results. A disease's early and correct diagnosis is crucial to a successful medical treatment. The process of illness diagnosis entails determining the nature and origin of the condition, as well as analysing the patient's antiquity and pertinent test statistics [1]. Health analysis must be rapid, precise, and exact, through as a couple of 'false findings' as achievable. Identification techniques that are very highly sensitive and specific assist in the initial analysis of diseases and give an improved forecast. Medical identification technology has a significant influence on overall population health care, and hence, progress in this area is one of applied science's most important goals [2].

In modern years, much bioengineering research has focussed on producing fast, accurate, movable, and affordable investigative equipment which may be used by patients to observe their own health. For diagnosis, a variety of assays and methods are available, including biosensing element, immunoassay, medical imaging and genetic-based testing. Enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR)-based genetic tests and discolouration substance for bacterial and viral illnesses such as Gram and Giemsa stains are among the most extensively used symptomatic substances [3]. Traditional diagnostic procedures have limitations, including low sensitivity, low specificity, and a long decision-making time. The use of nanotechnology in the development of diagnostic tools has proven revolutionary due to the enhanced sensitivity, specificity, and utility of the equipment [4, 5]. Biomarker sensing is typically related to expensive prices, extended wait times, and the use of sophisticated automated analysers in centralised laboratories. Nanostructured materials are promise in the development of improved diagnostics because they are cost-efficient, quicker, and more long-lasting. They might also be utilised to eliminate the need for time-consuming laboratory diagnostics and promote point-of-care identification. Nanotechnology, in conjunction with genomics, proteomics, and molecular machine systems, can aid in the development of onsite medical diagnostics that are effective, dependable, and fast. By making it simpler to create novel materials for medical diagnostic equipment, nanotechnology has transformed these modalities.

Lung cancer, ischaemic heart illness, cirrhosis of liver, and other infective diseases stay among the world's top sources of mortality [6]. A lack of early diagnosis thwarts effective and affordable treatments. Because of its user-friendly, cost-effective, trustworthy, and rapid sensing platforms, biosensors have gained appeal in the field of healthcare diagnostics [7]. In compared to traditional detection techniques such as spectroscopy or chromatography, biosensing technology provides a lot of advantages. These advantages include the removal of the requirement for highly trained operators, faster response times, mobility, and increased sensitivity [8]. With the aid of new biosensors, the detection period of diseases like anthrax has been lowered from 2–3 days to 5 min [9].

Nanomaterials are distinct as resources through at tiniest one dimension of 1–100 nm [10]. The mainstream of their element particles or molecules are positioned on the outward of the constituents because of tiny size, resulting in a striking difference in their important physicochemical belongings when associated to the substance of the same materials. The quantum properties originating from intermittent performance due to the quantum detention of delocalised electrons are another reason generating substantial variations in the properties of nanomaterials. Because there are supplementary elements on the outward of these nanoparticles than in the bulk, they have low energy and hence an inferior melting point. However, the physiognomies of these elements are determined by their form. Nanorods, on behalf of example, can have quite dissimilar physiognomies than nanospheres of the identical material [7]. Chemical reactivity is enhanced by around 1000-fold as a result of the increased surface area per unit mass [11]. Quantum dots, for example, are synthetic nanostructures that take use of quantum phenomena in nanoparticles. They work as artificial atoms since their electrical performance is extremely adjacent to the tiny particles or individual atoms, because the three-dimensional detention of electrons at the Nanoscale region creates a quantised energy scale. Nanoparticles have magnetic moments as well, owed to numerous unpaired electron turns from hundreds of particles, and exhibit their greatest performance at 10–29 nm diameters due to super magnetism, making them useful as dissimilarity agents in magnetic resonance imaging (MRI) [10–13]. Nanomaterials can be classified in a variety of ways as a result of all of these features. Based on their chemical makeup, nanomaterials may be categorised into three categories: (1) nanomaterials made of carbon allotropes, (2) inorganic nanoparticles, such as Au, Ag, and SiO_2, and (3) organic nanomaterials, such as polymeric nanomaterials. Various nanomaterials used as biosensors are schematically represented in Fig. 6.1.

Design of nanobiosensor is the trend of research in twenty-first century, which, in turn, critically depends on the development of novel materials and devices in lower dimension. Precisely, identification and detection of physical/living analytes is a very sensitive process, and, therefore, requires sophisticated instruments where reduced dimensional devices play pivotal role in determining weaker signals in near accurate form. Several synthesised nanodevices like semiconductor nanowire, carbon nanotube, quantum dot, nanowire field-effect transistor (FET) have already established their value in evaluating signals from living cells, virus, DNA, biomarker, etc. [1]. Due to its large surface-to-volume ratio, quantisation effect and tunable (subject to external excitation) discrete eigenstates, effective determination of living microorganisms is possible which are obviously in the equivalent dimension of artificial nanostructures [14]. In this present chapter, nanodevices based on inorganic materials are analysed, and corresponding sensing applications are mentioned. The work is a comprehensive review in this specific domain with a keen focus on recent developments as well as research trends in nanobiosensors.

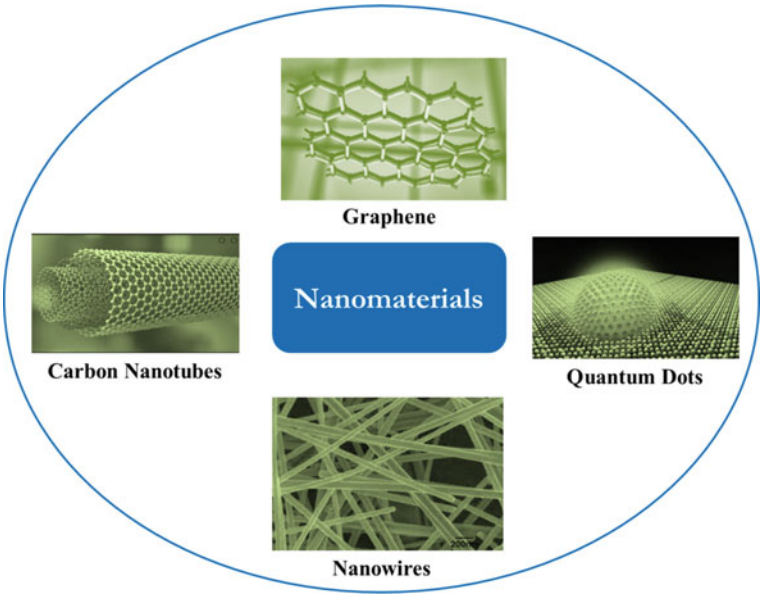


Fig. 6.1 Various types of nanomaterials used in biosensors

6.2 Application of Carbon Allotrope-Based Nano Biosensors

Carbon-based nanomaterials have received a lot of interest in the medical technology field in recent years. Carbon-based nanomaterials are extremely esteemed owed to the existence of a range of carbon-based nanomaterials like fullerenes, graphite, diamonds, and lonsdaleite, by way of more advanced forms like nanohorns, graphene, and nanotubes which are illustrated in Fig. 6.2 [15]. Each of these allotropes has distinct properties that have led to their distributed use in a mixture of biological applications, including cancer therapy, drug delivery, etc. in addition to bioimaging, biosensing, and other medical diagnostics [16, 17]. Carbon-based nanomaterials have an unequalled mix of electrical, optical, and mechanical physiognomies, resulting in diminished sensors through excellent enactment and reduced power consumption. These nanomaterials are tractable and thermally constant in environment, through high electron mobilities and a high strength-to-weight ratio [18]. Such biosensor materials can detect a wide range of chemicals that have uses in healthcare diagnostics and illness POC analysis [19]. Fullerenes, [20–24] nanotubes (CNT) [25–30], films of graphene and its derivatives [31–34], quantum dots [35–38], and nanodiamonds [39–44] are carbon allotrope-based nanomaterials that have played a significant part in recent biosensor developments. In the case of local exposure, such sensors provide improved three-dimensional resolution, as well as real-time and label-free non-devastating detection.

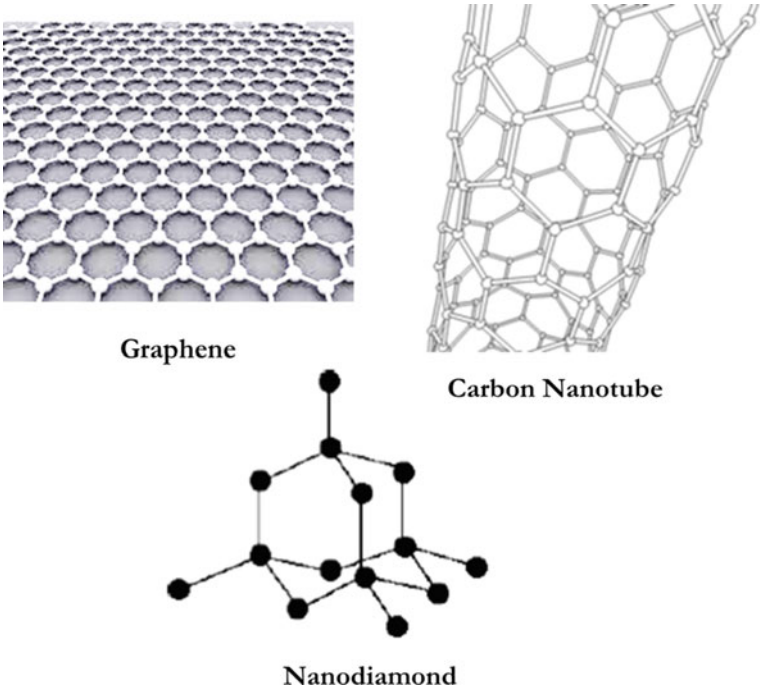


Fig. 6.2 Various crystalline allotropes of carbon

6.2.1 Carbon Nanotubes

CNTs are regarded as promising building blocks for biosensors because of their increased aspect ratio, large surface area, amazing optical and electrical physiognomies, excellent thermal and chemical robustness, and great mechanical strength [15]. Due to their higher sensitivity, lower background, decent SNR ratio, comprehensive preoccupation range, label-free disclosure, and real-time observing, CNTs provide biosensors as an advantage [19]. They act as scaffolding for the immobilisation of biomolecules, which improves signal transduction and identification [45]. Carbon nanotubes' semiconducting characteristic allows them to be used as tiny field-effect transistors (FETs) [29]. They might be used to construct top-of-the-line nanoscale electrodes due to their enhanced excellent conductivity along their length. CNTs' adjustable near-infrared emission, which reveals local dielectric function alterations while being resistant to photobleaching, is particularly interesting. They are also perfect for optical biosensing since they have a high luminous intensity and good luminous properties. [46–51]. CNTs have a density that is a sixth that of steel yet are 100 times powerful, making them suitable for use in piezoresistive sensors [52–55]. It's also feasible to make calorimetric sensors that trust on modification in

nanotube size induced by temperature changes [15, 56]. Various crystalline allotropes of carbon are shown in Fig. 6.2.

Several CNT-based biosensors for glycaemic indicators in diabetes mellitus must be developed. Hatada et al. developed a label-free chemiresistor-based affinity sensor for haemoglobin A1c (HbA1c) and a bacterial periplasmic protein (SocA) using single-walled carbon nanotubes (SWCNTs). Continuously, proteolytic hydrolysis method, HbA1c, produces fructosyl valine (FV), which the sensor can detect in an absorption variety of 1.2–1909 nM. Comba et al. used a CNT (CNT-muc) nanocompound halted on a platinum outward to create a long-lasting enzymatic biosensor for glucose. CNT's high surface area aided in the restriction of the GOx enzyme. Using chronoamperometry, the CNT-based sensor was capable to sense glucose in the variety of 0.002–3.2 mM, through a LOD of 3 M. Another research [57] used MWCNT scaffolds with cobalt functionalised MoS2 to obviate the need for the GOx. This scaffold has a low detection limit and may be used to detect glucose over a additive concentration range of 0.2–16.2 mM. (80 nM). Aryal and Jeong introduced a thermally reduced graphene oxide–MWCNT (TRGO-MWCNT) nanocomposite adapted with ambient plasma and -cyclodextrin for uric acid (UA) perception (CD) [58]. This sensor had a LOD of 0.06 M and could produce linear responses in the range of 10–300 M. Bollella et al. exploited the electron exchange capabilities of MWCNT and poly(methylene blue) (pMBexcellent) to develop a second-generation Au-based sensor for incessant lactate detection in epidermal interstitial fluids. After immobilising lactate oxidase (LOx) on the sensor, lactate disclosure between 10 and 200 M was possible with a degraded sensing limit (2.4 M). Shen et al. improved a label-free immunosensor for lucrative POC perception using SWCNT [30]. They employed non-covalent assembling communications using SWCNT and PCA to create a water-based ink capable of immobilising human serum albumin (HSA) antibodies. The SWCNT-based ink could detect 0.015–9.43 nM HSA while consuming a 1 pM LOD. Huang et al. developed an immune chromatographic test that allowed visual detection of rabbit immunoglobulin G using IgG antibodies (Ab1) powerless on MWCNT attracted with Fe3O4 (MMWCNT) (IgG) [51]. Visual detection in blood had a detection limit of 10 ng mL^{-1} and a linear dynamic range of 10–200 ng mL^{-1}. Other CNT-based nanoparticle (AuNP) nanocomposite through exposure of antibody (dAb) coating by way of a reporter probe was used to make another visual immune chromatographic biosensor accomplished of discovering carcinoembryogenic antigen (CEA), used as a biomarker for lung cancer [46]. The cotton thread-based approach allowed a straight information via the naked eye with a genuine response in the range of 10–500 ng mL^{-1} and a LOD of 2.36 ng $mL^{-1.}$ Meng et al. described a similar optical biosensor for human ferritin antigen (HFA) that employed MWCNT to achieve a linear concentration range of 100 to 5000 ng mL^{-1} with a LOD of 50 ng mL^{-1}. CNT-based surface plasmon resonance (SPR) optical sensors have also been reported. Pathak and Gupta developed a polypyrrole (PPy) MIP on carboxylated multiwalled carbon nanotubes (CMWCNT) with a permselective nafion membrane for the detection of dopamine (DA).

Biosensors made of carbon nanotubes are widely used to detect cancer and neurological diseases. For example, prostate-specific antigen (PSA), a chemiresistive-based CMWCNT biosensor was industrialised [59]. Anti-P-gp-SWCNT film twisted on a SiO2–Si layer was used in another CNT-based detector for the disclosure of P-glycoprotein (P-gp) for leukaemia biomarker.

6.2.2 Graphene

Graphene is a new form of carbon element composed of sp^2 hybridised carbon molecule configured in a hexagonal arrangement. At ambient temperature, the electrons in graphene give it unique characteristics including ambipolar like electric field properties, higher quantum hall effects and thermal conductivity. It has a two-dimensional artefact, which results in a large surface area and high porosity. As a result, graphene may adsorb a variety of gases, including methane, hydrogen, and carbon dioxide [7]. The number of layers and the stacking sequence of graphene can be denatured to alter its properties. It is extremely translucent, with a high modulus of elasticity and a good resistance to fracture. Furthermore, graphene is capable of physisorption interactions with a variety of biomolecules, making it an excellent option for biosensors [15]. Graphene derivatives, such as graphene oxide (GO), can exhibit fascinating properties such as fluorescence. GO, RGO, and graphene quantum dots are the most frequent graphene derivatives utilised in biosensing (GQDs) [60–62]. Cysteine [63–66], glycaemic biomarkers [67], cholesterol [68], neurotransmitters [69, 70], H2O2 [71], cancer cells [72], nucleic acids [73], pharmaceutical medicines [36], and pathogenic microorganisms [61–63] may all be detected using graphene-based biosensors. Graphene has been used to create a plethora of optical and electrochemical biosensors for the detection of different amino acids. Graphene-based biosensors serve an important role in the identification of incurable and lethal diseases such as diabetes and various forms of cancer. Jaberi et al. discovered and built an RGO–Au nanostructure-based electrochemical nanogenosensor for HbA1c exposure on a flexible and inexpensive graphite sheet (GS) conductor [73]. HbA1c levels can be altered by a variety of illnesses, including haemolytic anaemia and sickle cell anaemia etc., production it an inaccurate biomarker for diabetes mellitus diagnosis. Apiwat et al. elucidated this problem by replacing glycated HAS (GHSA) for HbA1c biomarker [74].

6.2.3 Nanodiamonds

Nanodiamonds (NDs) are the only carbon allotrope-based nanomaterials that include sp3 hybridised carbon centres, as opposed to the other carbon allotrope-based nanomaterials previously described. To individual's resultant by their higher exact area, which may approach 400 $m^2\ g^{-1}$ [75], NDs exhibit excellent features of diamond, like

comprehensive band gap electrical performance, chemical motionlessness, thermal conductivity, and exceptional mechanical things. Microdiamonds can be ground at high-pressure, high-temperature (HPHT) settings or carbonaceous explosives can be detonated to produce them (DND). After easy functionalisation through amines, thiol group halides, etc., they can connect covalently or non-covalently by bioparticles. HPHT diamonds have a lot of nitrogen impurities, which can be converted into vacancy-related colour epicentres, resulting in fluorescent nanodiamonds (FND). FNDs can be used as effective biosensing, bioimaging probes, and contrast agents due to the photophysical properties of the vacancy centres [76–78].

For the reason that of their glowing countryside and capacity to identify a variety of metal ions, NDs have been extensively engaged in biosensing. For the finding of Hg2 + ions, Shellaiah and colleagues produced photoluminescent cysteamine (CYA)-modified nanodiamonds [79, 80]. CYA has free thiol groups that can trap mercury ions and forms amide bonds with NDs. Heavy metal ions can also be quantified using NDs doped with nitrogen. Pb^{2+} and Cd^{2+} were detected concurrently using one-dimensional nitrogen-doped nanodiamond nanorods (N-DNR) for an electrochemical sensor.

NDs have been used to detect a variety of therapeutically relevant substances, containing neurotransmitters, medicines, and poisons, in addition to biomarkers for chronic diseases like diabetes [81]. Dai et al. electrophoretically dropped NDs on a boron-doped diamond (BDD) conductor and then enhanced it with Ni nanosheets for enzyme-free glucose sensing [82].

6.3 Applications of Inorganic Nanomaterial-Based Biosensors

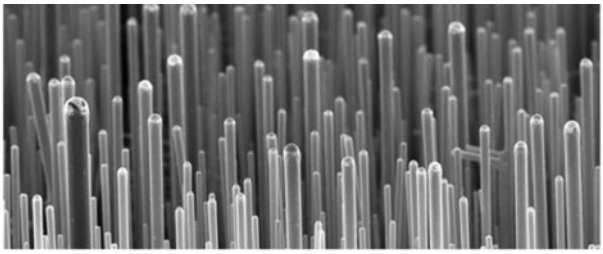
At the nanoscale, transitional metals and noble metals have remarkable characteristics. Unique quantum effects and optical characteristics result from the extra superficial particles combined through moderately occupied last but one or prepenultimate orbitals. They can not only be utilised to make excellent alloys, but they can also be similarly combined through other organic and carbon-based materials to create nanocomposites with a variety of diverse properties or totally new ones [7]. Anisotropies in inorganic nanomaterials might include triangular, round, and nanohole. Bimetallic alloys, core–shell architectures, nanotubes, and nanowires are only a few examples. Each of these nanomaterials can improve the biocompatibility and transduction characteristics of biosensors by utilising appealing interface and surface features. They can operate as immobilisation platforms, boost refractive index modification, catalyse interactions between substrates and chemiluminescents, magnify mass changes, and accelerate electron transfer [83–91]. Aside from immobilisation, such nanoparticle platforms might serve as electron wires in electrochemical sensors, translating biomolecular physicochemical changes into quantifiable signals.

Aside from their massive surface area, many inorganic nanomaterials, such as Fe_3O_4, have a magnetic nature and may be easily controlled by an external magnetic field, allowing for minor abstraction and buffer replacement. They similarly give a higher SNR ratio in biological examples [92–95]. They may be used to homogenise, trap, enrich, transport, and label analytes, which is very useful in POC testing. Microfluidic fraternisation, which is critical in lab-on-chip biosensing, may be accomplished using them. The majority of magnetic nanoparticles (MNPs) are made up of an amagnetic core made up of pure elements (such as Co and Fe), alloys (such as FePt), or iron oxides (such as maghemite -Fe_2O_3 or Fe_3O_4). This core is often coated with inorganic [96] or polymeric [97] molecules that act as biofunctionalisation sites. By embedding numerous MNPs in a non-magnetic matrix, superparamagnetic behaviour may be created [7, 92].

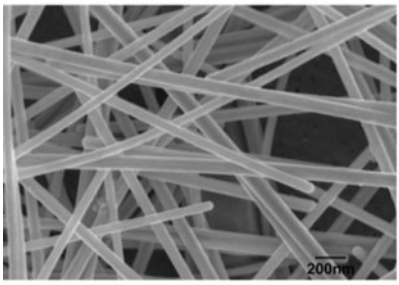
Biosensor development has recently focused on innovative inorganic structures like nanowires, nanocages, and nanoshells. Nanoshells are a new class of nanomaterials with high plasmon importance, consisting of a dielectric silica core surrounded in a highly conducting, ultrathin layer of silver or gold. This allows resources to be precisely wangled to equal the wavelength for specific usage, such as near-infrared (NIR) zones where optimal light penetration over tissue is required [7, 98]. Surface-enhanced Raman spectroscopy (SERS) is being investigated as a viable platform for in vivo exposure on nanoshell substrates [99–101]. Nanocages are heavy nanostructures with absorbent walls, often composed of decent metals [69, 102]. They have a lot of promise for biofunctionalisation and biomolecule immobilisation because of their large surface area. They have uncommon electrical and thermal physiognomies. Noble metal nanowires have SPR-like physiognomies which can be adjusted depending on their thickness [103–105]. Like nanoneedles, nanowire arrays may penetrate cellular lipid bilayers, allowing for cytosensing and other medical identification applications. Silicon oxide NW can be utilised as a substance for structure immobilisation in FET biosensors [7, 104]. Inorganic nanoparticles have been utilised in healthcare diagnostics in a number of ways. We will concentrate on the most widely used nanomaterials, like magnetic nanoparticles, quantum dots, and nanostructures like nanoshells, nanowires, and nanocages, in this study.

6.3.1 Nanowires

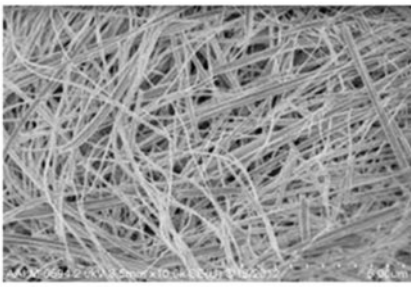
Nanowires (NW) are a type of mono-dimensional nanoparticle that comprises nanotubes, nanobelts, and nanorods, among others. NWs are at least 1000 times their diameter in length. NWs may be made from semiconducting materials like Si, InP, and GaN, as well as dielectric materials like TiO_2 and SiO_2 [37, 106]. The LSPR characteristics of noble metal NWs are thickness dependant. Because of its conductive characteristics, SiO_2 NWs are frequently used in FET biosensors and have a lot of potential in healthcare biosensing. The use of NWs as substrates on behalf of receptor control to allow requisite with different bioparticles leads in sensitive perception, fast analysis, and the possibility of shrinking [104]. Nuzaihan and coworkers



Silicon Nanowire



Silver Nanowire



TiO_2 Nanowire

Fig. 6.3 Various types of nanowires

recently disclosed a silicon nanowire (SiNW)-based microfluidic electrical sensor for sensitive detection of the dengue virus (DENV) DNA oligomer [107]. On a silicon wafer, the SiNW was created using a top-down method. Surface alteration, DNA immobilisation, and DNA hybridisation were then used to functionalise the SiNW. The sensor was able to reach a LOD of 2.0 fM because to its esoteric molecular gate control technique. Kim et al. developed a silicon nanowire FET biosensor with a honeycomb nanowire (HCSiNW) design for ultrasensitive detection of cTnI. [108]. The Debye effect gave the gadget excellent sensitivity and selectivity. cTnI antibodies were immobilised on the surface of the HCSiNW. Vertically oriented platinum nanowires had a greater density of AuNPs than a 2D planar modification. The nanowires boosted the electron compactness on the electrode surface, which improved the signal-to-noise ratio. Figure 6.3 depicts several nanowires utilised in sensor applications.

6.3.2 Nanowire-Based Sensors

The major research carried in the field of nanobiosensors deals with nanowire-based devices. Due to its extremely small power consumption and larger stable time

period, detection of biomolecules and chemical species [109] become advantageous. Working principle of these sensors critically depends on the fabrication methods, and electrical properties, as briefly outlined in the previous subsection. However, there are ample opportunities for its development for detecting subnanometric organisms with major fabrication challenges. Owing to large increase of surface-to-volume ratio compared to bulk counterpart, these sensors found in drug discovery and DNA detection. Carbon nanotube-based sensors are of special category for ultra-sensitive biomolecule detection [110]. It has another advantage if higher oscillator strength and extremely low thermal noise [111], which facilitates minute detection. This is the special type of one-dimensional nanostructure which is able to detect present of species electrically [112]. This type of detection also helps to detect label-free biosensors. Owing to higher sensitivity and scalability, these nanobiosensors have been appreciated from practical stand-points.

6.3.3 Quantum Dots

The traditional inorganic QD is a bimetallic substance centre via a shell region, such as chalcogenide. Despite the fact that the thickness of QDs is smaller than the Bohr radius of the electron-hole, quantum confinement effects dominate, resulting in distinct visual properties. Stokes shifts generated by NIR or UV electromagnetic radiation during eager electron relaxation to holes provide stronger photoluminescence than organic dyes. Proteins [113, 114], infections [107], lung cancer biomarkers [115], and nucleic acids [116, 117] are among the substances that have been detected using QDs. Nonetheless, the toxicity of cadmium, a frequent component in quantum dots, in addition to the propensity of defensive coatings to degrade in vivo are preventing broad in vivo usage of quantum dots [118]. Using QDs on a microfluidic device, a wide range of target molecules may be identified efficiently. A sensor like this was recently utilised to identify and subtype three influenza viruses (H_1N_1, H_3N_2, and H_9N_2) [119]. Streptavidin-coated quantum dots (Str-QDs) with immobilised biotinylated DNA served as fluorescent imaging labels, while capture probes were DNA-immobilised super paramagnetic beads (SMB). The sensor worked by using nucleic acid hybridisation on a microfluidic device with a regulated micromagnetic ground.

Another research on protein recognition found that metal oxide nanoparticles of Eu_2O_3 and CuO, in addition to decent metal nanoparticles of silver and gold, can destroy the fluorescence of CdSe QD [113]. The fluorescence movement of QDs was reinstated with the addition of analyte, since analyte–QD communications released QDs from the nanoparticle–QD complex. The fluorescence intensity was also enhanced by the communications between the proteins and the QDs. A combination of QD fluorescence with immunomagnetic separation (IMS) has been shown to provide favourable results in pathogen identification [107]. Magnetic nanoparticles with gold shells and biotinylated *E. coli* antibodies were used as detention probes for *E. coli*, while chit-coated CdTeQds were used as reporter probes in a sarnie

immunoassay. IMS was used to eliminate the bacteria from the sensing elucidation before moving on to fluorescence investigation.

6.3.4 Quantum Dot-Based Sensors

Quantum dot-based nanobiosensors make their mark for selective identification of diagnosis. Owing to their tunable optical properties, i.e. emission/absorption spectrum, biosensors made by semiconductor QD already exhibit superior sensitivity and also photo-chemical property [120]. For selection of specific DNA, protein, enzyme, mRNA, etc., quantum dot-based nanobiosensors are already proved their salient features as reported already in various literatures [121]. For antibiotic detection, quantum dot-based biosensors are researched [122] and proved efficient. Material parameters in this respect play a great role in determining the sensitivity of the sensor. Carbon quantum dots are used for early stage cancer biomarkers [123] compared to semiconductor QDs. It has the additional feature of better water solubility which is exhibited as the biocompatibility.

6.3.5 NWFET-Based Sensors

Nanowire field-effect-based transistors have been centre of attraction in last few years owing to possibility of making ultra-sensitive sensors for detecting mostly label-free specimens. Junctions are sometimes made of Schottky types in order to make to safe during operation [124] where devices are made on silicon-on-insulator wafers. Investigations at cellular levels are now become possible because of these sensors, thanks to the surface functionalisation and other transistor properties [125]. For different subcellular structural diagnosis, these sensors are proved as extremely useful, precisely because of their lower noise generation. Very recently, it is reported that grapheme FET can detect SARS-CoV-2 [126] in less time which may usher a new direction of Covid-19 treatment.

6.3.6 Magnetic Nanoparticles

Superparamagnetic nanoparticles include Fe_2O_3, Fe_3O_4, FePt, and a variety of other related nanoparticles. These particles can have a wide variety of sizes, ranging from 10 to 1000 nm, depending on their production method. MNPs are commonly used as either transducers or tags in biomolecule conjugation. MNP biosensors have applications in a variety of fields, including food science, medical diagnostics, and environmental research [127]. Before MNPs may be employed in healthcare biosensing, three prerequisites must be met: (1) to manage their mobility in blood without the

need for highly intense magnetic fields, MNPs must retain a greater intensity magnetisation. allowing MNPs to transfer close to the beleaguered tissue; (2) MNPs should be biocompatible and non-toxic; (3) MNPs should be between 10 and 50 nm in size to avoid combination or precipitation due to gravitational forces and to confirm colloidal constancy, especially in water at pH 7.0, resulting in a large superficial zone for a precise volume of the material [128, 129].

The use of MNPs in cancer detection at an early stage has shown great promise. Pal et al., for example, used monoclonal antibodies (mAbs) to multiplex MNPs for detecting ovarian cancer biomarkers (cancer antigen 125 (CA-125), Apo-lipoprotein A1 (ApoA1), and 2-microglobulin (2-M)) [130]. Polyclonal antibodies were used to create a sandwich test (pAbs). With the aid of magnetic force, the sandwiched elements were then detached from the sensing position. Simultaneously, the fluorescence change was monitored in real time against a standard concentration. Pathogens are frequently identified using MNPs.

6.4 Conclusion

Several nanomaterials and corresponding sensors are investigated some of which are highlighted in this chapter based on their spectra of application as well as their acceptance among scientific community. Properties of these sensors are critically depended on their electronic and optical properties, and therefore, material aspect is also taken care into consideration while making a detailed review. A comprehensive literature search is carried out on major nanobiosensors, and most favoured outcome from application stand-point is reported.

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Chapter 7
Nanomaterials and Nanodevices for Treating Human Infectious and Inflammatory Diseases: Bane or Boon for Human Health?

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Abstract The introduction of nanoscience and nanotechnology has been a path-breaking achievement of modern science and engineering. Development and fabrication of biocompatible nanoparticles, nanocomposites, nano-scaffolds, nanowires, etc., and their stable conjugation with biological molecules indeed came out fruitful in developing nanomaterials and nanodevices for diagnosing and treating life-threatening human diseases. Nano-sized smart materials possess several unique structural and functional traits like controlled bioactivity, superior penetration ability, and sustained action over the treatment time. All these properties have led to the inclusion of several metals and non-metal derived nanomaterials as well as hybrid nanocomposites for diagnosing different infectious and inflammatory complications of humans. Nowadays, nanobiosensors can efficiently trace the affected tissues or diseased organs while targeted delivery of nanoparticles or drug-loaded nano-cargo can provide a suitable outcome within less treatment schedule. However, several important parameters of the nanocomposites like size, drug-loading methods, dose of the drug/nanoparticle-drug conjugate, and bioavailability are of major concern to yield the desired efficacy of a nanoformulation. Alteration or disproportion in any of the aforementioned parameters could induce toxic side effects. Considering all the facts, the present chapter depicts a comprehensive overview of the properties,

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bioactivities, molecular mode of action, and toxicity of various nanomaterials and nanodevices having the potential to diagnose and treat various inflammatory and infectious diseases in humans.

7.1 Introduction

For the past three and half decades, nanomaterials (NMs) or nanoparticles (NPs) are considered to be one of the majorly essential and researched materials. NPs had also received attention as "material of the twenty-first century" attributed to their distinctive physic-functional arrangements [1]. Applications of NPs are wide and diverse and also exploratory with varied human and animal benefits. Some of the wide varieties are health-medical devices, engineering and manufacturing applications, biomedical relevance, electronics, sensor and diagnostics, and also the environment [2].

In recent years, additional interests have also been provided toward applications of NPs with novel implications on nanotubes, nanowires, fullerene derivatives, and quantum dots for the manufacture of new or improved analytical tools that can be applied in medical, bioscience, and biotechnology [3]. Considering the importance and applications and also enormous future aspects, nanomaterials are among the most studied worldwide. Most nanomaterials represent specific structures; however, they can only be included in this specific class of materials only if they possess less than 100 nm in one dimension minimum [1]. It also is kept in comprehension that the structural attributes of NPs require accurate experimental evidence and that too associated with the precise experienced expertise. Correct and elaborate diagnosis of the physical attributes of NMs is fundamental for the correlation of their biological effects in the application management [4]. For the application assessment of NMs, it is imperative to evaluate them with a specific objective, evaluating the requirement of the minimum amount, and definitely, the results or effectivity have to be reproducible. Furthermore, during the application evaluation which most of the time requires considerable time, the molecular markers needed to be stable [5]. NMs can have various applications; however, it is greatly significant to determine the most prolific application with higher specificity and sensitivity [6]. Additionally, steady inventiveness, detection, rational application objectives as well as exploring commercial possibilities, and clinical implementation can eventually reflect their acceptability.

Nanotechnology and nanoscience are continuously evolving as a stalwart in the field of application-based science and are attributed to the development of novel and enhanced biosensors for the detection and supervision of various diseases [7, 8]. The application of nanotechnology to design biosensors can significantly enhance the prospect of early cancer detection and thus can also achieve overall treatment betterment and mortality reduction [9, 10]. There is every possibility to further improve the existing diagnostic procedures and also provide new ones over the present diagnostic measurements. For this purpose, we have to critically assess the pros and cons of

the existing ones and the betterment of current technologies. Some diagnostic techniques do have a major drawback because of their shortcoming to fully and critically measure the biomarkers in all cases and even can be sometimes unobserved and un-identified [11]. NMs be able to be utilized during imaging to monitor and evaluate the altered, metamorphosed, or progressing diseased states many a time more sensitively and reliably than the existing compounds [12]. In this aspect, NPs that are utilized are liposomes, dendrimers, carbon nanotubes, etc., with the possibility of marked improvement in the imaging procedures of diseases [13]. Nanoscience applications can also result in manufacturing very small sensors, that can be moderately cheap with superior recognition capabilities of disease markers resulting in high-performance detection [7, 8]. Nanotechnology application during biosensors production can augment the probability of earlier cancer detection and lead to the betterment of overall treatment efficacy along with a reduction in mortality [10, 14].

We humans, in spite of boasting an efficient immune system and various severe and critical infectious and inflammatory ailments, do possess major concerns over our health and are liable for considerable mortality. Infections because by viruses (dengue, polio, hepatitis B and C, human papillomavirus, herpes simplex virus, Rotavirus, etc.), bacteria (*Salmonella typhi*, *Mycobacterium tuberculosis*, *Clostridium tetani*), fungus (*Candida Spp.*, *Aspergillus spp.*, etc.), and parasites both protozoan and metazoan (*Plasmodium falciparum*, *Entamoeba histolytica*, *Leishmania donovani*, *Fasciola hepatica*, *Wuchereria bancrofti*) affect in global morbidities and mortality [15, 16]. There are also complications or diseases mediated or modified by dysregulated immunity, viz. cancer, diabetes, rheumatoid arthritis, inflammatory bowel disease (IBD), different types of allergies, and autoimmune diseases, additively causing the death of millions of people [15, 17, 18]. Regretfully, despite several therapeutic measures like drugs, vaccines, and probiotics/prebiotics, the implication of therapeutic strategies is limited due to the lack/inefficiency, toxicity, and price [19–21].

7.2 Nanoparticles: Bioactivities and Molecular Mechanism(s) of Effectiveness

NMs are having dimensions in the range of 1–100 nm and also possess a superior surface-to-volume ratio due to their minuscule size. The higher surface-to-volume ratio is immensely important as well as the reason for many of the amazing functions and properties that NPs are extrapolative of. Some of the functions owing to this ratio are diagnosis maximum, imaging, prospects of enhanced drug delivery to diseased states, very confined and extrapolative places inside the body, or even sub-cellular organelles and environments that were earlier were unachieved and unreached [22, 23]. For diagnostic intentions specifically against malignancy, NPs are useful in capturing cancer biomarkers like cancer-associated proteins, circulating tumor DNA, circulating tumor cells, and exosomes [24]. NP facades can be efficiently enclosed

with antibodies, peptides, small molecules, aptamers, and some other molecules and thus can be extremely useful and important for a novel diagnostic purpose against specific cancer molecules as well as for therapeutic purposes. With NPs, the linking of ligands to the cancer cells can also be achieved to improve the diagnostic specificity and sensitivity attempt [25]. The downsizing of electronic transducers in NPs administrates them to become more biocompatible and nature-relevant and endorsed for the near-nature level sensitivity [26]. A neural circuit present at the cellular and subcellular level utilizes nanobioelectronic devices that resulted in negligibly invasive recordings [27]. Nanowire-nanotube heterostructures or probes proved to penetrate cell membranes spontaneously for a tough membrane seal of high resistance with the phospholipid to give the desired response [27]. Thus, intracellular sensing is also feasible, which is novel, non-invasive, and cost-effective during cancer diagnosis [27]. Microfluidic lab-on-a-chip (LOC) devices are an example [28] which are a cost-effective, compact, and easy-to-use device and also minimize the dependency on a laboratory's complexity. Quantum dots are also highly praised as a nanoapplication [29]. Nanocrystalline quantum dots show fluorescence, luminescence, and phosphorescence, in addition to many other properties, and some are also exclusive of the particle and can be widely applied to construct optical biosensors to screen malignancy [29]. Quantum dots release photons of diverse intensities, wavelengths, and spectra and thus prove effective in diagnosing and detecting various unique molecular elements [30]. Some of them in respect to medical applications are confirmation of cellular aberration, existence, and differentiation of certain bio- or disease markers and progress of chemotherapeutic applications [29]. Also, many applications are unexplored and provided they are being discovered incessantly, quantum dots hold great promise and advantage in biomedical applications in various ways [29]. Quantum dots can also be able to act as cite-specific delivery systems with therapeutic agents that can considerably reduce toxic effects and enhance pharmaceutical efficacy [31]. The invention of novel nanobiosensors is emerging as an interesting area of interdisciplinary research. NMs are also aided in the transformation of normal biosensors to ultra-sensitive biosensors with the application benefits of disease detection [32]. Improvements in the application due to better biocompatibility enhanced electrocatalytic activity with the high surface region of detection and finer electronic characteristics. In the coming years, it is anticipated that nano-diagnostics will emerge as incredibly handy to detect various biomarkers at the same time with precision and cost-effectively [33]. Nanobiosensors apart from being ultra-sensitiveness toward cancer biomarkers can also open new opportunities during the early detection of diseases which is critically helpful in allowing longer, less detrimental, and cost-effective treatment options. However, nanodevices with such applications are still in the developmental stages and are expected to be feasible [34]. Nanotheranostics is a comparatively new area of application and with precautionary approaches where nanodevices are engaged in persons before any obvious symptoms of illness or diseases and specific anticipated monitoring can be employed [35]. This nanotheranostics with this powerful approach of screening and scrutiny can help cure such maladies during the initial stages providing considerably helpful time for therapeutic intervention to the healthcare professionals. There is no doubt that

early detection and timely treatment are immensely beneficial and utmostly sought for patient survival and NMs can be very helpful in this purpose. Future preference in disease diagnostics as research on NMs suggests likely will to replace biochip technology with the nanoscale array [36]. Analysis of biofluids by nanobiosensors can be the pioneer. Nanobiosensors are expected to be ultra-sensitive toward the detection of cancer biomarkers and that too in their primitive stages. However, nanodevices for such applications are still not achieved [37]. Table 7.1 summarizes the available NPs against cancer while nanomedicines currently employed for treating viral infections are listed in Table 7.2.

"Nanopharmaceuticals" surround and include the discovery, development, and application of reformulated chemotherapeutics (nano-conjugated) or drug-conjugated NPs for medicinal intention [39]. Nanopharmaceuticals also engineer the amalgamation and size diminution of active bio-substances or molecules previously or currently in use and by doing so enhance the competence and effectiveness as well as reduce toxicity [40, 41]. The augmented particle surface-area ratio in the NPs with the decreased particle volume enhances as well as endows with comprehensive or special physio-chemical-bio properties (biochemical, electronic optical, and magnetic characteristics) against the native original conformations [5]. In the

Table 7.1 Nano-based approach for the treatment of cancer

Nanoparticle	NP formulation	Cancer targets	Trial name, status
Doxil®, Myocet®, Caelyx®	Liposome packed Doxorubicin	Breast cancer, ovarian cancer, Kaposi's sarcoma, multiple myeloma,	FDA approval 1995 [38]
DaunoXome®	Liposome packed Doxorubicin	Kaposi's sarcoma	FDA approval 1996 [38]
Abraxane®, ABI-007	Nanoparticle albumin-bound paclitaxel	Breast, lung, melanoma, pancreatic	FDA approval 2005 [38]
Nanotherm®	Iron oxide nanoparticle	Glioblastoma	EU approval 2010
Marqibo®	Liposome vincristine	Acute lymphoblastic leukemia	FDA approval 2012 [38]
Onivyde®	Liposome packed Irinotecan	Metastatic pancreatic cancer	FDA approval 2015
Vyxeos®	Liposome packed Daunorubicin and Cytarabine	Acute myeloid leukemia	FDA approval 2017
SPIO MRI/Ferumoxytol®	Super-paramagnetic iron oxide nanoparticles and MRI	Pancreatic cancer	Phase IV (2008–2017) [38]
NBTXR3	Crystalline NP and radiation	Soft tissue sarcoma	Phase II//III (2015–2020)

Table 7.2 Nanomedicines for the treatment of viral infections

Name	Company	Description	Mechanism of action	Pathogen	Approval year/stage
InflexalV®	Crucell (former Berna Biotech Ltd.)	Virosomal (150 nm liposomes) vaccine	Liposomes coated with highly purified surface antigens of strains A and B of the influenza virus mimic the native antigen presentation process which produces high immunogenicity	Influenza	Phase 3 clinical trial (number: NCT01310400)
Epaxal®	Crucell (former Berna Biotech Ltd.)	Inactivated virosomal (liposome) vaccine	Formalin inactivated HAV (strain RG-SB) adsorbed to the surface of special liposomes (virosomes), which mimic the natural antigen presentation process and enhance immunogenicity	HAV	1999
PegIntron®	Merck	PEGylated interferon alfa-2b	Improved stability of protein through PEGylation	HCV	2001
Pegasys®	Genentech	PEGylated interferon alfa-2b	Improved stability of protein through PEGylation	HBV, HCV	2002
Influvac® Plus	Solvay pharma/abbott	Virosome vaccine	Containing influenza surface proteins neuraminidase and hemagglutinin	Influenza	2005
Fluquit™ (STP 702)	Sirnaomics Inc.	Short interfering RNA (SiRNA) therapeutic	Gene silencing	H5N1 and H1N1 influenza	Preclinical evaluation
Cervisil® (STP 909)	Cervisil® (STP 909)	Short interfering RNA (siRNA) therapeutic	Gene silencing	HPV	Preclinical evaluation

(continued)

Table 7.2 (continued)

Name	Company	Description	Mechanism of action	Pathogen	Approval year/stage
VivaGel® (SPL 7013)	Starpharma	Dendrimer	Lysine-based dendrimer with sodium 1-(carboxymethoxy) naphthalene-3,6-disulfonatesurface groups	HIV, HSV	2006
DermaVir	Genetic immunity	Therapeutic vaccine	Immunological induction to HIV-specific precursor/memory T cell pool by expressing synthetic pDNA for 15 different HIV-specific antigens	HIV	Phase 3 development as a therapeutic HIV vaccine
Doravirine (MK-1439)	Merck & Co., Inc.	Solid drug nanoparticle formulation	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	HIV	Clinical trial (number: NCT02549040)
ARB-001467 TKM-HBV	Arbutus biopharma	Wet lipid nanoparticle	Lipid particle containing three RNAi therapeutics that target three sites on the HBV genome	HBV	Phase 2a clinical trial (number: NCT02631096) completed in 2018

HAV Hepatitis A virus; *HBV* Hepatitis B virus; *HCV* Hepatitis C virus; *HIV* Human immunodeficiency virus; *HPV* Human papillomavirus; *HSV* Herpes simplex virus

year 1974, Norio Taniguchi popularized the phrase "nanotechnology" and postulated it as materials of nanoscale dimension [42]. The effectiveness of conventional medicine can also be improved several times by conjugating them with the NPs [5]. Alongside, limitations like insufficient effectiveness, limited biodistribution, and bioavailability being deficient in spatial selectivity can also be improved with NPs and thus can be considered advanced therapeutic speculation [43]. More importantly, most of the time the toxic side effects of a chemotherapeutic can be reduced with NPs when administrated either directly or passively [44]. Fascinatingly, the dosage of the conventional therapeutics can be reduced significantly with nanopharmaceuticals without altering the basic way of its mechanism of action or site specificity [45–47]. The compact size of the engineered "nano" conjugated with drug/medicine with suitable delivery systems is advantageous having improved stability, solubility, absorbance, dissolution potential, decreased dose, reduced toxicity, rapid action, etc. [23, 48, 49]. Interestingly, nanopharmaceuticals can be of comparable dimension to the biomolecules (receptors, antibodies, and nucleic acids) and consequently appropriate for definite targeting and superior bio-imaging applications as well [50]. Moreover, these therapeutics when applied go through different intricate interactions within the body [45]. Therefore, sometimes nanoformulations intended for specific preferred purposes resulted in unwanted outcomes beyond control for the complex interactions within the biological system [51]. The complex interactions are mostly because of the particle size, shape, and surface chemistry of the synthesized NPs [52]. Therefore, if possible, consequences or activities like cellular uptake, biodistribution, and removal from the targeted sites should be considered during NP formulation [53, 54]. NPs in size less than 10 nm are cleared through the kidneys, and NPs larger than 10 nm in size are removed from the body by the liver and mononuclear phagocyte system [54]; therefore, upon the clearance strategies NPs can also be formulated [55].

Ag-NPs are being applied in medicinal devices, medicine, biotechnology, pharmacology, engineering, electronics, magnetic fields, energy, and environmental remediation [56–58]. Ag-NPs also have proficient antibacterial activity, and therefore, in industrial sectors including textiles, consumer products, food, medicine, etc., it is attaining attractiveness [59]. (Fig. 7.1 graphically depicts the possible way of NPs-mediated bactericidal functions). Presently, Ag-NPs are comprehensively used in paints, women's hygiene products, the food industry, clothing, electronics, medical devices, biosensors, cosmetics, sunscreen, etc. [60, 61]. The high surface-area-to-volume ratio augments the interaction of NPs with serum, saliva, mucus, and fluid components of the lung lining and is possibly accountable for their enhanced effectiveness [62, 63]. Complications or challenges are also there, and the strong oxidative property of Ag-NPs liberates silver ions, which can mediate immunological responses, cytotoxicity, genotoxicity, and also cell death [59, 62]. The need for continuous research and exploration of remedial opportunities is viable with Ag-NPs application as the use of Ag-NPs is associated with unwanted erratic concerns because of their unwanted or unexpected communication with biological systems [59, 62, 64].

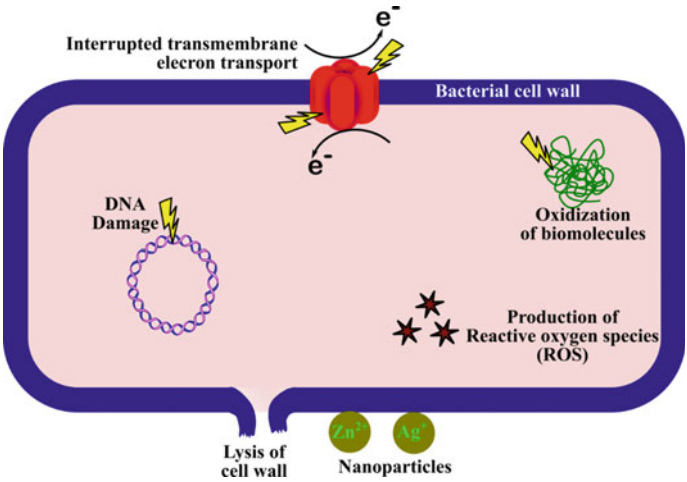

Fig. 7.1 Nanoparticles mediated killing of bacteria cells. Certain NPs and their ions produce free radicals that lead to the formation of reactive oxygen species (ROS). ROS induce damages in bacterial proteins and nucleic acids and eventually lead to bacterial death

Therefore, the vast application possibilities of Ag-NPs are considerably restricted and scrutinized over human exposure. Ag-NPs can also traverse the blood–brain barrier (BBB) by the process of transcytosis of capillary endothelial cells and also get access to various other important parts of the body or tissues. This efficient passage of Ag-NPs unfortunately can also be liable for many unwanted misfortunes among the recipient and is subject to a major concern [65, 66]. Among many modes of bio-effectiveness of Ag-NPs, a more elaborated one is apoptosis which is confirmed by loss of mitochondrial membrane potential, down-regulation of Bcl-2 (anti-apoptotic protein), upregulation of BAX (pro-apoptotic protein), and cytosolic release of cytochrome c. Bcl-2 down-regulation is influenced by JNK (Jun amino-terminal kinases). JNK MAPK family member participates during apoptosis through Bcl-2 phosphorylation leading to Bcl-2 inactivation. Cytochrome c after being released into cytosol instigates a cascade involving activities of apaf-1 and caspase 9 to ultimately activate caspase 3, the apoptosis executioner [67, 68].

7.3 Current Scenario of Nanomaterials-Based Treatment

Chances of drug resistance among pathogens over time are always a grave concern and which results in efficiency decrement of the chemotherapeutics previously well advised and sooner or later deter the optimum pharmacological activity of them at the preferred site of action [19]. The efficacy of many compounds is found to be extremely reduced because they have to move a long trail inside the body to reach their place of action and even can be hindered by poor bioavailability [69].

In recent decades, incredible improvements are carrying out in the field of health care-associated nanoscience [42]. Innovative drug delivery systems with precisely engineered chemotherapeutics to achieve better and more competent penetrability and absorbance (tight epithelial junction, blood–brain barrier) potential are being explored to counter different complaints [70]. (How the NPs can overcome the drug resistance is depicted in Fig. 7.2.)

Research efforts from many scientists established the fact that NMs can be very effective against various ailments in humans [50, 70, 71] and they also can effectively

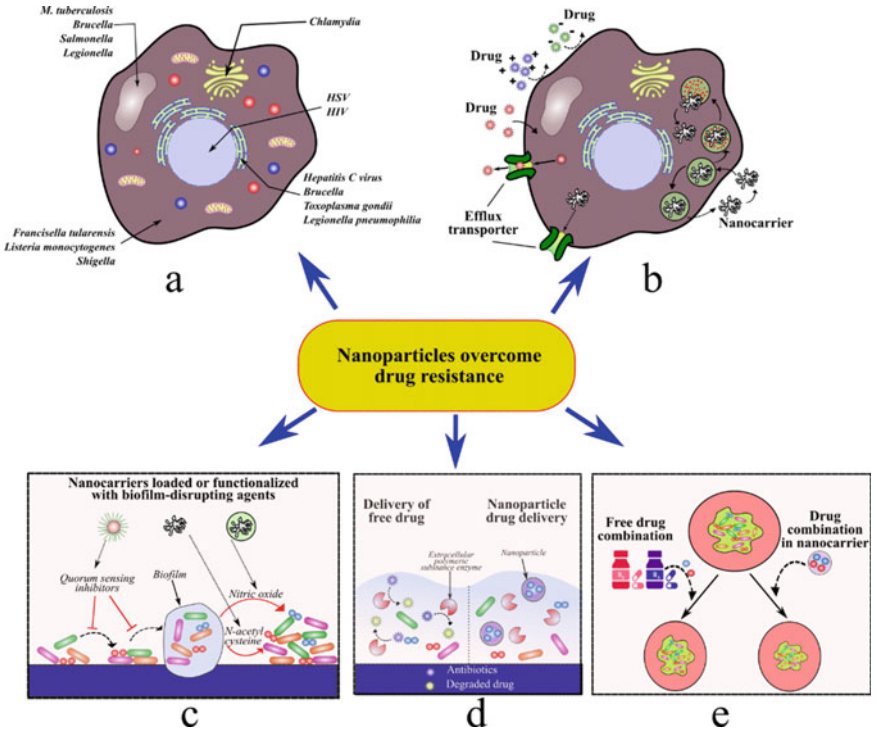

Fig. 7.2 Nanocarriers to overcome drug resistance. Nanocarrier systems can be designed to evade physical and chemical mechanisms for microbial drug resistance. **a** Pathogens can develop resistance to conventional drugs by hiding themselves in intracellular foci that are poorly accessible to drugs. **b** The drawbacks of conventional drugs (such as low cellular uptake of high polar drugs and frequent removal of drugs by efflux transporters) lead to incomplete removal or development of drug-resistant strains of intracellular pathogens. Nanocarriers have been used to improve cellular uptake of drugs and improve their activity. **c** Nanocarriers can be loaded or surface-functionalized with moieties that disrupt quorum sensing or the biofilm matrix. **d** Nanocarriers in the biofilm can enhance the effect of drugs by protecting the drugs from drug-degrading enzymes. **e** Co-encapsulation of drug combinations in nanocarriers has been shown to produce improved efficacy over co-administration of the free-drug combination

avoid the toxic side effects on the host [50, 70]. Drug delivery systems are actually of immense importance to achieve maximum effectiveness, best pharmacological benefits, and minimum undesirable reactions in the human body. Drug delivery systems are also important particularly considering that they can prevent or minimize the denaturation/inactivation/degradation of chemotherapeutics by productively and efficiently transporting therapeutic molecules to the target sites [53, 72, 73].

Therefore, polymeric drug delivery systems can be persuaded to augment the potential of the drugs at much lower concentrations and also can restrict the side effects [5, 72]. As it has been described that targeting the pathogens in different parts of the body where they reside by employing complex and sustained interactions involving immunological, physio-biochemical, spatiotemporal, and physical means can be troublesome. Therefore, inventing novel therapeutic means is necessarily hoping that they possibly overcome this problem [15, 56, 74]. A targeted and controlled site-specific drug delivery system can be immensely beneficial as it is associated with amended bioavailability and pharmacokinetics [72]. NMs having better penetrance to biological systems, site specificity, and slow release with maximum retention time with minimum toxicity are desired chemotherapeutic option against various pathogenic complications [45, 74].

Liposomes are confined microscopic vesicles (diameter ranging from 20 nm to micrometers) and comprise phospholipids with surplus water over their transition temperature [75]. Among the major forms, unilamellar ones are having a single phospholipid layer around the aqueous core, whereas the multilamellar form is with concentrical phospholipid bilayers separated by aqueous layers [76]. Interestingly, because of their physio-chemical attributes liposomes can house lipophilic molecules in the lipid bilayers as well as hydrophilic molecules simultaneously in the aqueous spaces [76]. Liposomes are evaluated as a therapeutic option against several pathogenic infections with variable success, namely influenza, human immunodeficiency virus (HIV), dengue, tuberculosis, malaria, lymphatic filariasis, etc. [77, 78]. Liposomes were also reported as the first nanocarriers to enhance the effectiveness of antifilarials [40], and immunoliposomes are used to clear circulatory microfilariae with considerable success [47, 79]. For this purpose, immunoliposomes have been used DEC and there are also reports of the effectiveness of immunoliposomes against other infectious diseases [77, 80]. To add, though there are noteworthy advancements in liposome-mediated drug delivery owing to better target selectivity, further investigations are necessary to speculate and advocate for the extensive use of these nanopharmaceuticals.

Biodegradable polymeric NPs for controlled drug delivery have now been widely documented [49, 81, 82]. The foremost concerns regarding them are their biocompatibilities [82]. Additionally, and importantly, these nanopharmaceuticals are stated to penetrate the blood–brain barrier (BBB) and ophthalmic barrier for a prolonged presence as well as the sustained release of the required drug [5, 83]. The drug release can be initiated from the polymeric NPs by diffusion and/or dissociation into monomeric structures precisely based on the kind of drugs used [84]. The nanodimension of the polymeric NPs can also be subjected to better pharmacokinetics and administered in various and different ways and regions of the body [85]. Promisingly,

recent reports also provided evidence that polymeric NPs formed by biocompatible polylactic acid (PLA) or glycolic acid (PLGA) can be released from the body of the administered subjects through the normal metabolic pathways [86]. However, these polymeric NPs are reported to frequently promote an opsonization response that can initiate phagocytosis by the immunological cells; however, polyethylene glycol can sometimes minimize this function when coated externally [53].

Polymeric NPs-based delivery systems had widely been reported against parasitic infestations and the potential of nanopharmaceuticals for being better antifilarial drug delivery systems relying on their access to the lymphatics [84, 87]. Polymeric nanoparticles were found to be efficient in regulating the growth of the Plasmodium falciparum K1 strain in vitro [88]. Moreover, halofantrine-loaded PEG-coated polylactic acid (PLA) nanocapsules were also found to be effective against malaria [89]. Additionally, primaquine-loaded polyalkylcyanoacrylate (PACA) NPs are also effective against amastigote stages of *Leishmania donovani* and nifurtimox-loaded polyethyl cyanoacrylate nanoparticles against *Trypanosoma cruzi* [90, 91]. Interestingly, polymethyl methacrylate caused stabilization and controlled release of pentamidine at lower pH with improved transport potential [92]. Poly (lactide-co-glycolide), poly (L-lactic acid), polyhexylcyanoacrylate, and polymethylmethacrylate can be useful to access drugs in lymphatics, therefore, recommended for targeting antifilarials [93]. The polymeric nanopharmaceuticals are also advised to be in a range to be maximally utilized in the lymphatic system [45, 93]. Different studies confirmed that particle diameters of 20, 45, and 100 nm of polyethylene glycol (PEG)-stabilized polypropylene sulfide are appropriate for lymphatic uptake with 20–70 nm diameter being the most suitable [94, 95]. Hyaluron, folate, dextrin, L-selectin, lectin, and Lyp-1 are considered important ligands for antifilarial drug developments [96]. Additionally, a sugar-mediated drug delivery system can be of novel strategy against lymphatic filariasis [97]. It was also reported that Wolbachia (a bacterial endosymbiont of selected filarial parasite) surface-exposed mannose receptors can be targeted with lectin-coated NPs [98]. (Diagnostic properties explored by NPs are graphically presented in Fig. 7.3).

Solid lipid nanoparticles (SLNs) are immensely evolving as novel therapeutics with a potential of diverse clinical relevance as they are unique size-dependent NPs with high lipophilicity [99, 100]. SLNs do have considerable protection from the harsh chemical and enzymatic constituents inside the host body, and this property is also protecting the associated chemotherapeutic molecules [99]. SLNs are proposing a considerably stable core to load both hydrophobic and hydrophilic chemotherapeutics and can even carry two active agents and can consider relatively easy to synthesize [99]. Importantly, SLNs are decomposable and increase the bioavailability of the associated drug several folds to reduce the dosage frequency. Thus, SLNs promote controlled, sustained, and slow release of drugs. These NPs can also avert unwanted immune responses, minimize resistance possibility, and increase drug retention which cumulatively assist in an advanced therapeutic index, reduced toxicity, and improved treatment efficiency [101]. Figure 7.4 depicts the use of NPs to induce immune responses for enhanced vaccination.

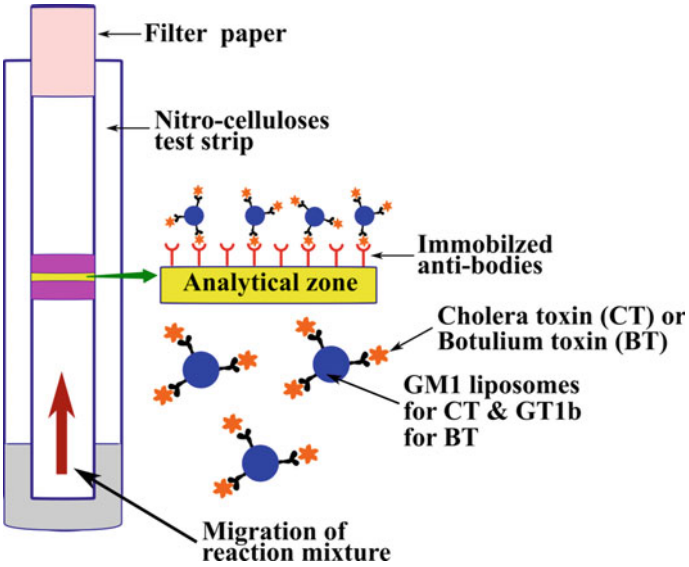

Fig. 7.3 Test strip assay format. Toxins are captured by the gangliosides attached to the liposome exterior. The toxin-bound liposomes travel through the nitrocellulose test strip. Toxins bound to the liposomes are seized by anti-toxin antibodies in the analytical region, producing a color change that indicates the existence of toxins

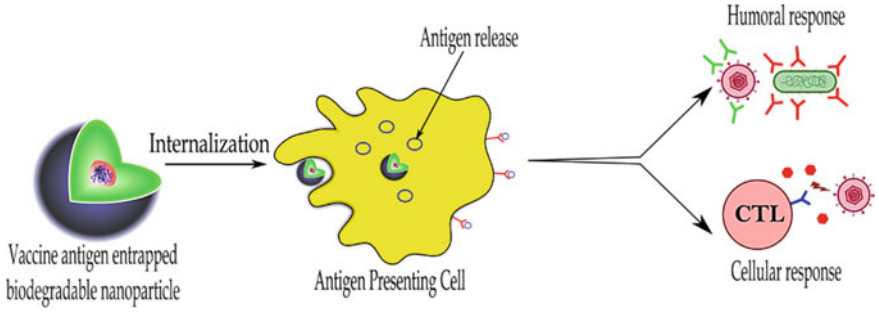

Fig. 7.4 Use of nanoparticles to induce immune responses for enhanced vaccination

Studies have shown that doxycycline-loaded SLNs can intensely affect *Brucella melitensis* even when they are inside the macrophages compared with free doxycycline [102]. Similarly, the efficiency of rifampicin-loaded SLNs on *Brucella abortus* is considerably enhanced [103]. The therapeutic efficiency of rifampicin against tuberculosis is because of the mannosylated formulations acting as lectin receptor ligands on macrophages [104]. Additionally, SLNs are also effective against quite a few parasitic diseases affecting the brain, e.g., Trypanosoma or malaria specifically during their infection of the brain or central nervous systems (CNS) [105]. Transferring-conjugated SLNs with quinine dihydrochloride were explored with

success against cerebral malaria having upgraded presence in the brain [106]. Lipid conjugated diminazene diaceturate is reported to be useful against trypanosomiasis [107]. The SLNs can cross the lymphatic barrier efficiently and thus propose a better drug delivery system against lymphatic filariasis [97]. It was also shown that Efavirenz SLNs are competent in lymph targeting when controlling HIV infection [108]. Additionally, corroborative in vivo and in vitro experiments advocated for praziquantel-loaded SLNs for better intestinal lymphatics [109]. Apart from lymphatic targeting, mitoxantrone (MTO)-loaded SLNs were found to be more effective against lymph node metastases and breast cancer [110]. Further studies have revealed that SLNs are also beneficent to increase the concentration of chemotherapeutics at the target site, particularly when in lymphatics [111]. Cumulatively, SLNs propose themselves for better therapeutic and drug delivery system options for lymph-dwelling diseases including filariasis [111]. The effectiveness of the SLNs against different bacteria also makes them a possible candidate to target the Wolbachia endosymbiont of various filarial parasites [112]. Extended retention characteristics and site-specific deliberate release of drug molecules can be an added advantage for the SLNs to be an effective antifilarial drug carrier system [5, 96]. (Table 7.2 and Table 7.3 comprise a detailed view of the available nano-based treatment options against viral infections and parasitic infections, respectively, whereas Table 7.4 summarizes the NP's treatment options against bacterial infections).

Nanoemulsions are of submicron size (10 to 1000 nm) and by function are colloidal nano-drug carriers. having lipophilic solid spheres which is (−)Ve charged and amorphous surface [114]. Nanoemulsions are reported to improve the therapeutic potency and restrain the unwanted toxic effects of chemotherapeutics [114]. Nanoemulsions are reported to be majorly explored against the reticuloendothelial system (RES) infection, liver enzyme replacement therapy, cancer, and improved vaccination [115]. Thermodynamically, nanoemulsions are considered unstable and therefore required to be stabilized by emulsifying agent [116]. Nanoemulsions are transparent and can be broadly subdivided into three types, namely (1) oil in water, (2) water in oil, and (3) bi-continuous [114].

For their functional attributes, nanoparticles interact with specific or different biomolecules available on the cell exteriors and interiors. Additionally, their functional properties as potential drug delivery systems with assistance during non-invasive imaging are other causes of their relative acceptance over conventional chemotherapeutic options. However, there are some troubleshoots over full-fledged NP administration; namely, the systems must be biocompatible, stable, and site-specific after systemic administration [45, 53].

NPs can also be beneficial as they can allow simultaneous attachments of multiple therapeutic substances, and thus, a cumulative concentration of therapeutic and diagnostic substances at the pathological site is increased [5, 117]. Also, the concentration and dynamics of the attached compound can be changed by changing the size of the NPs [1, 45]. The surface adjustment of NPs helps in immune evasion and can be beneficial for circulating for considerably longer periods in the blood [45, 53] and also facilitate imaging probes.

Table 7.3 Nano-based method for the treatment of protozoan and helminthic neglected tropical diseases

Type	Nanoparticle	Drug or mode	Disease/Parasite
Metal	Gold NPs [113]	Quercetin	Leishmaniasis
		Couroupita guianensis reduced Au NPs	Malaria larvae
		Kaempferol	Leishmaniasis
		Ciprofloxacin	*Plasmodium falciparum*
	Silver NPs [113]	MSP10 Oligonucleotides	*Plasmodium vivax* in urine diagnosis
		Thermotherapy	Leishmaniasis *Giardia lamblia* cysts Leishmaniasis *Entamoeba histolytica Cryptosporidium parvum*
		Catharanthus roseus extract	*Plasmodium falciparum* Fascioliasis
	Ag_2O	–	Leishmaniasis
	Copper(II) nanohybrid solids [113]	–	Inhibition of plasmepsin II
	Copper oxide NPs [113]	–	*Entamoeba histolytica* and *Cryptosporidium parvum*
	TiO_2 NPs		Leishmaniasis
Organic NPs	Andrographolide nanoparticles	Andrographolide	Leishmaniasis
	Chitosan NPs	S-nitroso-mercaptosuccinic acid	Leishmaniasis
		Siparuna guianensis essential oil	*Aedes aegypti* larvae *Giardia lamblia* cysts
		Chloroquine	*Plasmodium berghei*
		Albendazole	*Echinococcus multilocularis*
	Beeswax-Copaiba oil NPs	Diethyldithiocarbamate	Leishmaniasis
	Poly(D, L-lactide-co-glycolide) NPs	Amphotericin B	Leishmaniasis

(continued)

Table 7.3 (continued)

Type	Nanoparticle	Drug or mode	Disease/Parasite
	Lipid nanoparticles	Curcuminoid	Malaria
	Liposome	Amphotericin B	Leishmaniasis
	Liposome	Andrographolide	Leishmaniasis
	Solid lipid NPs	S-benzyl dithiocarbazate (H2bdtc)	*Trypanosoma cruzi*

7.4 Toxicology of Bioactive Nanomaterials

The particle size difference is one of the foremost explanations against the Ag-NPs mediated cytotoxicity, and the particle size can influence exposed cell mortality, lactate dehydrogenase (LDH) activity, and ROS production [59]. Different physio-chemical properties like surface area, volume, and surface reactivity of NPs also be altered by their dimensions [1]. Other physio-biological properties like attachment, sedimentation, mass diffusivity, and deposition velocity are also dependent on NPs size and dimension and can control their interaction with mammalian cells [62, 94]. The size and shapes of Ag-NPs are dependent prominently on the synthesis processes, and we can have immense possible structures, e.g., spherical, triangular, square, cubic, rectangular, rod, oval, and flower-shaped [1, 142]. From the toxicological opinion, it is still debated if particle shape has a somewhat important outcome on the biological system and potentially be multi-factorial. To elaborate, alveolar epithelial cells (A549) when exposed to diverse forms of Ag-NPs and Ag+ are becoming clustered in the cytoplasm [59]. The shape of the Ag-NPs possibly can affect the cellular uptake and modulate the cytotoxicity. However, spherical particles showed the least unfavorable or toxic effects as examined in different cytotoxic parameters in A549 cells, and instead, wires are the least favorable based on cellular safety [59, 62]. When various cell lines, namely macrophages (RAW 264.7, J774.1), A498, A549, HepG2, and neurons (Neuro 2A), were exposed to different concentrations of Ag-NPs (5–43 nm), it was observed that at 2.0 mg/L concentration Ag-NPs interact mostly and macrophages displayed maximum sensitivity [62]. Macrophages' internalization of Ag-NPs is mediated through the scavenger receptor pathway, and the cytotoxicity is mediated by the release of Ag+ [27, 143]. To summarize Ag-Nps mediated death of RAW 264.7 cells, 0.2 ppm Ag-NPs caused 80% of cell death, whereas at 1.6 ppm, the death is 60% [144]. A similar tendency was observed for human Chang liver cells. Both Ag-NPs and $AgNO_3$ are cytotoxic for human lung cells [59]. HepG2 and $CaCO_2$ cells expressed dose-dependent toxicity and cell proliferation when exposed to Ag-NPs and elaborated experimental explanations were oxidative stress, mitochondrial injury, and DNA damage with dose-dependent activation of mitogen-activated protein kinase (MAPK) [145]. Bio-synthesized spherical Ag-NPs around 50 nm in size inhibited cell survival, cell proliferation, and migration involving caspase-3 activation and Akt phosphorylation inhibition in bovine retinal endothelial cells (BRECs) at a 500-nM concentration [146]. In most of the cases if

Table 7.4 Different nanoparticle-based methods for identification and treatment of infectious bacterial diseases

Specific properties	Nanocomposition	Targeted organism	References
Targeted antibiotic delivery	Uncoated liposomes and PEGylated liposomes	*Staphylococcus aureus*	[118, 119]
	Super-paramagnetic iron oxide nanoparticles (SPIONs)	*S. aureus*	[120]
	Cationic triblock polycarbonates	Methicillin-resistant *S. aureus* (MRSA)	[121]
	Polymeric nanoparticles coupled with mannose-specific or fucose-specific lectins	*Helicobacter pylori*	[122]
	Aptamer-magnetic nanoparticles (MNPs) conjugates	*Salmonella typhimurium, Mycobacterium tuberculosis*	[123, 124]
	Ligand (such as mannose, maleylated bovine serum albumin, and O-stearoyl amylopectin) attached nanoparticles	Various intracellular microorganisms	[125]
Environmentally responsive antibiotic delivery	pH-sensitive charged polymer tagged or ion coated liposomal drug delivery	Various intracellular microorganisms (such as *Salmonella enterica, and Listeria monocytogenes*)	[126, 127]
	Cationic liposomes attached with negatively charged gold nanoparticles	Various skin pathogens (such as *Propionibacterium acnes*)	[128]
	Anionic liposomes stabilized by positively charged gold nanoparticles	Gastric pathogens (such as *H. pylori*)	[129]
Combinatorial antibiotic delivery	Liposome mediated combined (hydrophilic and hydrophobic) drug delivery	*Pseudomonas aeruginosa* *Burkholderia cepacia* *Klebsiella pneumonia* *S. aureus* *M. tuberculosis* *M. avium* *H. pylori*	[130–133]
Nanoparticle-enabled antibacterial vaccination	Antigen conjugated nanoparticle	Activation of B cell	[134]
	Antigen encapsulated and toll-like receptor (TLR) agonist embedded nanoparticle	Activation of T cell	[135]

(continued)

Table 7.4 (continued)

Specific properties	Nanocomposition	Targeted organism	References
	Cationic nanogel	*Clostridium botulinum*	[136]
Nanoparticle-based bacterial detection	Silica nanoparticles encapsulated fluorescents	Bacteria detection	[137]
	Semiconductor quantum dots (QDs)	Bacteria detection	[138]
	Ligand-conjugated QDs	Bacteria detection	[139]
	Magnetic nanoparticles coated with pathogen-specific antibodies	Isolation of antigen from the body	[140]
	Paramagnetic iron oxide-mediated magnetic resonance imaging (MRI) systems	Ultra-sensitive bacteria detection	[141]
	Iron nanoparticle-mediated micro-NMR system	Identification of bacterial 16s rRNAs	[141]
	Gold nanoparticles adsorbed with fluorescent polymers (such as poly-paraphenyleneethynylene and polylysine)	Bacteria detection	[133]

not all, the toxicity of the Ag-NPs is primarily dose-dependent and associated with oxidative stress, anti-oxidants, intracellular calcium reduction, and caspase-3 activation although the cell membrane damage is not always associated [59]. Moreover, Ag-NPs displayed augmented toxicity in stem cells; e.g., Ag-NPs exposed to murine spermatogonial stem cells displayed reduced viability, LDH leakage, and apoptosis [147]. The toxicity of NPs is a cumulative event where the particle size, particle crystallinity, medium, temperature, and surface functionalization all played their role [148]. Toxicity properties of Ag-NPs are well explored, and although considerably less, the toxicity of other NPs against different cells and organisms is well explored. As a case, titanium dioxide (TiO2) NPs induce ROS which mediate lipid peroxidation, protein dysfunction, and DNA degradation and cause harm to the mouse brain [149].

7.5 Prospects and Challenges

Novel technologies like nanotechnology when explored against various biotargets and potentially explored for diverse applications in biomedical applications and detections can be potentially helpful for lots of people. Considering these points in mind, the convenience of effective therapeutics with the aid of NPs exploring

different layers of application can be exciting as well as helpful for society. The future seems to be optimistic as well. The medical application of NPs can be easier with knowledge of the epigenetic regulations, physiology, biochemistry, chemistry, and bioinformatics and thus opens for collaborative and cooperative research ventures. NPs also focus on and correct the difficulties of different biological systems in healthy as well as diseased individuals. NPs also encourage novel research goals to explore the valid reasons for biological, physical, and chemical complexities and extrication of facts. NPs also have enormous potential in the areas of therapeutics and diagnosis and have the latent to entirely revolutionize clinical applications.

The use of nanopharmaceuticals can be very challenging as well as thought provocating and hence needs extensive exploration of probable possibilities. Various tested delivery systems should therefore also be taken into consideration especially in treating infectious diseases to evaluate their potential of feasible and preferred drug delivery moieties against these ailments. However, these nanopharmaceutical drug delivery systems are accomplished enough to prioritize the drug efficiency at maximum [5, 45, 142].

For effective therapy and recovery of patients, precise, cost-effective, and prompt medication is what we need. We should always consider that the final therapeutic application should be easy, penetrating, and non-invasive with whatever system we may use. Mostly, these qualifications can be met with nanomaterials and this is why more and more research and ventures are promoting NPs. Again, novel therapeutic NMs, on the other hand, must be compatible with new challenges against various existing, emerging, and re-emerging pathogens in present times. It is always advised that the implementation of new therapeutics can be the best indicator of success and achievement for any emerging technology. In recent times, healthcare cost is considerably raising and is a matter of apprehension, especially for the underprivileged people located in low-income areas. In this respect, it is suitable to mention that parasitic diseases largely prevail in tropical and sub-tropical countries with lower per capita income [150, 151]. Despite the vast apprehension of nanomedicine and nano-drug delivery system, precise application in the healthcare system is underachieved. Hopefully, uncovering the molecular mechanism of action, evaluation of toxicity and pharmacokinetics will eventually promote nanomedical applications against different and diverse ailments and health conditions.

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Chapter 8
Design and Analysis of One-Dimensional Photonic Crystal Biosensor Device for Identification of Cancerous Cells

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Abstract The present chapter highlights one-dimensional photonic crystal (1D PhC) and its vital applications. The remarkable scientific progress in PhC has been able to draw the attention of researchers for novel bio-sensing applications. With the advancement in technology, different defect-based PhCs have been successfully fabricated with extensive analysis of propagation characteristics and tested for various sensing applications like blood, gas, salinity, DNA, alcohol, liquid, food, hormones, enzymes, cells, urine, glucose, chemicals, etc. The transfer matrix method is the most suitable method to study the spectral characteristics of 1D PhC structure. The sensing principle is based on the study of alteration in the resonant mode wavelength according to the modification in the analyte refractive index. This chapter deals with the study of defect-based 1D PhC cancer cells sensor, where TMM is employed to detect basal, cervical, and breast cancer cells. In order to enhance the sensitivity, a thin graphene layer is deposited at the side wall of the defect layer. A complete optimization of geometrical parameters has been performed to envisage high performance. The 3D colormap plot is studied to clearly show the variation in the properties of the defect mode with change in the incident angle. Moreover, signal-to-noise ratio, Q-factor, resolution, and figure of merit of the sensor are measured meticulously. The noteworthy sensing performance can open an avenue to effectively detect the cancer cells in the early stage.

8.1 Introduction

Most of the technological innovation have brought up by deeply perceiving the nature. There are numerous examples which validate the existence of periodic nature

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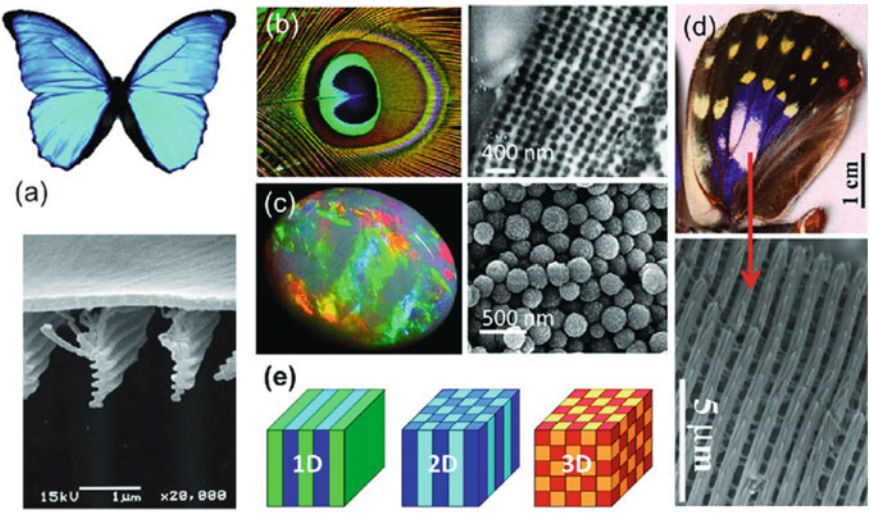

Fig. 8.1 Natural photonic crystals **a** morph butterfly, **b** multi-colored peacock feather, **c** opal gemstone, **d** wing of Sasakia Charonda with their microscopic images, **e** 1D/2D/3D PhCs

of variations in nature. For example, wings of butterflies, opal of the bracelet, feathers of birds, and barbules of colorful birds contain a systematic periodic arrangements, which resemble with the property of photonic crystals [1–3]. The color changes with respect to the angle of observation, which is primarily owing to the interaction between the light and the above-mentioned material's natural design. Figure 8.1 demonstrates some nature-based multilayer effects.

The study on periodic multilayer structures by E. Yablonvitch and S. John in the early 1987 is considered as the flagship research on photonics, which ignited the minds of the researchers to explore different applications using photonics principle [4, 5]. In their work, the authors revealed the effect of periodicity in two dimensions and three dimensions. As far as research on photonic crystal is concerned, it can be realized in 1D, 2D, and 3D forms. Among these, 3D PhCs are facing fabrication feasibility issues, and still research is going on to find an effective fabrication technique to produce low-loss 3D PhCs [6, 7]. On the other hand, 2D PhCs can be successfully fabricated by etching technique resulting in triangular, square, and honeycomb structures. The 2D PhCs can be designed in the form of slab, which can be organized in two ways: arrangement of dielectric materials with air as the background and arrangement of holes on a dielectric slab [8–10]. Defect-based PhCs have brought the revolution in the photonics research community. The defects can be created by omitting a series of air holes or by altering the properties of air holes along a particular shape in the PhC, which makes it behaves as a photonic crystal waveguide (PCW) [11–13]. The light signals fall within the PBG can be effectively guided and trapped in PCW, which enables the manipulation of electromagnetic

waves in the nano-structure. Nonetheless, several efforts have been given by scientists and researchers to mimic 2D and 3D PhCs, but 1D PhCs are the utmost explored structures and investigated from theoretical to experimental aspects due to their fabrication feasibility, high compatibility, and broad application domain [14–16]. The 1D PhCs are blessed with an elegant characteristics called as photonic band gap (PBG), which appears owing to the periodical arrangement of different dielectric materials along the stacking direction [17]. The PBG reflects that wavelength band which is restricted to propagate along the multilayer structure. The PBG characteristic of the 1D PhC has a significant effect in envision of many novel applications such as laser applications, sensing applications, filter, optical mirror, polarizer, communication applications, biomedical applications [18–20]. PhC must be structurally altered to produce a resonant mode in the spectral characteristics in order to be used as a biosensor. The finest technique to create such a resonant mode is to insert a defect in the design [21]. If the input wavelength and the defect mode wavelength matches each other, a discrete spike is formed in the spectral characteristics. The light (photons) are strongly localized near the defect layer [22]. A slight change in the surrounding refractive index leads to a significant modification in the location of the defect mode. Till now, 1D PhCs are widely used for a variety of purposes [23–26]. Moreover, 1D PhCs are highly reliable pertaining to temperature fluctuations, offer fast operation, and possess higher lifetime compared to the high-dimensional PhCs.

In the last decade, researchers have explored numerous novel 2D materials, which be integrated with photonic devices to improve the performance. Graphene has come up as a promising material for the design of various optical devices and therefore evolved as a center of attraction of researchers worldwide [27, 28]. A. Geim and K. Novoselov first introduced graphene as a 2D material, for which they received the prestigious Nobel Prize in the year 2010 [29, 30]. Graphene possesses a unique lattice configuration and is considered as a novel material due to its outstanding electronics properties. Graphene has hexagonally arranged lattice structure of sp2 hybridization having noteworthy electronic properties. Most importantly, graphene has a higher conductivity of 10^6 s/m and a very low resistivity of 10^{-6} Ω·cm, which makes it convenient to work in a broad applications domain compared to the conventional materials [31]. Moreover, graphene demonstrates zero bandgap properties and excellent carrier mobility. Even though, the approximate thickness of graphene is only 0.34 nm, but still it shows a remarkable absorption property [32]. It has been experimentally observed that a monolayer of graphene can absorb 2.3% of light in a wide wavelength band. Owing to its high absorption property, graphene shows distinguished reflectance for TE and TM modes under total internal reflection, which is very sensitive toward a small deviation in the RI of the contacting surface [33]. Due to the aforementioned properties, graphene has successfully entered the photonics industry to realize different electromagnetics applications. The properties like flexibility, durability, robustness, high conductivity, excellent mobility, etc. make graphene a right candidate for design of photonic devices [34–36]. Notably, the conductivity of graphene can be adjusted by controlling the chemical potential across the graphene sheet. So by suitably controlling the chemical potential across the graphene sheet, the optical properties of the integrated graphene photonic devices can

be varied according to the user requirement. From the energy band transition point of view, two types of interactions are noticed between graphene and light signal, namely inter-band transition and in-band transition. The inter-band transition is mainly seen in visible to NIR wavelength range, whereas the in-band transition is observed in the far-infrared wavelength regime. In case of in-band transition, graphene behaves as a free electron which is capable of exciting the surface. Nevertheless, a monolayer of graphene bestows high light absorption, but it shows a poor absorption of only 2.3% as material, which demands a more deep research to boost the interaction between graphene and electromagnetic signal.

Since the last two decades, 1D PhCs are dominating in the field of designing biosensors. A 1D PhC sensor is proposed by W. Nouman et al. for detection of brain lesions within the refractive index range of 1.3333 to 1.4833 and achieved an excellent sensitivity of 3080.8 nm.RIU^{-1} [37]. Z. Zaky et al. reported a Tamm plasmon structure to detect various gases. Ag plays a vital role in generating the plasmonic modes at the interface between the metal and 1D PhC [38]. A thorough analysis is carried out on the characteristics of the defect modes, where it is seen that the cavity resonance tends to decrease with an escalation in the RI of the targeted gases [39]. A. Ahemad explored a Psi-based photonic crystal including a metal layer and investigated Tamm plasmon polariton (TPP) resonant modes for sensing liquid analytes. The authors studied the shifting nature of the defect mode by infiltrating the cavity with liquids of different refractive index [40]. A. H. Aly manipulated TMM to examine the transmission spectrum in a defect 1D PhC for sensing the creatinine concentrations in blood [41]. A. Panda et al. studied the absorption spectrum and transmission spectrum in both symmetric and asymmetric 1D PhC structure to detect various viruses present in the drinking water, which find a suitable application in rural area [17]. A hemoglobin sensor is realized by M. Abadla and his team to sense a wide concentrations of hemoglobin. After optimizing numerous structure parameters, they attained a sensitivity of 167 nm per RIU [42]. A glucose sensor is designed using defect-based 1D PhC through the analysis of reflectance characteristics [43]. A steady and low-cost sensor is reported by Elsayed et al. [44], to sense various types of biodiesels. The bandgap properties of a 1D PhC comprising a single layer of graphene sandwiched between the dielectric materials is inspected by J. Fu et al. [45]. A graphene-based 1D PhC is investigated by Fan et al., where the authors explored the dependence of optical properties of graphene on its chemical potential [46]. By varying the chemical potential, the authors measured the change in the defect mode frequency. A novel protein sensor is investigated on the ground of 1D PhC. Although the authors used the optimized parameters, they found a sensitivity of only 170 nm per RIU, which is not up to the mark [47].

Cancer, the leading reason of fatality worldwide, has evolved as a precarious diseases. As per the information of IARC, around 19.3 million population around the globe are contrived by the cancer, and nearly 10 million fatalities have occurred in the year 2020 [48]. The primary reason behind the growth of cancer cells in human body is the interaction between the genetic factors of the body with different external agents like physical carcinogens, chemical carcinogens (tobacco, aflatoxin), and biological carcinogens (bacteria, viruses) [49]. Due to this effect, the cells grow in uncontrolled

manner by absorbing relatively more protein and nutrients from the body [50]. The main point of concern is that till date there is no fully guaranteed treatment of cancer in medical science. Probably, the single approach to combat the situation is the early detection of this diseases. So, a label-free and point of care cancer testing device is indispensable to effectively fight with cancer. In the last decade, researchers have greatly relied on photonics technologies to successfully detect different cancer cells with high accuracy and less time. A cancer cell sensor is proposed by Bijalwan et al., which is consisted of alternating layers of SiO_2 and TiO_2. The authors used TMM technique to achieve a sensitivity of 73 nm/RIU [51]. A. Aly et al. optimized the geometrical parameters of a 1D PhC and tested the spectral characteristics of the structure by filling different cancer cells in the defect layer. The authors are settled with a sensitivity of 2200 nm/RIU [52]. The transmission spectrum of a defected photonic crystal is analyzed in detail by Ramanujam and his team, where a very low sensitivity of 43 nm/RIU is achieved, which is very low [53]. A point defect-based 2D photonic crystal waveguide is studied for detection of different types of cancer cells. In their work, the authors rely on the electric filed analysis in the defect region and measured the reflected wavelength, which differentiated the normal cells from the cancer cells [54]. N. Ayyanar et al. investigated a dual core photonic crystal fiber to detect various cancer cells like cervical, basal cells, etc. [55]. Sani et al. studied the bandgap characteristics in a 2D photonic crystal to differentiate cancer cells from the normal cells [56]. Jabin et al. attempted to integrate a metal layer in the core of the photonic crystal fiber and studied the plasmonic behavior of the device. Further, the authors tested their device to distinguish cells infected with cancer cells in a broad wavelength band of visible to NIR regime [57].

8.2 Theoretical Formulation

This chapter explores a 1D PhC, which is realized as a regular periodic arrangements of MgF_2 and ZnSe. A cavity/defect layer is formed at the center of the arrangement. A monolayer of graphene sheet is sandwiched between the defect layer and the dielectric layer. The alignment of the whole structural arrangement is $(MgF_2/ZnSe)^N$/graphene/defect/graphene/$(MgF_2/ZnSe)^N$, which is represented in Fig. 8.2. The thickness of the layer MgF_2, ZnSe, and graphene is represented as d_A, d_B, and d_G, respectively. The cavity is loaded with different cancer cells. A 589 nm light, produced from a laser source, is made to strike the proposed structure normally along the x–z plane.

The constituent material RI plays a significant part in finding the spectral response of the entire configuration. The RI of MgF_2 and ZnSe can be measured with the help of Sellemier equations [58, 59],

$$n_{\mathrm{ZnSe}}^2 = 4 + \frac{1.90\lambda^2}{\lambda^2 - 0.113} \tag{8.1}$$

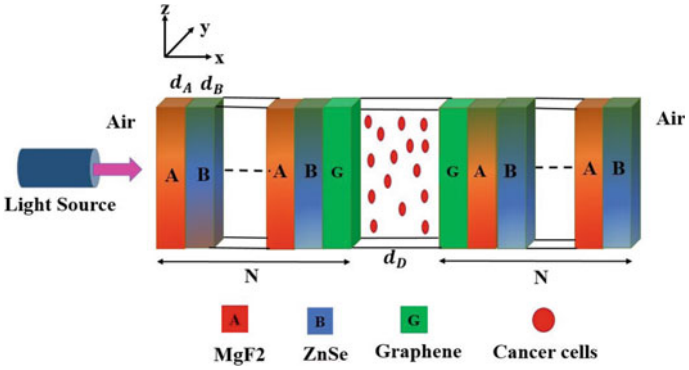

Fig. 8.2 Schematic of the proposed sensor

$$n^2_{\mathrm{MgF2}} = 1 + \frac{0.48755\lambda^2}{\lambda^2 - 0.04338^2} + \frac{0.39875\lambda^2}{\lambda^2 - 0.09461^2} + \frac{2.31203\lambda^2}{\lambda^2 - 23.7936^2} \tag{8.2}$$

The dielectric permittivity of graphene is stated as [60],

$$\varepsilon_G = \begin{bmatrix} \varepsilon_{\mathrm{Gt}} & 0 & 0 \\ 0 & \varepsilon_{\mathrm{Gt}} & 0 \\ 0 & 0 & \varepsilon_{G\downarrow} \end{bmatrix} \tag{8.3}$$

The terms $\varepsilon_{\mathrm{Gt}}$ and $\varepsilon_{G\downarrow}$ denote the normal and tangential component of permittivity, respectively. $\varepsilon_{G\downarrow} = 1$, and $\varepsilon_{\mathrm{Gt}}$ is expressed as below,

$$\varepsilon_{\mathrm{Gt}} = 1 + i\frac{\sigma(\omega)}{\varepsilon_0 \omega d_G} \tag{8.4}$$

where ω signifies the angular frequency, d_G denotes the graphene thickness, and ε_0 is the permittivity of air. The surface conductivity (σ_ω) regulates the physical properties of graphene. The transfer matrix representation of the graphene layer takes the form, $M_G = \begin{bmatrix} 1 & 0 \\ -\sigma_\omega & 1 \end{bmatrix}$, where σ_ω can be mathematically expressed by Kubo formula [61] as written below,

$$\sigma_\omega = \sigma_\omega^{\mathrm{intra}} + \sigma_\omega^{\mathrm{inter}} \tag{8.5}$$

$$\sigma_\omega^{\mathrm{intra}} = \left(\frac{ie^2}{8\pi\hbar}\right)\left[\frac{16K_BT}{\hbar\omega}\log\left(2\cosh\left(\frac{\mu_c}{2K_BT}\right)\right)\right] \tag{8.6}$$

$$\sigma_\omega^{\mathrm{inter}} = \left(\frac{e^2}{4\hbar}\right)\left[H(\hbar\omega - 2\mu) - \frac{i}{2\pi} \times \left(\log\left(\frac{(\hbar\omega + 2\mu)^2}{\hbar\omega - 2\mu^2 + (2K_BT)^2}\right)\right)\right] \tag{8.7}$$

where, H denotes the Heaviside step function, K_B represents the Boltzmann constant, ω be the angular frequency, e is the electron charge, μ_c represents the chemical potential, and T is the temperature in kelvin scale.The field components can be written as,

$$\begin{pmatrix} E_p \\ H_p \end{pmatrix} = \begin{pmatrix} \exp(-\mathrm{i}q_p y) & \exp(iq_p y) \\ -n_s \exp(-\mathrm{i}q_p y) & n_s \exp(iq_p y) \end{pmatrix} \begin{pmatrix} A_p \\ B_p \end{pmatrix} \tag{8.8}$$

All the terms in Eq. (8.8) are defined in Ref. [41]. The field components between the adjacent layers of p and $p+1$ are stated as [17],

$$\begin{pmatrix} E_p \\ H_p \end{pmatrix} = \frac{1}{2} \begin{pmatrix} [\exp(iq_p\alpha_p) + \exp(-iq_p\alpha_p)] & \left(-\frac{1}{\gamma_p}\right)[\exp(iq_p\alpha_p) - \exp(-iq_p\alpha_p)] \\ -\gamma_p[\exp(iq_p\alpha_p) - \exp(-iq_p\alpha_p)] & [\exp(iq_p\alpha_p) + \exp(-iq_p\alpha_p)] \end{pmatrix} \times \begin{pmatrix} E_{p+1} \\ H_{p+1} \end{pmatrix} \tag{8.9}$$

where $\alpha_p = d_p \cos\theta_p$. Here, d_p and θ_p signify the thickness and incident angle, respectively. The widely accepted transfer matrix method (TMM) is manipulated for studying the spectral characteristics of the projected 1D PhC. The TMM describes the discrete layer p in matrix form, which can be stated as [17],

$$M_p = \begin{bmatrix} \cos\sigma_p & \left(-\frac{i}{\emptyset_p}\right)\sin\sigma_p \\ -i\emptyset_p \sin\sigma_p & \cos\sigma_p \end{bmatrix} \tag{8.10}$$

For TE mode, σ_p and $\emptyset_p$. are defined as,

$$\sigma_p = \frac{2\pi}{\lambda} d_p n_p \cos\theta_p \text{ and } \emptyset_p = n_p \cos\theta_p \tag{8.11}$$

The TMM of the entire structure can be calculated by multiplying the characteristics matrix of the each layer and can be written as [44],

$$M = (M_A M_B)^N M_{\mathrm{MXene}} M_D M_{\mathrm{MXene}} (M_A M_B)^N = \begin{bmatrix} M(1,1) & M(1,2) \\ M(2,1) & M(2,2) \end{bmatrix} \tag{8.12}$$

The transmission and reflection coefficients are stated as [43],

$$t = \frac{2\gamma_0}{(M(1,1) + M(1,2)\gamma_1)\gamma_0 + (M(2,1) + M(2,2)\gamma_s)} \tag{8.5.13}$$

$$r = \frac{(M(1,1) + M(1,2)\gamma_s)\gamma_0 - (M(2,1) + M(2,2)\gamma_s)}{(M(1,1) + M(1,2)\gamma_s)\gamma_0 + (M(2,1) + M(2,2)\gamma_s)} \tag{8.5.14}$$

where $\gamma_0 = \sqrt{\mu_0/\varepsilon_0} n_0 \cos\theta_0$ and $\gamma_s = \sqrt{\mu_0/\varepsilon_0} n_s \cos\theta_s$.

Lastly, transmittance (T) and reflectance (R) are numerically expressed as [17],

$$T = \frac{\gamma_s}{\gamma_0} \left| t^2 \right| \text{ and } R = |r|^2 \tag{8.15}$$

Absorbance can be computed as [42],

$$A = 1 - T - R \tag{8.16}$$

8.3 Results Analysis

In this chapter, different cancer cells like breast, basal, and cervical cells are considered, which are received from different body parts in liquid form. The sensing principle relies on the contrast in index value of the normal and infected cell. The experimental refractive index (RI) data collected from references [62, 63] are enumerated in Table 8.1.

The geometrical parameters of the designed structure are properly optimized to accomplish a sharp and high-intensity resonant mode inside the bandgap. We consider $d_{\mathrm{MgF2}} = 150\,\mathrm{nm}$, $d_B = 150\,\mathrm{nm}$, $d_G = 0.34\,\mathrm{nm}$, $\varepsilon_{\mathrm{MgF2}} = 1.90$, $\varepsilon_B = 6.8$, and $N = 5$. TMM is employed to analyze the transmission spectrum by changing the width of the cavity region, angle of incidence, and chemical potential across the graphene sheet. Initially, an attempt is taken to show the effect of graphene on the transmission characteristics. As shown in Fig. 8.3a, by including the graphene layer, the intensity of the resonance mode increases, which is suitable for sensing purpose. With the presence of graphene, the overall transfer matrix is updated, which is the reason behind the red-shifting of defect mode wavelength. Figure 8.3b demonstrates the study of the defect mode characteristics with reference to different period (N) of the photonic crystal. As, it can be seen, at $N = 5$, maximum intensity with lowest FWHM is obtained, which is considered as the most apposite result. For higher values of N, the FWHM is relatively higher and therefore not suitable from sensor design point of view.

By setting $D = 500$ nm, $\theta_{\mathrm{in}} = 0°$, $\mu_c = 0.2\,\mathrm{eV}$, we simulated the proposed multilayer structure in COMSOL Multiphysics software to study the electric field

Table 8.1 Cell's refractive index

Cell type	RI
Normal	1.35
Basal	1.38
Cervical	1.392
Breast	1.399

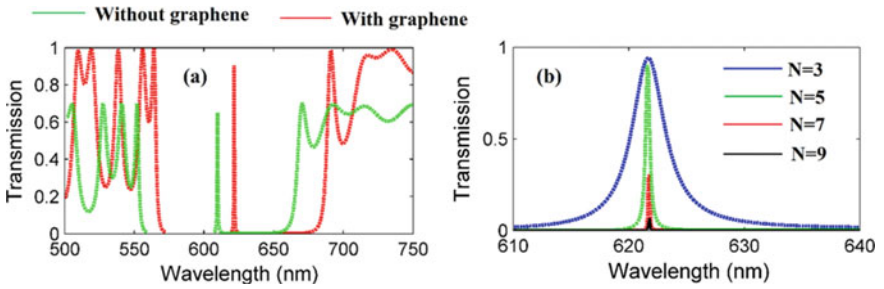

Fig. 8.3 **a** Effect of graphene layer on the transmittance spectrum, **b** variation in the period of the crystal

propagation in the structure, which is represented in Fig. 8.4. Owing to the presence of graphene layer, most of the light is confined inside the defect layer thereby increasing the interaction between light and infiltrated cancer cells. An electric field intensity in the order of 10^3 V/m is observed inside the cavity layer and gradually decays to both the sides of the defect layer.

For understanding the nature of variation of the defect mode, a colormap plot has been studied in Fig. 8.5, which shows the change in the transmission characteristics with respect to the wavelength and incident angle. In this figure, the high-intensity defect mode can be clearly seen inside the bandgap.

The transmission spectrum is examined for numerous selected cells at varied cavity layer thicknesses, which is depicted in Fig. 8.6. A substantial shift in the resonant mode is observed from normal to cancer type cells. In particular, the resonant wavelength (λ_{res}) experiences a red-shift as we infiltrate the cavity layer from the normal cells to the cancerous cells. This shifting nature closely follows the standing wave condition [26]. At $d_D = 500$ nm, the λ_{res} is moved from 621.6 to 633.8 nm

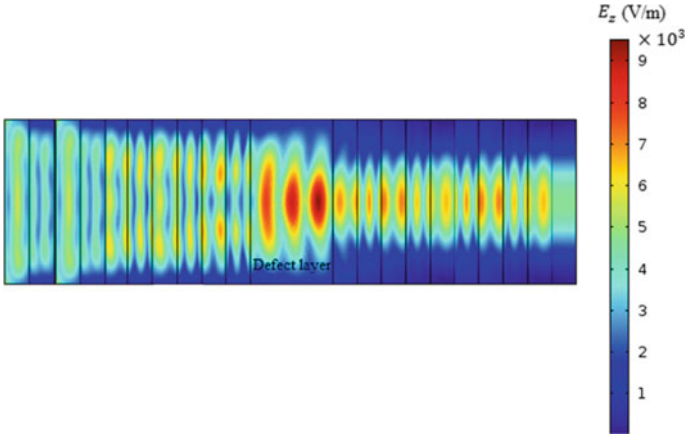

Fig. 8.4 Electric field distribution in the proposed structure

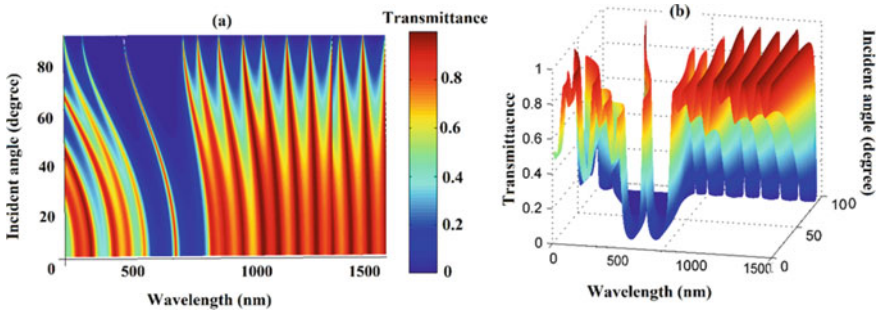

Fig. 8.5 Study of the colormap plot

as the cell type is changed from normal to breast cancerous cell. At $d_D = 600$ nm, the λ_{res} experiences a total shift from 612.3 to 625.5 nm, at $d_D = 700$ nm, the λ_{res} undertakes a total shift from 604.8 to 618.8 nm. Similarly, at $d_D = 800$ nm, the λ_{res} experiences a total shift from 898.8 to 613.3 nm.

Afterward, incident angle is varied, and the change in the defect mode properties is studied as demonstrated in Fig. 8.7. By increasing incident angle (θ_{in}) from 25° to 50°, the defect mode position experiences a blue-shift. This analysis is carried out at all the considered values of d_D. The blue-shifting nature of the defect mode is in accordance with the Bragg condition [26].

A thorough analysis on transmission spectrum is performed at $d_D = 500$ nm and $\theta_{in} = 0°$, by varying the number of graphene layers (L) from $L = 1$ to $L = 4$, which

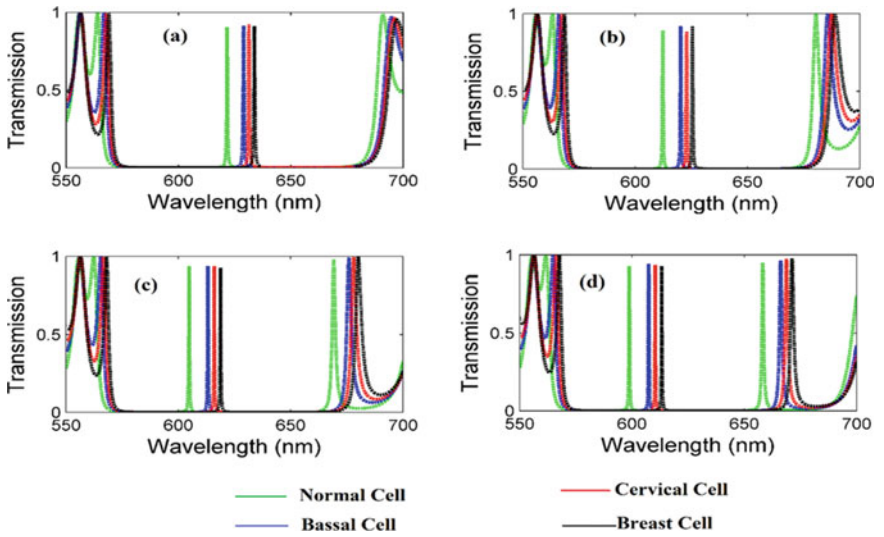

Fig. 8.6 Transmittance spectrum of different cells under normal incidence at **a** $d_D = 500$ nm, **b** $d_D = 600$ nm, **c** $d_D = 700$ nm, **d** $d_D = 800$ nm

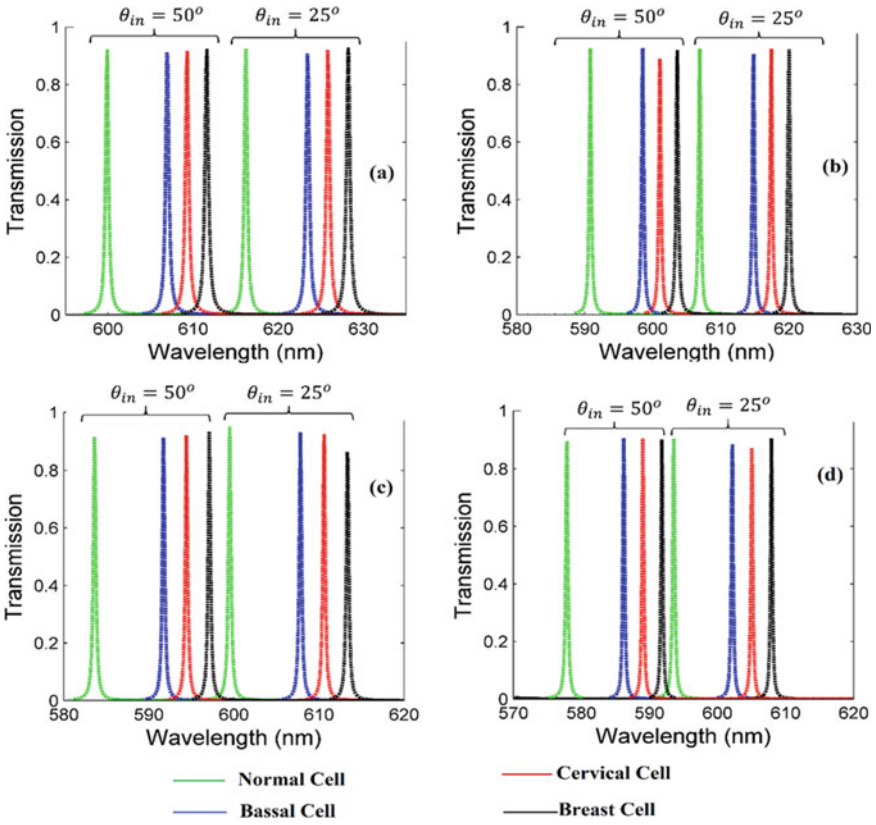

Fig. 8.7 Shift in the resonant modes for $\theta_{in} = 25°$ and 50° for **a** $d_D = 500$ nm, **b** $d_D = 600$ nm, **c** $d_D = 700$ nm, **d** $d_D = 800$ nm

is illustrated in Fig. 8.8a. By incrementing the L value, it is perceived that λ_{res} is moved to higher wavelength. On the other hand, with an increase in L, the intensity of the resonant mode goes on decreasing. As L increases, the effective thickness of the proposed design increases, which in turn escalate the geometrical path difference. Due to this reason, the wavelength is red-shifted. Based on this analysis, we chose monolayer of graphene ($L = 1$) as the optimized condition, where the most desirable characteristics are attained. Further, the effect of chemical potential (μ_c) across the graphene sheet can have a noteworthy effect on the device performance, which is examined in Fig. 8.8b. Here, it is perceived that at a constant L, the λ_{res} is red-shifted with rise in the chemical potential. With a variation in the chemical potential, the permittivity of the graphene sheet is changed, hence the red-shifting nature is observed in the λ_{res}.

In Fig. 8.9, the solid line and the dashed line indicate the transmission characteristics at $\mu_c = 0.3$eV and $\mu_c = 0.4$eV, respectively. From this figure, it is affirmed that by increasing the μ_c, the defect mode wavelength is moved to higher

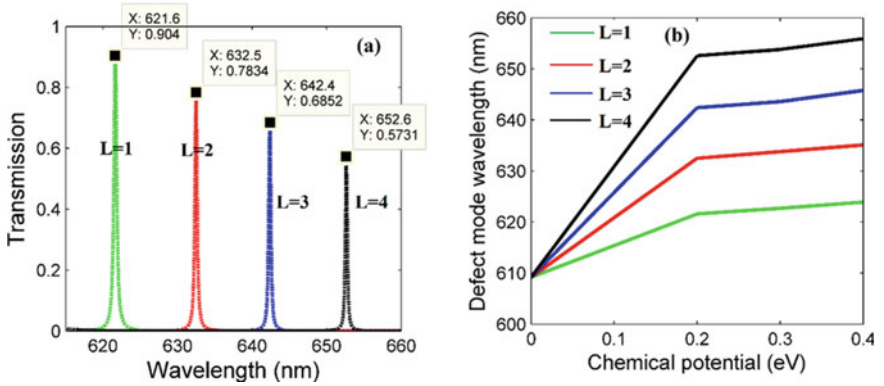

Fig. 8.8 **a** Transmittance as a function of L and **b** effect of chemical potential on the defect mode position

wavelength value for the normal as well as the cancer cells, so μ_c can greatly affect the sensing performance. As the defect modes are formed within the bandgap, there is possibility of absorption with different cells, and the same is analyzed in Fig. 8.10. As we change the cell type from the normal cells to the high refractive indexed cancer cells, the defect mode wavelength is red-shifted. Additionally, the absorption intensity increases for high indexed cancer cells.

Evaluation of sensitivity is outmost important to judge the performance It is explained as the ratio of shift in the defect mode wavelength with respect to different

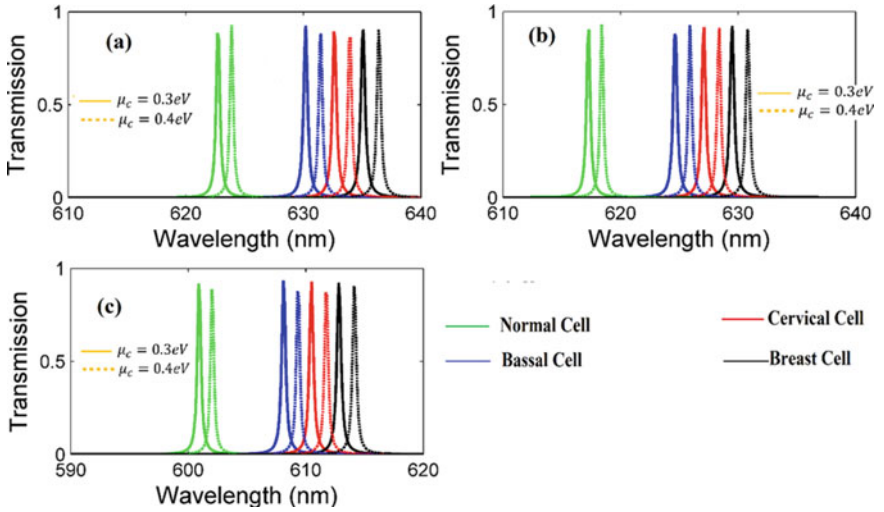

Fig. 8.9 Shifting the defect mode position w.r.t. μ_c at $d_D = 500$ nm for **a** $\theta_{\text{in}} = 0°$, **b** $\theta_{\text{in}} = 25°$, **c** $\theta_{\text{in}} = 50°$

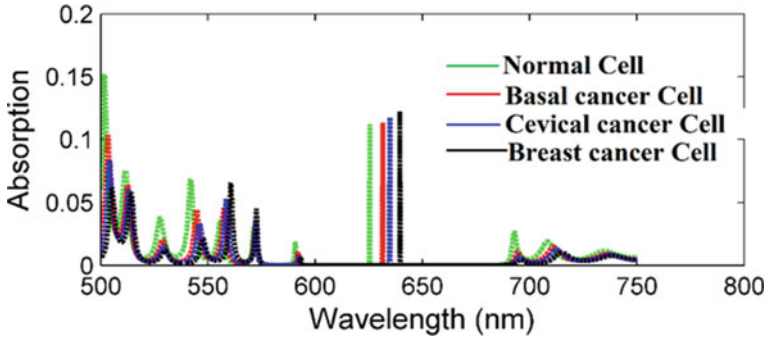

Fig. 8.10 Analysis of absorption spectra of different cells

cells under consideration. Sensitivity is written as [26],

$$S(\text{nm/RIU}) = \frac{\Delta\lambda_{\text{res}}}{\Delta n} \tag{8.17}$$

The sensitivity analysis at different incident angles is presented in Fig. 8.11. It is concluded that the sensitivity shows a declining trend with rise in the incidence angle and the sensitivity rises upon increasing the d_D. With the designed parameters $d_D =$ 800 nm and $\theta_{in} = 0°$, the maximum sensitivity of 290 nm/RIU is accomplished.

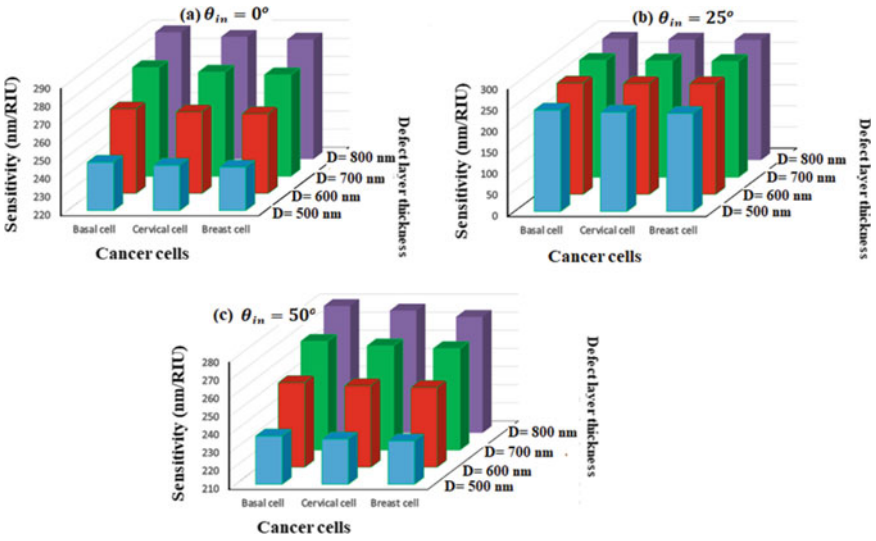

Fig. 8.11 Sensitivity analysis

Table 8.2 Measurement of sensor performance parameters

Cancerous cells	d_D (nm)	FoM (1/RIU)	QF	SNR	R (nm)
Basal	500	649.1	1640.5	19.4	0.121
Cervical		644.7	1646.3	25.7	0.113
Breast		642.1	1652.1	32.1	0.107
Basal	600	784.2	1808.2	23.5	0.103
Cervical		779.4	1815.8	31.1	0.096
Breast		776.4	1823.5	38.8	0.091
Basal	700	903.2	1960.6	27.0	0.090
Cervical		895.1	1969.6	35.8	0.084
Breast		890.3	1978.0	44.5	0.080
Basal	800	1074.0	2238.3	32.2	0.075
Cervical		1064.81	2255.7	42.5	0.070
Breast		1059.2	2270.7	52.9	0.066

Finally, other important performance parameters such as SNR, QF, resolution, and FoM are computed for different values of d_D at normal incidence and summarized in Table 8.2.

8.4 Conclusions

This chapter presents a novel graphene integrated photonic crystal configuration for the identification of numerous cancer cells. The layer thickness and incident angle can greatly control the spectral characteristics. The transmission spectrum is systematically scrutinized using TMM. The impact of chemical potential across the graphene sheet and the number of layers of graphene are studied on the sensing performance. The sensitivity is evaluated by assessing the defect mode wavelength shift between the normal cell and infected cancerous cells. A notable sensitivity of 290 nm/RIU, Q-factor of 2270.74, FoM of 1074.07 RIU^{-1}, SNR of 52.96, and resolution of 0.0668 have been accomplished with the designed structure. So, the authors are confident that the projected sensor can be the suitable candidate as cancer cells sensor.

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Chapter 9
Dielectric-Modulated Biosensor Based on Vertical Tunnel Field-Effect Transistor

Vandana Devi Wangkheirakpam, Brinda Bhowmick, and Puspa Devi Pukhrambam

Abstract This chapter discusses the application of vertical tunnel FET (VTFET) as dielectric-modulated label-free biosensor. Various features of this biosensor are presented by focusing the analyses on enhancement of sensitivity, its lower limit of detection and response time. The concept of sensing is formulated on the dielectric modulation where the incubation of the biomolecules is represented by an insulator whose dielectric constant value is equal to the dielectric constant of target biomolecule since different biomolecules have unique dielectric constant. The proposed sensor design is deployed using a Technology Computer-Aided Design (TCAD) approach by incorporating relevant physics-based simulation models. Keeping in mind the practical consideration of the device, the study has been extended to analyze its performance under non-deal conditions like steric hindrance, irregular orientation. In the end, the status of the proposed sensor is highlighted by presenting the comparison of different sensing parameters of some significant work on TFET-based label-free biosensor available in the literature.

9.1 Introduction

The philosophy behind the Moore's law has been the guiding principle for the exponential growth of the semiconductor industries [1–3]. The continued scaling down of

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the device dimension is performed to obtain good processing speed, low operating voltage and increase the number of transistors (MOSFETs) on chip so as to increase the device functionality [4, 5]. Scaling down of supply voltage leads to a reduction in dynamic power supply consumption which is desirable for power constraint applications. However, prolonged scaling down causes MOSFETs to encounter some serious issues known as the short channel effects (SCEs) [6–9]. Such issues arise due to the charge sharing between source and drain with the reduction in channel length, and as a result of this, various phenomenon such as velocity saturation, hot electron effects, DIBL effect are observed to be occurring [10–15]. Apart from these effects, the MOSFET technology faces another issue called the CMOS power problem. Decreasing the supply voltage (V_{DD}) at the time of scaling is required to maintain the power utilization of chip after the addition of a greater number of transistors [16–19]. But with the reduction of V_{DD}, the threshold voltage (V_{th}) should also be minimized in order to maintain the same ON state current, i.e., the overdrive voltage should be maintained. Therefore, to get the higher value of overdrive voltage, it is required to either keep V_{DD} at higher voltage or reduce V_{th} more forcefully [20–22]. The subthreshold swing (SS) of the state-of-the-art MOSFET cannot go down below 60 mV/dec due to its thermal limit, and this becomes a limiting factor of threshold voltage scaling. Also, keeping V_{DD} high leads to an increase in power consumption and thus causes the power crisis problem of CMOS. Hence, threshold voltage scaling can be done. In this scenario, it is found that tunnel field-effect transistor (TFET) has the ability to operate at low voltage with steeper SS and becomes a capable candidate which can replace MOSFETs in the near future. The fundamental fabrication methodologies of TFET are similar to MOSFET, and due to this, TFET has gained a concentrated focus for low applications as an alternative solution to scaled CMOS. Unlike the thermionic emission of MOSFETs, TFET conducts current through band-to-band tunneling (BTBT) mechanism resulting in low leakage current and steeper subthreshold swing [25, 26]. However, TFET experiences low ON state current and ambipolar behavior. Different modified structures of TFETs are proposed till now to overcome the limitation of conventional TFET geometry. Some of the widely used structures include heterojunction TFET, multiple gate TFET, III-V TFET, negative gate capacitance TFET, nanowire TFET, SOI TFET, etc.

TFETs are mostly popular for low-power digital circuits and memory applications. In the recent past, TFETs are observed to be widely used as a dielectrically modulated label-free biosensor. The sensitivity analysis is performed by observing the shift in drain current after the incubation of target molecules inside the sensing area. This current is affected by the dielectric constant and charge density of the target biomolecules. Section 9.2 of this chapter presents the concept of dielectric modulation in TFET, the geometry of TFET-based biosensor and its simulation strategies. Section 9.3 describes the proposed designed of VTFET biosensor. Section 9.4 discusses the sensitivity measurement methods. The non-idealities of TFET biosensors are analyzed in Sect. 9.5. Section 9.6 studies different sensing parameters considering the impact of charged biomolecules. Section 9.7 concludes the chapter.

9.2 Dielectric-Modulated Vertical TFET Biosensor: Concept, Geometry and Simulation Strategies

9.2.1 Working Methodologies

The conventional TFET has an insulator-gated reverse biased p-i-n geometry having three terminals, namely source, gate and drain. The source and drain regions are degenerated p and n-type, respectively. The channel remains intrinsic or doped lightly with p/n-type material, and it is sandwiched between the source and drain areas creating a p-i-n geometry. This arrangement provides a narrow depletion layer and forms a favorable condition for the carriers to tunnel from valence band (E_V) of source region to conduction band (E_C) of channel region. The fundamental working methodology of tunnel FET is Zener tunneling [27]. In TFET, BTBT is mechanism of electron transport. Depending on the polarity of the applied voltages, a TFET can be classified into p channel and n channel modes. In nTFET, the source terminal is connected to ground and the drain terminal is positively biased (V_{DS}) [28].

The application of positive voltage (V_{GS}) at the gate terminal causes band bending at the channel region. Further increase in this gate voltage causes more and more band bending resulting in the reduction of the tunneling width, i.e., the area between the E_V of source and E_C of channel where the bending is taken placed [29]. As V_{GS} increases beyond a particular limit known as the threshold voltage (V_{th}), carrier tunneling starts facilitating from the filled state E_V to the empty state Ec of source and channel, respectively. This can be understood from Fig. 9.1 where nature of E_C and E_V of the conventional TFET is plotted.

The tunneling probability of the energy barrier governs the current conduction of TFET. This probability is modeled through WKB approximation assuming a triangular barrier and is given by,

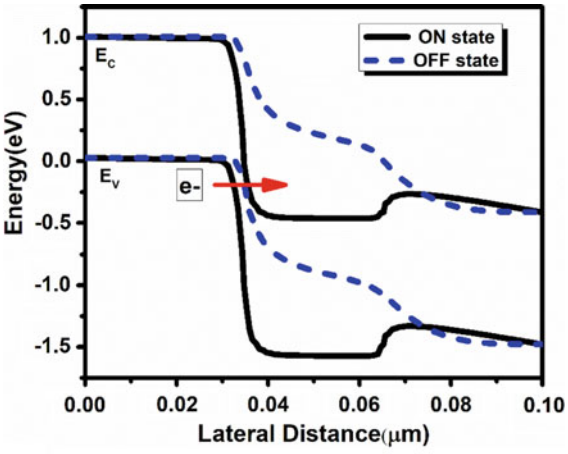

Fig. 9.1 Energy band diagram of nTFET showing the behavior at ON state and OFF state

$$T(E) = \exp\left(\frac{-4\lambda\sqrt{2m^*}E_g^{3/2}}{3q\hbar\left(E_g + \Delta\emptyset\right)}\right) \tag{9.1}$$

where m^* represents the effective mass, E_g denotes the forbidden energy gap, $\Delta\emptyset$ represents the energy of the area at tunnel junction where bands are overlapped, λ is the tunneling width, q and $\hbar$ are the value of an electronic charge and reduced Planck's constant, respectively. λ is defined as,

$$\lambda = \sqrt{\varepsilon_s/\varepsilon_{ox}}t_{ox}t_s \tag{9.2}$$

where $\varepsilon_s and \varepsilon_{\text{ox}}$ correspond to the dielectric constants of substrate and oxide layer respectively, t_{ox} and t_s respectively represent the oxide and substrate thickness. It is clearly seen from Eqs. (9.1) and (9.2) that the drain current is a function of tunneling width whose value depends on material's dielectric constant (k). The gate and channel pairing are more for the higher value of k. This improves the tunneling probability giving higher value of I_D. The concept of dielectric modulation is applied in the TFET-based biosensors by incorporating embedded nanogaps at the regions above two junctions [30]. The drain current is found to be increasing when the analytes are incubated within the cavity regions due to the value of k possessed by the captured biomolecules. Drain current shift is measured to analyze the sensitivity of the sensor [32]. Different biomolecules have unique dielectric constant such as APTES = 3.57, uricase = 1.54, streptavidin = 2.1, protein = 2.5.

9.2.2 Geometry

As mentioned in previous section, the implementation of dielectric modulation in biosensing application requires the modification of the device geometry in order to incorporate a region where the molecules will be captured. The following are the fundamental requirements for such structural modification.

- The analytes should capture the target biomolecule and hybridized them in the region between the gate and channel acting like a gate dielectric. This can be realized by creating a nanogap after etching a part of the fixed dielectric material. The modified structure, therefore, has dual gate dielectric where one is the fixed high-k material (HfO2) and the other is the one possessed by the incubated biomolecules.
- The height nanogap cavities must be sufficient enough for the analyte to enter easily. In most of the analyses done for nanoscale TFET, this height is usually fixed at 10–15 nm.
- The structure can also be modified to increase the capture area of the biomolecules. This includes the utilization double gate and also creating nanogaps on either side of the fixed oxide layer.

- The structure must be designed in such a way that it can show significant change in the drain current even for low dielectric constant since some biomolecules have dielectric constants close to 2 or 3.
- The doping level of drain must be kept lesser than the source to prevent the ambipolarity effect to some extent.

9.2.3 Simulation Strategies

The dielectric-modulated biosensor can be conveniently analyzed on computational platform where the device architecture is employed with different physics-based models for its performance analyses. The simulation process is carried out using 2D Synopsys TCAD tool [31]. In the simulation process, the conditions when the cavities are incubated with target biomolecules are represented by an insulator with the same dielectric constant. For those biomolecules which carries charges, the impact of charge is taken in account by considering its effect at the oxide–semiconductor interface. Non-local band-to-band tunneling model is adopted to create the inter-band tunneling process as it looks after the gradient of the energy band as well. The bandgap narrowing model and Fermi–Dirac statistics are necessary since the geometries consist of highly doped source and drain. Field-dependent mobility models are also required.

9.3 Vertical TFET as Dielectric-Modulated Biosensor

This section presents a vertical TFET design for biosensing application and discusses some of its sensing parameters. This particular biosensor is designed using an n-type vertical tunnel FET (VTFET) via dielectrically modulated technology for label-free detection of biomolecules. The sensor design proposed in Fig. 9.2 is simulated in the Synopsys TCAD tool. An n+ pocket is incorporated in addition to the conventional design of TFET. This modification is done to boost the current when the device is in operation mode overcoming the limitation of conventional TFET. The SiGe pocket has 30% Ge concentration, and it is doped with 1×10^{19} cm^{-3} arsenic atoms. Another line tunneling normal to the gate stack is possible through this pocket in addition to the tunneling that occurs in the lateral direction resulting in enhanced drain current characteristics (I_D). Furthermore, the gate is modified by utilization dual metals with $\varphi_{m1} = 3.8$ eV (Al) near source end and $\varphi_{m2} = 5.1$ eV (Au) near drain end. The arrangement is done to control the ambipolar behavior encounter in the TFET and also to improve the I_{ON}/I_{OFF} ratio. Source (p-type), i-channel and drain (n-type) have the doping concentration 5×10^{19} cm^{-3}, 1×10^{15} cm^{-3} and 1×10^{18} cm^{-3}, respectively. The 10 nm long and 10 nm high HfO2 is kept as a constant dielectric material. The presence of nanogaps on both side of this fixed oxide allows incubation of more molecules inside the cavity. An SiO2 layer with

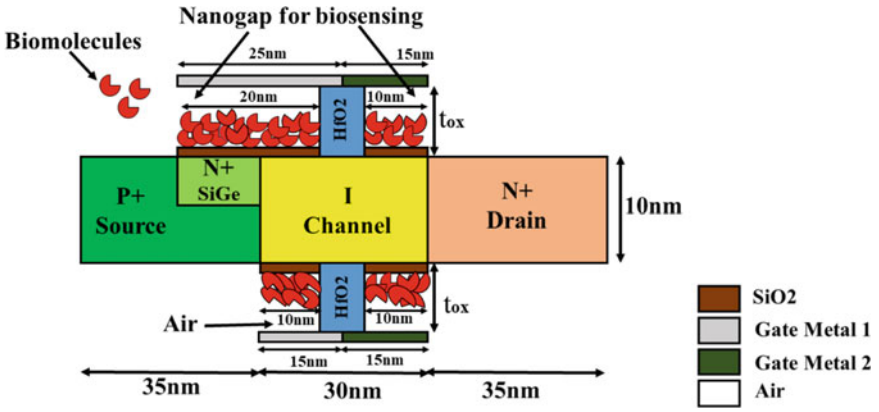

Fig. 9.2 Schematic image of n-VTFET biosensor

thickness 1 nm is introduced within the cavities representing the glass slides where the receptor molecules are immobilized, and this also minimizes the leakage current and degradation of sensitivity. The entire process of detection is considered to occur in a dry environment.

9.4 Sensitivity Measurement

The most common method of analyzing the sensitivity is the measurement of the change in its electrical parameters. Sensitivity calculation based on the change in drain current and threshold voltage is used as the most convenient technique. The sensitivity of the sensor is measured with reference to the value of the desired electrical parameter obtained when the nanogap is devoid of biomolecules, i.e., assuming that it is occupied by air ($k = 1$). The mathematical formula for the calculation of drain current sensitivity at fixed gate bias is given by,

$$\text{Sensitivity, } S_I = \left.\frac{I_{D(k=\text{BIO})}}{I_{D(k=\text{AIR})}}\right|_{V_{GS}} \tag{9.3}$$

where $I_{D(k\,=\,\text{BIO})}$ and $I_{D(k\,=\,\text{AIR})}$ respectively represent the value of drain current for the filled state and empty state. The threshold voltage (V_{th}) of a device is also a function of dielectric constant and oxide–semiconductor interface charges. Hence, V_{th} can also be used as a parameter for measuring the sensitivity. The mathematical relation is

$$\text{Sensitivity, } S_{V_{\text{th}}} = V_{\text{th}(k=\text{AIR})} - V_{\text{th}(k=\text{BIO})} \tag{9.4}$$

where $V_{\text{th}(k=\text{AIR})}$ and $V_{\text{th}(k=\text{BIO})}$ respectively define the threshold voltage at empty state and filled state. It is possible to extract V_{th} in a numerous way using transfer characteristics curve. The most commonly used methods are constant current method and the linear extrapolation (LE) method.

9.5 Non-ideal Hybridization of Biomolecules Inside the Nanogaps

In the simulation process, it is favorable to consider the nanogaps are completely filled with the analytes. However, in practice, this assumption is not always true. The conditions like steric hindrance and irregular orientation of receptors/probe are resulting in different partially filled profiles of the nanogaps.

9.5.1 Steric Hindrance

Steric hindrance is the condition arises when already hybridized biomolecules restrict the entry of the new one before the cavities are completely filled. This results in a different cavity profile and consequently alters the sensitivity. This partial hybridization condition is implemented in the TCAD simulation process by considering step profiles including increasing, decreasing, concave and convex. This is designed by defining various height of the dielectric material inside the nanogaps mimicking the pattern of partially filled molecules as shown in Fig. 9.3.

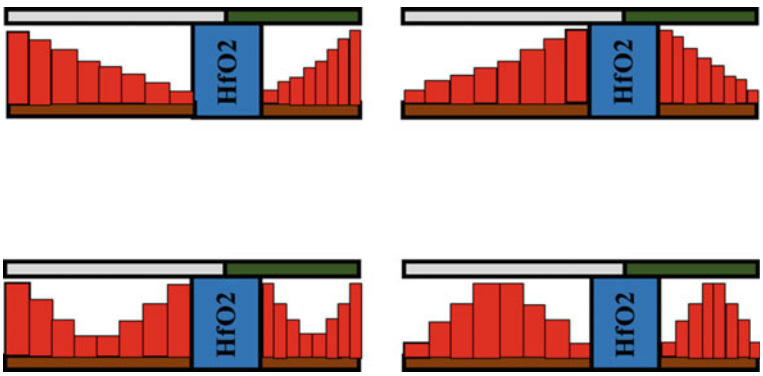

Fig. 9.3 Probable step profiles of the cavity introduce due to the steric hindrance

9.5.2 Receptor Placement

The receptors or probes are immobilized on the sensing surface usually a glass slide in order to capture the target biomolecule and hybridized them inside the cavity. The receptor molecules have an antigen binding site which will recognize the target biomolecule. If these receptor molecules are not properly aligned, then it will not be able to capture the antibody. This may result in the partially filled cavity profiles. Also, the placement of these receptors may not be continued throughout the cavity. In the simulation process, this is defined by inserting air gaps in between the insulators representing the biomolecules.

9.6 Sensing Parameters of VTFET Biosensor

This section observes the influence of different factors like charged molecules, step profiles, etc. on the sensitivity of the VTFET biosensor. Other sensing parameters such response time and lower limit of detection are also discussed in this section.

9.6.1 Biomolecules Carrying Negative Charge

It is known that the sensitivity is altered by the charges of the biomolecule. Assuming the nanogaps to be fully filled, the impact of the magnitude of negative charges on the sensitivity of the VTFET biosensor is investigated for four different values of k (5, 7, 10 and 12) representing four different biomolecules. The result shown in Fig. 9.4a provides the gradual degradation of sensitivity as the number of negative charges rises. The negative charges of the biomolecules present near the SiO2 surface prevent the further depletion of the p-type channel thereby increasing the V_{GS} needed for channel inversion. This can be explicated using the following equation.

$$V_G = \Psi_S + \Phi_{MS} - \frac{qN_{bio}}{C_{ox}} \tag{9.5}$$

Since the sensitivity is measured at fixed V_{GS} and V_{DS}, the potential balance equation given in Eq. (9.5) will be satisfied only if the value of Ψ_S is reduced as the magnitude of negative charge increases. Accordingly, the drain current degrades giving reduced sensitivity. Figure 9.4a also demonstrates the increase in sensitivity with k value. This is due the increase in the gate-channel coupling as the value of k increases. Figure 9.4b depicts the I_D-V_{GS} characteristics visualizing the impact of positive and negative charges of the analytes incubated inside the nanogap cavities. It is evident that the curve shifts upward as the value of charges changes from extreme negative to the extreme positive. The curve for $k = 1$ represents the result obtain when

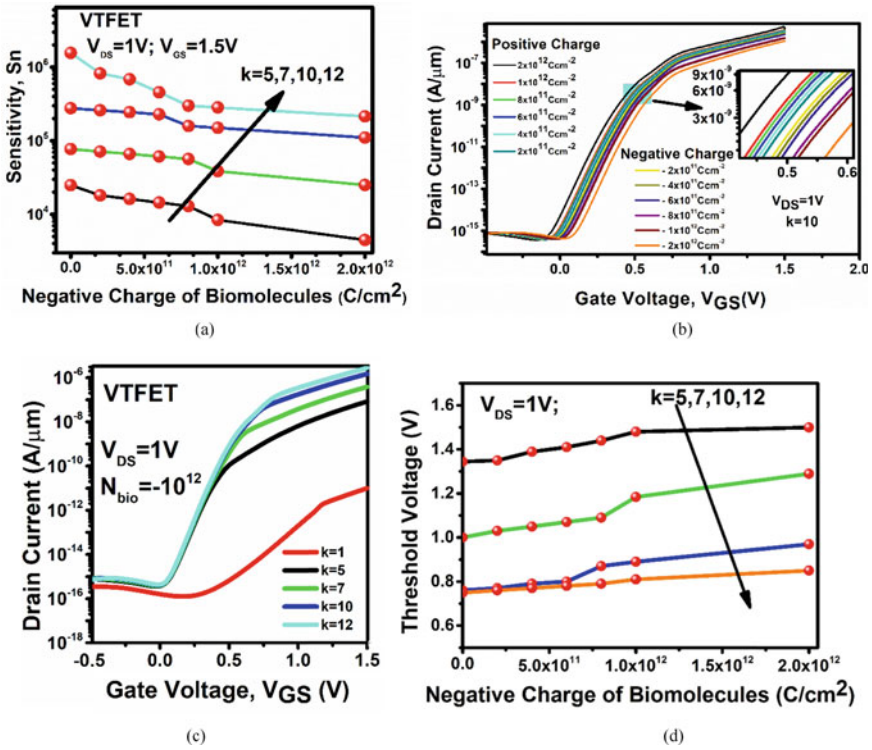

Fig. 9.4 **a** Sensitivity variation plot due to the influence of biomolecules carrying negative charge, **b** transfer characteristics curves showing the impact of various negative and positive charges, **c** I_D-V_{GS} curve of VTFET biosensor for $k = 1$, 5, 7, 10 and 12 at $N_{bio} = -10^{12}$ C/cm^2, **d** V_{th} versus negative charge of biomolecules at $k = 5$, 7, 10 and 12

the nanogap is devoid of biomolecules, i.e., the condition when it is filled with air. The presence of nanogaps on either side of the fixed dielectric (HfO2) increases the area where the target biomolecules can be captured. Figure 9.4c shows the I_D-V_{GS} curve of VTFET biosensor for $k = 1$, 5, 7, 10 and 12 at fixed negative charge $N_{bio} = -10^{12}$ C/cm^2. The shift in drain current as an after effect of the impact of charge density impact also explains the change in threshold voltage. The threshold voltage obtain for each case is plotted in Fig. 9.4c. V_{th} increases as the magnitude of negative charges increases.

9.6.2 *Biomolecules Carrying Positive Charge*

Similar to Sec. 9.6.1, various plots for positive charge biomolecules are presented in this section. Figure 9.5a shows the sensitivity plot for various magnitude of positive charges. The biomolecules possessing positive charge which are present at the interface enhance the depletion of channel and improve the quantum mechanical tunneling of electron. As a result, the sensitivity rises. The trend of plots is analogous to Sect. 9.6.1. Figure 9.5b shows the I_D-V_{GS} curve of the VTFET biosensor for various value of at $N_{bio} = 10^{12}$ C/cm^2. The corresponding threshold voltage for each value of positive charge density is plotted in Fig. 9.5c.

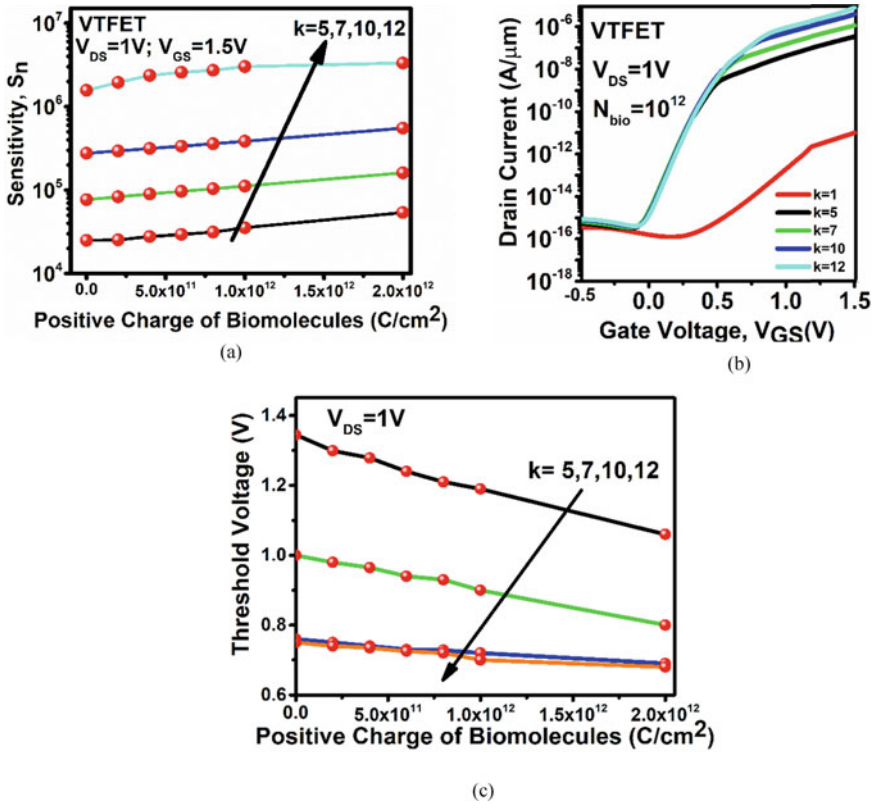

Fig. 9.5 **a** Sensitivity obtained at various positive charge densities of the biomolecules **b** I_D-V_{GS} curve of VTFET biosensor for $k = 1$, 5, 7, 10 and 12 at $N_{bio} = 10^{12}$ C/cm^2, **c** V_{th} versus positive charge densities of the biomolecules

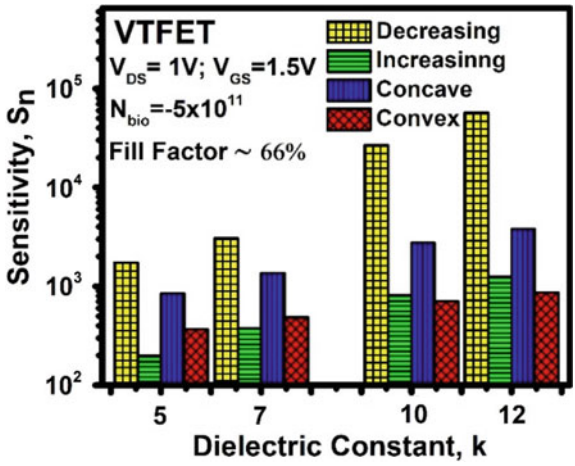

Fig. 9.6 Sensitivities of different step profiles of the biomolecules inside the nanogaps of VTFET biosensor

9.6.3 Step Profiles of Biomolecules Inside the Nanogaps

The comparison of the sensitivities for four different step profiles of biomolecules, viz., increasing, decreasing, concave and convex is shown in Fig. 9.6. Due to the steric hindrance, only 66% of nanogaps are occupied by biomolecules. The decreasing and concave profile response well, but the remaining two show very less sensitivity. This is because the decreasing and concave profile have highest steps close to the source-channel junction where tunneling takes place. Whereas in case of increasing and convex profiles, the highest step lies farther from the tunneling junction and this reduces the gate-channel coupling.

9.6.4 Response Time and Lower Limit of Detection

The time taken to rise to 90% of final value measured from onset of step input change is defined as the time response. The response time of VTFET biosensor is measured from Fig. 9.7a, and it is found to be $t_{\mathrm{pLH}} = 12$psec. Thus, this biosensor has very less response time which means it will response faster. This is because of the steeper SS characteristics of TFET.

The lower limit of detection (LOD) is calculated from the signal-to-noise ratio (SNR) measured using a combination of noise and *I*-*V* characterization. The SNR for FET-based biosensor in terms of number fluctuation model is presented as [33],

$$\mathrm{SNR} = \frac{g_m}{\sqrt{S_{\mathrm{id}}}} = \frac{1}{\sqrt{S_{VFB}}} = \sqrt{\frac{WLC_{\mathrm{ox}}^2 f}{\lambda kTq^2 N_T}} \tag{9.6}$$

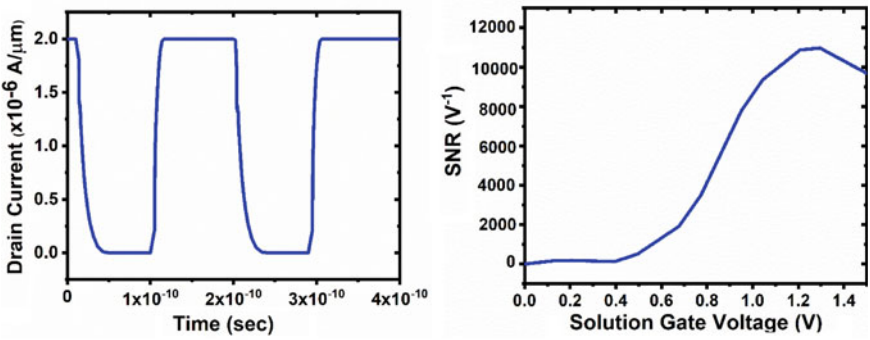

Fig. 9.7 **a** Drain current response w.r.t. time of VTFET biosensor at $k = 12$, **b** SNR versus solution gate voltage

where W and L respectively represent the width and length. T is the temperature, λ is the tunneling parameter, f is the frequency at which noise density power (S_{id}) is evaluated, and C_{ox} gives the oxide capacitance. Density of trap states such as interface traps/defects and surface states is given by N_T. In dielectric-modulated FET biosensors, the LOD is obtained by measuring the minimum change in the surface potential that can be detected which is obtained from 1/SNR. This value is restricted by the fluctuation in flat-band voltage caused by the traps and interface states [33]. Figure 9.7b shows the SNR plot with respect to the applied gate voltage. The value of peak SNR is 11200 which interprets to a minimum detectable voltage of ~89 μV. Hence, for this significantly low minimum detectable voltage, the LOD obtain is significantly less.

9.6.5 Status of VTFET Biosensor

This section shows the status of VTFET biosensor among various simulated, analytically modeled and fabricated FET-based biosensors available in the literature. Table 9.1 presents the sensitivity comparison of various geometries of DM TFET biosensors and highlights the status of the proposed VTFET biosensor.

9.7 Conclusion

This chapter presents a brief idea of the application of TFETs as dielectrically modulated label-free biosensor. Various aspects of TFET-based biosensor are studied through TCAD simulation with main focus on design and development of higher sensitivity sensor. The study has been extended to analyze the practicality of the

Table 9.1 Overview of various TFET-based biosensors

S. No.	Biosensors	Approx. sensitivity	ΔP
1	Conventional TFET [17]	50	12.5
2	Nanowire TFET [17]	9×10^3	2.25×10^3
3	DM FET ($L_{gap} = 200$ nm, $H_{gap} = 15$ nm, $k = 2.1$) [18]	1×10^4	2.5×10^3
4	DM FET ($L_{gap} = 100$ nm, $H_{gap} = 15$ nm, $k = 2.1$) [18]	3×10^4	7.5×10^3
5	Lateral DM TFET ($L_{gap1} = 10$ nm, $H_{gap} = 5$ nm, $k = 2$) [23]	10	2.5
6	Vertical DM TFET ($L_{gap1} = 10$ nm, $L_{gap2} = 15$ nm $H_{gap} = 5$ nm, $k = 2$) [23]	40	10
7	Full gate DM TFET ($L_{gap} = 10$ nm, $H_{gap} = 5$ nm, $L_{gate} = 42$ nm, $k = 4$) [19]	1×10^5	2.5×10^4
8	Short gate DM TFET ($L_{gap} = 10$ nm, $H_{gap} = 5$ nm, $L_{gate} = 20$ nm, $k = 4$) [19]	1×10^6	2.5×10^5
9	DM FET ($L_{gap} = 30$ nm, $L_{gate} = 100$ nm, $H_{gap} = 9$ nm, $k = 10$)[20]	5	1.25
10	DM FET ($L_{gap} = 75$ nm, $L_{gate} = 250$ nm, $H_{gap} = 9$ nm, $k = 10$) [20]	7	1.4
11	DM PNPN TFET ($L_{gap} = 30$ nm, $L_{gate} = 100$ nm, $H_{gap} = 9$ nm, $k = 10$) [20]	3×10^6	7.5×10^5
12	SiGe source DM PNPN TFET with 0% Ge composition ($L_{gap} = 15$ nm, $L_{gate} = 100$ nm, $H_{gap} = 9$ nm, $k = 2.1$) [22]	4×10^3	1×10^3
13	SiGe source DM PNPN TFET with 10% Ge composition ($L_{gap} = 15$ nm, $L_{gate} = 100$ nm, $H_{gap} = 9$ nm, $k = 2.1$) [22]	6×10^3	1.5
14	SiGe source DM PNPN TFET with 20% Ge composition ($L_{gap} = 15$ nm, $L_{gate} = 100$ nm, $H_{gap} = 9$ nm, $k = 2.1$) [22]	5×10^3	1.25×10^3
15	Dual metal gate SiGe pocket vertical TFET ($L_{gap1} = 20$ nm, $L_{gap2} = 10$ nm $H_{gap} = 5$ nm, $L_{gate1} = 25$ nm, $L_{gate1} = 15$ nm, $k = 12$) [This work]	2×10^6	5×10^5

Sensitivity of biosensor = ($\Delta P \times$ Sensitivity of MOSFET-based biosensor)
Approximate sensitivity of MOSFET-based biosensor [24] = 4

sensor by considering some non-idealities. A vertical tunnel FET-based biosensor is proposed in this chapter, and it shows interesting results in terms of sensitivity, response time and lower limit of detection. Eventually, an overview of various FET sensors is presented to highlight the status of the VTFET biosensor.

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Chapter 10
Electrochemical Biosensor Designs Used for Detecting SARS-CoV-2 Virus: A Review

Riya Titus, Mukti Mandal, and Gorachand Dutta

Abstract With new mutations of the COVID-19 or SARS-COV-2 virus being reported worldwide, one wishes to have access to a reliable, low-cost early diagnostic device that can be used at home. Biosensors able to detect these viruses have been sought out as point-of-care (POC) devices, which can be used in such situations and are hence gaining popularity. Recently, many research works are focused on this sector due to the pandemic. The electrochemical biosensors are a subcategory of biosensors based on the transducer used. The electrochemical means allow for more low-cost, efficient, and portable POC products. Different designs for a POC biosensor have been developed to detect different biological analytes of importance over the years. This review gathers information about the important designs of electrochemical biosensor devices currently being used and advances possible for COVID-19 virus detection. The information provided can be used for further design developments in the field of electrochemical biosensors that can be used to detect such viruses.

10.1 Introduction

The year 2019 marked the start of a pandemic that is still evoking fear worldwide [55]. The SARS-COV-2 virus, a member of the B lineage of the genus Betacoronavirus (β-CoV), is more similar to the SARS-COV than the MERS virus. The various variants that are arising due to its mutation are causing havoc and concern among the public. SARS-CoV-2 is a virus of ~3 kb length that is single-stranded and possesses a

Riya Titus and Mukti Mandal are equal contribution.

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positive RNA genome [56]. A virus is an intracellular parasite that uses host cellular mechanisms to replicate itself for its survival. A genomic material (DNA or RNA), a protein capsid for protecting the nucleic acid, and often an envelope for the capsid made of lipid, make up the essential components of the virus structure [54].

From the nineteenth to the twentieth, the turn of the century witnessed the emergence of formal studies on viruses. In 1933, the first Influenza virus (Flu) was isolated in a laboratory. The years 1918–1919 saw the Spanish Flu pandemic caused by a respiratory virus [25]. The respiratory tract of humans has physical barriers composed of epithelial cells and mucus and the alveolar macrophages in the lungs that protect us from most respiratory infections. The viruses usually attach to the receptors of the target-cell surface and penetrate the cytoplasm of the cell. The uncoated viral nucleic acid gets replicated and, upon assembly and maturation, becomes the infectious virus that leads to the visible symptoms. RNA viruses tend to mutate faster, such as respiratory syncytial virus (RSV), coronavirus (MERS and SARS-CoV), and Flu. These respiratory viruses cause concern in the public health sector because such mutations can lead to them being more virulent and potent [47]. SARS-CoV belongs to the subfamily coronavirinae of the family coronaviridae of the order Nidovirales. There are four genera within coronavirinae which are the alpha-, beta-, gamma-, and delta coronaviruses. Though widespread among mammals and causes mild respiratory or enteric infections, they were not a cause of much concern until the outbreak of severe acute respiratory syndrome (SARS) in 2002. The Middle East witnessed another coronavirus outbreak in 2014, caused by Middle East respiratory syndrome coronavirus (MERS-CoV) [41]. The SARS-CoV-2 or COVID-19 pandemic, which started toward the end of 2019, was declared a pandemic at the beginning of 2020. This time, it was caused by the coronavirus 2 (SARS-CoV-2) [50]. In viruses like SARS-CoV-2 and SARS-CoV, spike glycoprotein enters the host by binding to the angiotensin-converting enzyme 2 (ACE2) receptor found in several human body organs [38]. The whole genome analysis showed that β-CoVs encode several nonstructural (2/3rd of SARS- CoV-2's RNA) and mainly four structural proteins (spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) (1/3rd of the genome) [37]. ORF1ab or ORF8 regions and the E, N, S, and RdRP genes of SARS-CoV-2 are the often genomic target regions of primers and probes. The main proteins (antigens) that trigger an antibody response in humans are S and N [44].

Cross-reactivity is a problem that can occur during diagnosis. This occurs when the test shows false positivity for any other species of coronavirus in humans. This happens due to the antibodies created against the proteins of one type of virus occasionally binding weakly to the proteins of another closely related virus, causing cross-reaction [9]. We can detect the viral infection either by directly detecting the virus or by the reactions or immune responses that occur in the body during the viral infection and detecting the products thus released during those reactions. Methods used for virus detection can be classified broadly as immunological assays, amplification techniques, and biosensors [42]. Immunological assays may include enzyme-linked immunosorbent assay (ELISA) and lateral flow immuno assay (LFIA), which can provide relatively fast on-site results, but some disadvantages exist like a high false-positive rate (5–11%) and relatively low sensitivity. These serological methods also

face difficulties in detection due to the infection differences like being symptomatic or asymptomatic, immune response differences, and time duration of viral infection from patient to patient [3]. Amplification techniques include polymerase chain reaction (PCR), nanopore-targeted sequencing (NTS) [53], and recently the use of CRISPR technologies. Though they provide a relatively accurate result, some disadvantages exist. This is a slow process involving many steps for processing. The high mutation rate of RNA sometimes renders these processes inaccurate in detecting the mutated virus's target genome [3]. The gold standard for immunoassays is radioimmunoassay (RIA), and the most commonly used immunoassay technique includes the enzyme-linked immunosorbent assay (ELISA) test [34]. The biosensors have proved themselves as a potential device to complement the ELISA tests in detecting several infections and diseases [17]. Especially now, upon the rise in COVID cases, different biosensors have been developed to detect the same.

10.1.1 Biosensors

Biosensors have mainly two parts: Bioreceptor and transducer. A biosensor is a device that can be used to detect biological analytes. The fear of the virus has driven the public to stay at their homes for safety. Thus, a device available for point-of-care (POC) applications like a biosensor catered to detecting SARS-COV-2 provided an option for the health sector to keep the COVID cases in check. Biosensors can be classified based on the analyte or reactions they can detect. They can be immunosensors (antigen–antibody interaction), enzymatic biosensors (enzyme-target analyte interaction) [18], DNA biosensors (hybridization), and whole cell biosensors as per this classification. Biosensors can also be of different types based on the transducers they use, such as electrochemical, optical, piezoelectric, calorimetric, or scanning probe microscopies. Optical biosensors use the principles of absorbance, fluorescence, chemiluminescence, or refractive index. Similarly, piezoelectric, calorimetric, and scanning probe microscope biosensors use the principles of affinity interactions, thermal characteristics, and atomic-level forces [30], respectively. Meanwhile, electrochemical biosensors use amperometric, potentiometric, conductometric, and impedimetric principles. If a biosensor uses an electrode system to convert any biological recognition event into an electrical signal, the thus obtained device is called an electrochemical biosensor [2]. Thus, electrochemical biosensing allows for comparing the change in the values of current, voltage, resistance, or capacitance due to any identifiable biological or chemical change. Commonly, these biosensors use three types of electrodes, namely working (electrode where the concerned reaction takes place), e.g., carbon, platinum, gold, etc., reference (the electrode whose potential remains constant against throughout the reaction), e.g., silver/silver chloride, saturated calomel electrodes, etc., and auxiliary electrodes or current-carrying electrodes (electrodes for making voltammetric and impedimetric measurements), e.g., inert solid electrodes like Pt, graphite, etc. These have low detection levels,

inexpensive running costs, and simple instrumentation, making it easier for miniaturization. Based on the biological recognition elements and working principles also the electrochemical biosensors can be classified as enzymatic electrochemical biosensors, bioaffinity-based electrochemical biosensors, microbial biosensors, and nanobiosensors [2, 15].

10.1.2 Designs and Principles

There have been attempts to make simpler designs of these biosensors, and thus, microfluidics seemed a promising field as they are also based on total analysis system (TAS) [20]. Similarly, lab-on-chip designs apply microfluidics principles and integrate several laboratory steps into a single chip or circuit. Methods like lithography, laser machining, and xurography have been popular in recent times and modified to manufacture microfluidic devices such as lab-on-chips [26]. Electrochemical biosensor technologies and lab-on-chip principles, if appropriately implemented in a device, can reduce the cost, effort and increase the reliability of the detection kit thus developed [14]. As a result, much research has been initiated during these recent years to develop a biosensor design that will detect COVID-19 at high accuracy and precision with greater sensitivity and specificity [21]. There are different types of electrochemical biosensing techniques. Some of them are cyclic voltammetry, potentiometry, amperometry [22], electrochemical impedance spectroscopy [16], and conductivity. Cyclic voltammetry consists of changing different voltages and measuring the respective current values. Equipment used to detect phenolic antioxidants in chocolate food items uses this principle. Potentiometry measures the intrinsic voltage generated by the working electrode that is sensitive to the analyte versus the reference electrode insensitive to the analyte (Example: pH meter). Amperometry includes the applying of voltage and measuring the current value (Example: Glucose sensor). Electrochemical impedance spectroscopy is a non-destructive technique used to detect the time response of chemical systems at various voltage frequencies using low-amplitude alternating current (AC). An example of a system based on this principle is a microfluidic-based electrochemical biochip for diffusion-restricted DNA hybridization detection [4, 19], and conductivity is used for measuring salinity. Another type of popular principle used is that of Bio-FET. The (bio)molecules that bind to the substrate cause a surface potential change that leads to the device being gated. It is a type of field-effect transistor (FET) operating as an intrinsic amplifier. They can cause significant changes in the current when even small changes in the surface potential occur without any additional circuitry requirement. Here the design is such that a change in conductance occurs when biomolecules bind to the FET dielectric or gate electrode. As a result, it changes the dielectric material (FET gate dielectric), changing the underlying semiconductor material charge distributions.

An example of this is the label-free microfluidic integrated DNA FET on a printed circuit board by Xu et al. [58] to be used as a sensing FET and as an electrophoretic

electrode to immobilize probe DNA at specific sites. Sometimes the principle of open-circuit potential (OCP) that uses net charge changes of an electrode surface and electrochemical impedance spectroscopy (EIS) is implemented together to create detection techniques that can detect MicroRNAs [28] and a microfluidic DC-biased AC electroosmotic vortex integrated DNA biosensing chip [19, 57]. Another principle we can add to an electrochemical biosensor to enhance its properties is carbon nanotubes. Carbon nanotubes show excellent conductivity, superior strength and sensitivity, remarkable physicochemical properties and chemical stability, and good biocompatibility. There are mainly two types of CNT based on the layers of walls: single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT). Carbon nanotube-based electrochemical biosensors are a type of nanobiosensor that can be broadly classified on the basis of the enzymes or biomolecules they detect. They are oxidase-based biosensors (e.g., glucose oxidase-based biosensors and cholesterol oxidase-based biosensors), dehydrogenase-based biosensors, DNA aptamer-based biosensors [36], CNT-based biosensors coated with antibodies for the detection of biomarkers, and other biosensors developed for biomedical applications based on carbon nanotubes [23].

Detection systems can be label-based systems or lab-free systems for biosensors. Label-based systems can be colorimetric, fluorescence, or optical fiber transducer-based. Colorimetric-based systems are fast, easy to use but have weak sensitivity. Fluorescence systems are of high efficiency, accuracy, fast labeling but costly. Optical fiber-based are controllable in sensing and sensitivity. Label-free systems can be electrical or conductance transducer-based or cantilever, or SPR based. Electrical and conductance transducer-based are of low cost, easy to use, and compatible with bioMEMS. Cantilever and SPR systems are too sensitive and can be affected by the environment.

Different materials can be used for designing a microfluidic biosensor. These materials can be inorganic, polymers, paper microfluidic chip, and hydrogels. Inorganic materials include silicon microfluidic chips, glass microfluidic chips, and ceramic microfluidic chips. Polymer materials include elastomeric materials like PDMS microfluidic chips and thermoset polyester (TPE) microfluidic chips. Polymer materials also include thermoplastic polymers like polystyrene (PS) microfluidic chips, polycarbonate (PC) microfluidic chips, polymethyl methacrylate (PMMA) microfluidic chips, polyethylene glycol diacrylate (PEGDA) microfluidic chip, microfluidic chip made of teflons like perfluorinated compounds (PFEP/PFA/PFPE), and polyurethane (PU) microfluidic chips. Composite materials such as cyclic olefin copolymer (COC) microfluidic chips and paper/polymer hybrid microfluidic chips can also be used [51].

When considering a suitable recognition element for detecting a virus, the nucleic acid-based biorecognition elements are more advantageous to use than antibodies due to their more stable nature. Designing any diagnostic device involves the proper selection of the biorecognition molecule/antigen that will finally be detected either qualitatively or quantitatively or both qualitatively and quantitatively by the device. In detecting the COVID-19 virus, we need a biorecognition molecule that one can detect easily and has the chance of least mutation so that we may be able to detect the virus

even if specific mutations occur while spreading worldwide, making the diagnostic test a reliable one. It was found in the SARS-CoV-2 samples taken from India that ORF1ab is the most mutated region. After ORF1ab, the most mutated region was the N-gene, then the S gene, and ORF8 [43]. Thus, the ideal antigen/biorecognition molecule that can be taken for the diagnostic test can be either the spike protein or ORF8.

10.2 Some Designs of Electrochemical Biosensors for SARS-CoV-2 Detection

10.2.1 Electrochemical—Amperometry

The making of this sensor involved cutting a 1.5 × 1.5 cm Ti sheet from a G1 grade Ti sheet. Any formed oxide layer was removed by polishing with 600 grit polishing paper. To avoid any exposure to the electrolyte composed of n 96.5 mL (CH2OH)2, 3 mL DI H2O, and 0.505 g NH4F, the unpolished side was masked with Kapton tape. With the Ti foil (working electrode) kept 3 cm apart from a platinum foil (counter electrode) in a standard two-electrode configuration in a teflon beaker, the electrochemical anodization was carried out. Rinsing with DI H2O and baking in an oven for 4 h at 120 °C followed anodization. The sample was annealed after the tape had been removed from the baked sample. Thus, TiO2 nanotubes (TNTs) were synthesized, and then, using an incipient wetting method (a wet ion-exchange process), it was functionalized with cobalt. To detect the S-RBD Protein of SARS-CoV-2, amperometry was performed at a bias voltage of −0.8 V. It can detect the S-RBD protein in a 14–1400 nM concentration range in a very short time of ~30 s. In addition to this sensor's evident rapid diagnostic application, it can also be modified with appropriate metallic elements to functionalize TNTs to detect other respiratory viruses. Figure 10.1 represents this electrochemical biosensor [52].

10.2.2 Electrochemical—Paper-Based Amperometry

This design is a simple, rapid, quantitative, easy to implement, selective, and low-cost paper-based gold nanoparticle-mediated electrochemical sensor chip. A simple two-step procedure was used to fabricate the chip that does not require any amplification of genes as a step. First, a graphene suspension was coated on filter paper, forming a conductive film providing high carrier mobility. Then as the next step, a gold electrode was deposited with a predefined design. The special feature of this design is that four different types of highly specific antisense oligonucleotides (ssDNA) are used to cap the gold nanoparticles, which are used to target two domains of the viral nucleocapsid phosphoprotein (N-gene). This allows for a multimodal approach that

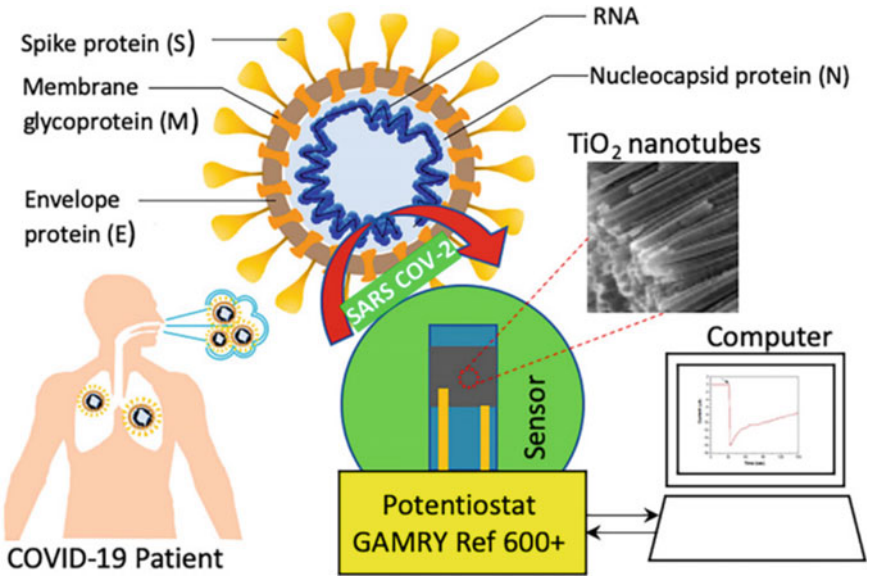

Fig. 10.1 An illustration of the working of the cobalt-functionalized TNT-based SARS-CoV-2 detecting platform. Reprinted (adapted) with permission from [52] from the Special Issue Detection and Diagnosis of the New Coronavirus under Open Access. Copyright © 2020 Sensors

helps to increase the reliability, sensitivity, and feasibility of the test even if some mutations occur in the viral gene. This type of electrochemical biosensor design is represented in Fig. 10.2 [1].

10.2.3 Electrochemical—Differential Pulse Voltammetry (DPV)

The design used here is that of a super-sandwich-type electrochemical biosensor which makes use of a capture probe (CP), label probe (LP), auxiliary probe (AP), and target sequence. Nucleic acid amplification and reverse transcription are not needed in this method. For detection, the use of two premixes is required. Premix A was prepared by immobilizing CPs labeled with Thiol on the Au@Fe3O4 nanoparticle surfaces forming CP/Au@Fe3O4 nanocomposites. Then, Premix B was prepared. For this, Toluidine Blue (TB) was enriched for the SARS-CoV-2 detection by functionalizing graphene (SCX8-RGO) using p-sulfocalix[8]arene (SCX8). Au@SCX8-TB-RGO-LP biconjugate was formed by immobilizing the host–guest complexes (SCX8-TB) on RGO. Thus, producing the sandwich structure (CP-Target-LP). Long concatemers were formed due to the introduction of AP. The detection step involves the extraction of the target viral RNA (ORF1ab), then incubating it with Premix A

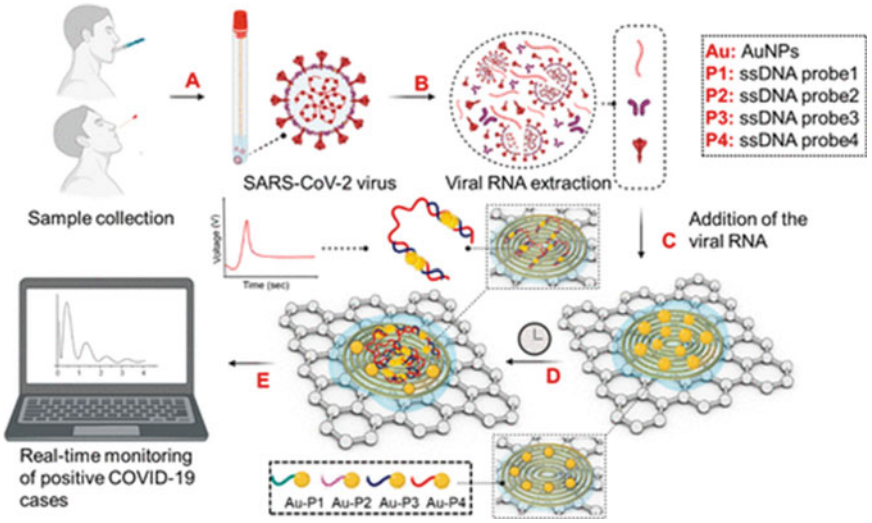

Fig. 10.2 A diagrammatic representation of a graphene-ssDNA-AuNP platform from sample collection, extraction, and getting added to the platform to incubation and recording of the digital electrochemical output. Reprinted (adapted) with permission from [1]. Copyright © 2020 American Chemical Society

for 1 h, and then next incubating this with Premix B for 2 h. Then detection occurs on the treated screen printing carbon electrode (SPCE), which is linked to a smartphone to show the results based upon the differential pulse voltammetry (DPV). This biosensor is reported not to show any cross-reactivity. This type of electrochemical biosensor design is shown in Fig. 10.3 [59].

Printed circuit board (PCB) technology was used to make electrodes for a low-cost electrochemical DNA biosensor, each costing just USD $0.55 (i.e., approximately INR ₹40) was used to detect COVID-19 virus N protein from wastewater samples using portable PCR instruments like miniPCR, without the requirement for qPCR reagents. Methylene Blue-DNA complex adsorption principles were used to increase the differential pulse voltammetry measurements. The PCB electrodes can also be reused after wiping them with lint-free wipes soaked with isopropyl alcohol. These electrodes were designed using Autodesk EAGLE software, and the standard ENIG plating process was used to form the gold electrodes on the PCB. Underneath the gold lies the nickel and copper layers of the electrode, respectively. PalmSens EmStat3 Blue potentiostat was used to perform voltammetry. PSTrace software was used to obtain peak values for DPV peak current and cathodic peak current in cyclic voltammetry voltammograms. The information thus obtained was used for the preparation of the required graphs. This type of electrochemical biosensor detection is represented in Fig. 10.4 [31].

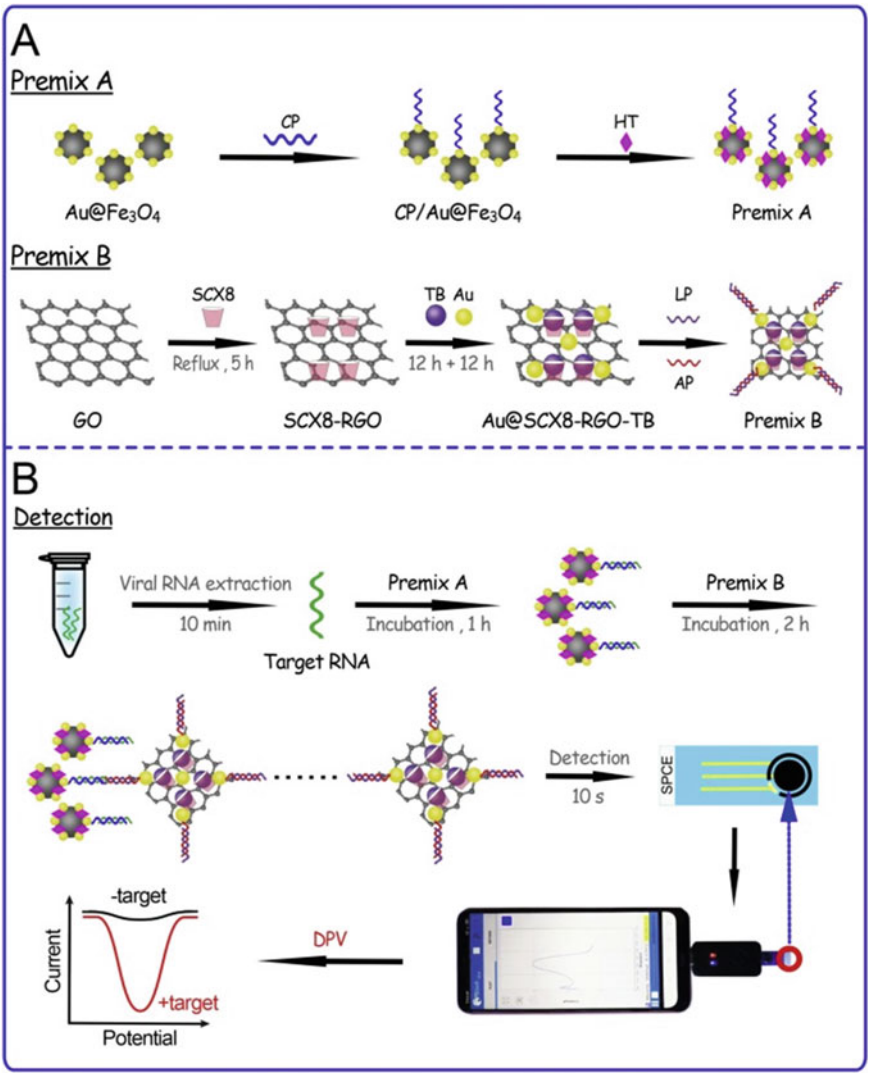

Fig. 10.3 A diagram representing SARS-CoV-2 detection using a type of super-sandwich-type electrochemical biosensor and its detection with the help of smartphones. Reprinted from [59], Copyright 2021, with permission from Elsevier

10.2.4 Electrochemical—Electrochemical Impedance-Based Sensing (EIS)

Another research team hypothesized that the binding kinetics between the anti-SARS-CoV-2 antibody and the SARS-CoV-2 spike protein receptor-binding domain

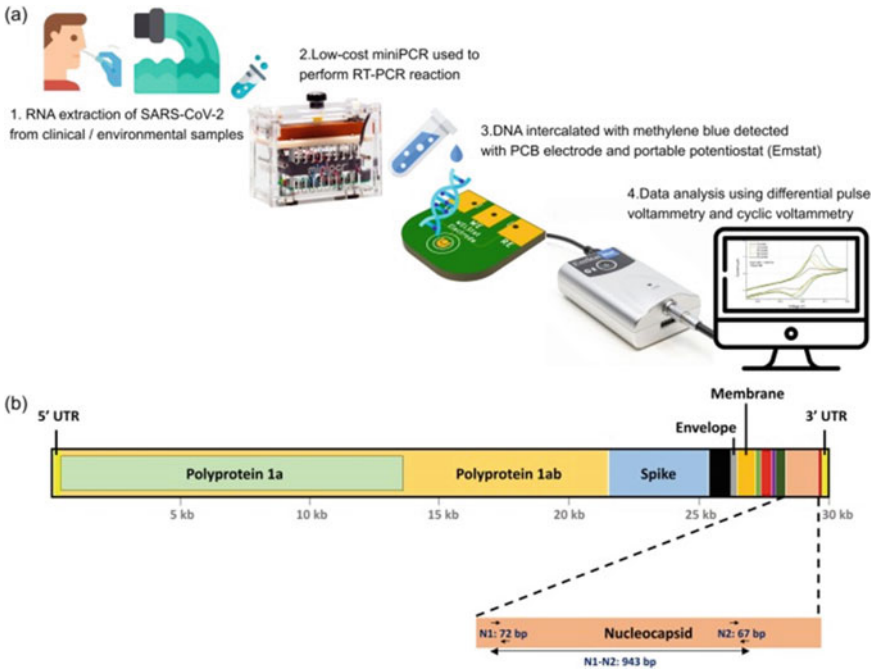

Fig. 10.4 A schematic illustration of the workflow for detecting SARS-CoV-2 using the PCB-based electrochemical biosensor and the coding genes of major proteins used in this study. Reprinted from [31], Copyright (2021), with permission from Elsevier

(RBD) mainly governed the detection mechanism. They introduced a design that uses a 16-well-plate xCELLigence system (RTCA S16) with integrated electrodes from ACEA Biosciences. This system design majorly allows for the non-invasive EIS detection of cell proliferation, morphology change, and attachment quality. Each well consists of an array of specially designed interdigitated electrodes fused to polyethylene terephthalate. Single-frequency measurements (10 kHz) are acquired every few seconds by each well, and the plate interface independently sees these. The interface attached to the well-plate is connected to the laptop via a USB. The timing measurements of independently addressable wells are controlled using a software; this type of electrochemical biosensor design is shown in Fig. 10.5 [45].

The 3D-printed COVID-19 test chip (3DcC) device has a working electrode (WE), a counterelectrode (CE), and a reference electrode whose base layers consist of coats of chromium and gold. Microdroplets containing gold nanoparticles were aerosol jet (AJ) printed on WE as micropillar arrays. The organic, non-polar solvent was used in the AJ printing technique, and this evaporated, leaving the dry nanoparticles and binders solidified. The printed structures were sintered to form the gold micropillars of the electrode. Shadow mask was used to coat thin silver or silver chloride layer on the RE. The replica-molding method was used to fabricate a PDMS microfluidic channel. For this, a poly(methyl methacrylate) (PMMA) mold and a

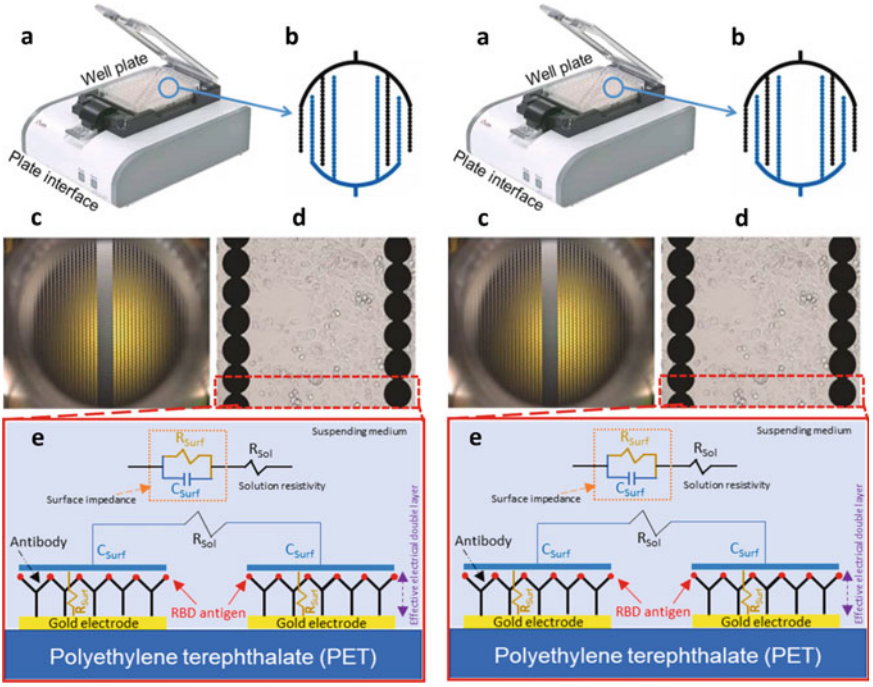

Fig. 10.5 96-well platform of ACEA Bioscience with the schematic layout of its electrode (**a**, **b**) and images of the electrode (**c**) and its magnified version (**d**), and illustration of electrochemical impedance-based sensing circuit (**e**) solution protein/antibody equivalent. Reprinted from [45], Copyright (2021), with permission from Elsevier

poly(dimethylsiloxane) (PDMS), reverse mold was used. The micropillar array and the CE and RE electrodes on the glass slide were covered manually using the PDMS housing. There are also tubes out of the microfluidic channel to input the fluid into the channel. This followed the micropillar electrode array (3D-printed) functionalization using reduced graphene oxide (rGO) nanoflakes that enhance viral antigen bonding, increasing the antibody detection from the introduced fluid. The binding of the antibodies to the corresponding electrodes with the respective antigens increases the impedance due to the increasing thickness of the double-layer capacitance (Cdl) detected using the electrochemical impedance spectroscopic (EIS) measurements. This detection occurred in seconds, and regeneration of the electrodes for further use was also possible within 1 min in low pH chemistry using elution of antibody–antigen immunoaffinity. The results could be visualized on a smartphone-based platform, making it one of the best options for POC applications. Thus, the respective SARS-CoV-2 antibodies (S1 and receptor-binding-domain (RBD)) were detected. Figures 10.6a, b show the steps involved in the construction of these types of electrochemical biosensors [3].

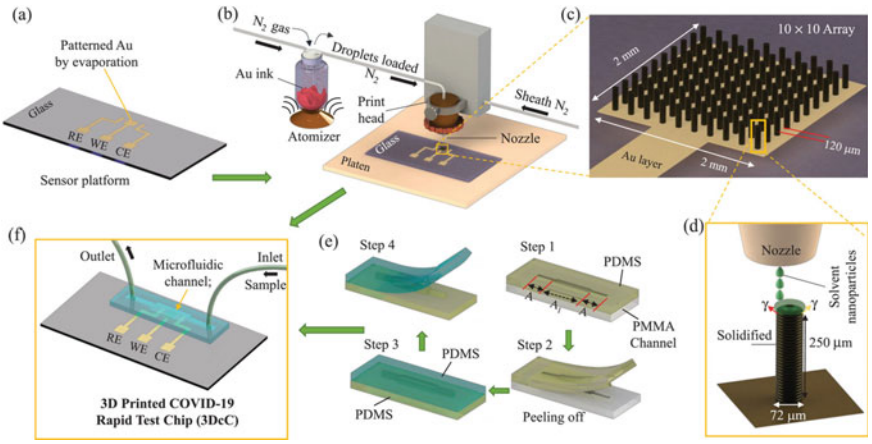

Fig. 10.6 An illustration of the process of manufacture of the 3D-printed COVID-19 test chip (3DcC) using aerosol jet nanoparticle 3D printing. Reprinted from [3], Copyright © 2020 Wiley–VCH GmbH, with Open Access permission from Advanced Materials, Wiley Online Library

10.2.5 Electrochemical—Semiconductor Analyzer

This was designed as a graphene-based electrochemical biosensor using the principles of field-effect transistors (FETs). The wet transfer method was used to deposit the graphene layer onto a SiO2 substrate. Channels were constructed using photolithography and reactive ion etching. Thin-film deposition and lift-off methods were used to add the metal electrodes. The graphene layer is soaked with PBASE solution. The PBASE acts as a linker, especially a pyrene group of the compound that non-covalently attaches to graphene through pi-pi stacking. At the other end of the compound is the activated ester that reacts with the amines. Thus, the SARS-CoV-2 S protein antibody reacts with the linker to form a chemical bond. The sensing area dimensions were set as 100 × 100 µm2 ($L \times W$). The device was also passivated with SU8-2010 so that the interferences during electrical measurements may be reduced. Also, the device showed no measurable MERS-CoV antigen cross-reactivity. Figure 10.7 shows the basic GFET design used for this type of detection method [48].

10.2.6 Electrochemical—Field-Effect Transistor (FET)

This device is an example of a FET nanobiosensor that uses a high-purity semiconducting (sc) single-walled carbon nanotube (SWCNT) for the detection of the SARS-CoV-2 spike protein and nucleocapsid protein. A specific antibody is used to functionalize the sc-SWCNT, enabling them to detect the two SARS-CoV-2 structural proteins from the nasopharyngeal swab samples. The high-purity sc-SWCNTs

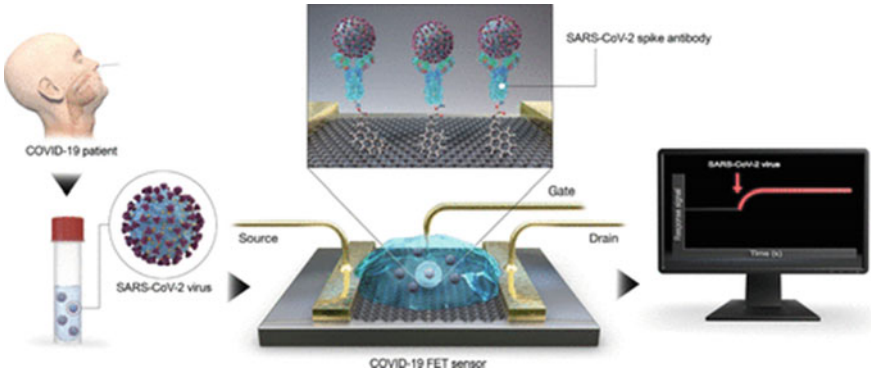

Fig. 10.7 A diagrammatic representation of the graphene FET sensor for detecting the SARS-CoV-2 spike antibody. Reprinted (adapted) with permission from [48]. Copyright © 2020 American Chemical Society

increase the sensitivity to detect the target analyte compared to unsorted SWCNT and graphene. Photolithography was performed to pattern the interdigitated gold electrodes on a Si/SiO2 substrate. Thus, the formation of microchannels of 10 μm took place, and dielectrophoresis (DEP) was used to deposit the sc-SWCNTs between the gold electrodes. Some treatments are done to activate the carboxylic groups and functionalize the SARS-CoV-2 antibody on SWCNTs. The devices were rinsed before and after soaking with a blocking buffer using nanopure water. This prepared the device for the FET measurements as nanopure water acted as the gating electrolyte. Detection of the SARS-CoV-2 antigens is done by studying its FET transfer characteristics in the liquid-gated FET device configuration. This type of electrochemical biosensor detection is shown in Fig. 10.8 [48].

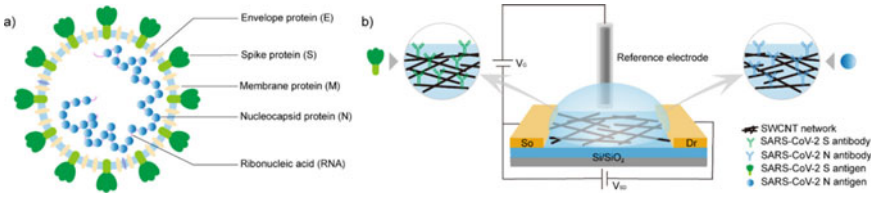

Fig. 10.8 Diagram representing the structure of SARS-CoV-2 and schematic illustration of the basic working of the SWCNT FET biosensor. Reprinted (adapted) with permission from [49]. Copyright © 2021 American Chemical Society

10.2.7 Electrochemical—Square Wave Voltammetry (SWV)

The design of this electrochemical immunosensor for the coronavirus related to the MERS consisted of an array of gold nanoparticles modified carbon electrodes (DEP). It had eight working electrodes. Two control electrodes were used for comparative purposes. For the MERS-CoV antigen, four electrodes were used, while for human coronavirus (HCoV), two electrodes were used. This allowed for duplicate measurements and testing of cross-reactivity for each sample on the same chip. Due to the design having eight electrodes, multiplexed detection can be done for eight different CoVs by immobilizing their antigens on each of them. The spiked nasal samples give electrochemical measurements recorded as SWV using the ferrocyanide/ferricyanide as a probe [35].

10.2.8 Electrochemical—Magnetic Force-Assisted Immunoassay (MESIA)

Sampinute™ COVID-19 Antigen MIA is a product by Celltrion, USA Inc. It is a magnetic force-assisted electrochemical sandwich immunoassay (MESIA). This is used for detecting the SARS-CoV-2 receptor-binding domains (RBDs) spike proteins qualitatively present in the nasopharyngeal swab specimens either collected directly or through the means of a viral transport media. The product comes with 25 test cartridges. While in use, the dispersion of the sample into the sample inlets of the cartridges takes place via a pipette or a reagent tube. The sample then flows through the microfluidic channel. They form complexes with the anti-SARS-CoV-2 spike protein antibodies conjugated to magnetic nanoparticles (MNPs) if the sample contains SARS-CoV-2 spike proteins. Otherwise, no complexes are formed. In such an electrochemical sensor, the working electrode coated with anti-SARS-CoV-2 spike protein antibodies is encountered with these complexes, which also bind with the electrode. The principle of magnetism is used to actively control the antigen–antibody reactions, ensure that thorough mixing occurs between the MNPs and the antigens, and remove the unbound MNPs. The device makes use of a detection buffer, after which the electrochemical measurement step is done where an electric current is induced. This is because of the electrochemical oxidation and reduction of gold on the MNPs induced due to the voltage applied initially. The electric current quantity measured above a specific cut-off value indicates if the test result is positive (SARS-CoV-2 spike protein antigen present) or negative (SARS-CoV-2 spike protein antigen absent). The results are then displayed on screen by the Sampinute Analyzer device [7].

10.3 Comparison Table and Future Perspectives

From the designs mentioned above, we can see the common essential elements of a biosensor listed in Table 10.1. Some of the designs focused on solving the problems that existed before while they were used to detect other organisms other than SARS-CoV-2. The paper-based amperometry electrochemical biosensor uses the change in output voltage to address the issue related to signal amplification methodology and is working on how to integrate the technology with a portable mobile platform [1]. Some were novel technologies developed to overcome the shortcomings of PCR-based RNA assays. Ultrasensitive super-sandwich-type electrochemical sensors aim to develop high-throughput diagnostics through microfluidic-based cartridges in the future [59]. Future aims of the team that developed the printed circuit board electrodes include identifying optimal primers for electrochemical sensing and PCR amplification, assay integration with electrochemical sensing and onboard thermocycling, and also at consistent potentials to enhance the stability of the reference electrode for achieving redox peaks [32]. The electrochemical impedance-based sensing (EIS) using capacitive immunosensing assay established the possibility of detecting SARS-CoV-2 antibodies at clinically relevant concentrations utilizing a quantitative EIS method using widely accessible equipment. Such techniques might allow for more fast screening of patient samples, larger serological surveys to measure community anti-SARS-CoV-2 antibody levels, and possibly improved vaccination activity evaluation [45]. The biosensing technologies used will allow for early infection identification and isolation, potentially saving lives. Some of the test platforms like functionalized TiO2 nanotube-based [52] and the aerosol jet-printed 3D electrode biosensors [3] are general, which means they may be used to identify biomarkers for other diseases, including Zika, HIV, and Ebola. Finally, the platform will serve as a valuable tool for studying the infection and after recovery immune response dynamics. The sc-SWCNT FET detection method also allows for multiplex detection of viral antigens as well as antibodies that recognize these antigens [49]. Thus, we can see that new technologies and improvements made in either the fabrication or the working of these biosensors increased their utility and robustness. 2D materials like graphene and black phosphorus have also been utilized in biosensors for POC diagnostics [10]. Instead of the traditional and commonly used carbon-based screen-printed electrodes (SPCE) used in the super-sandwich-type electrochemical biosensor [59], screen-printed graphene electrodes (SPGE) can be synthesized and used. Reports suggest that SPGE have superior electrochemical properties than SPCE [10]. Similar studies are also being done on black phosphorous (BP), which shows excellent electrochemical properties due to their inherent redox properties. They have been used in an aptamer-functionalized and nanostructured label-free electrochemical biosensor [32]. BP-based biosensors also seem to show higher sensitivity and specificity in detecting IgG or IgM against the SARS-CoV-2 virus in blood samples compared to reduced graphene oxide (rGO) [10]. Also, it is seen that the detection sensitivity of the EIS method depends on various factors. Often there is an unaccounted change in impedance due to these factors. Thus, it has been found that the

application of machine learning algorithms considering these factors can help predict the change in impedance more accurately [24].

10.4 Conclusion

Electrochemical biosensors used to detect several diseases have a huge potential due to their low cost, portable nature, easy instrumentation, and miniaturization (especially in making MEMS devices). Due to their electrochemical nature and signals that can be detected using a current/voltage change detector, they can be linked with other devices like smartphones to make customizable applications. These applications providing interactive and easy-to-use and detect platforms make diagnostic tests truly point of care (POC) in nature. Point-of-care tests should have the features according to the ASSURED guidelines. Thus, they have to be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free (no complex equipment), and delivered to end-users [27]. Apart from electrochemical biosensors, chip-based, microfluidic-based [33], paper-based, or some material-based like film-based [8] and textile-based [40] biosensors or a combination of these can be developed for the detection of COVID-19 virus. The film-based biosensors can be used to detect pathogens from a crude sample. The textile-based biosensors can be cloth-based, thread-based, or fabric-based. These biosensors seem to provide ways to make the least complex ones that reduce cost, increase sensitivity, and provide a possible diagnostic method to detect the antibodies against the SARS-CoV-2 virus.

In the pandemic scenario, when we need regular testing and vaccination drives, releasing a massive amount of biomedical waste, one must focus on creating an ecological solution that causes the minimum amount of impact on the environment while providing the best utility. Thus, biodegradable alternatives for materials that we can use to make these devices must be considered. At last, the ultimate product we get must cause the least harm to the environment and be easy to dispose of. Simple bioinspired technologies or techniques can also be used in these test kits to make sample collection and processing easier. New innovative ideas are being used to develop these diagnostic kits, such as smartphone-based detection tests [39], using a collection pad on a lollipop stick to collect saliva samples [6], Carbon NanoTubes (CNT), and CRISPER-CAS technologies. Some of the current POC devices also incorporate CRISPR technologies for the diagnostic test process. Most of these tests, for sample preparation, made use of commercially available RNA extraction kits [5, 12] or made use of several pipetting steps for simplified lysis for the detection reaction [29, 39]. One notable achievement by Helena et al. (De Puig et al.) [11] involved developing the only diagnostic that can detect various variants of SARS-CoV-2 at nearly the CDC RT-qPCR standards. This device is called minimally instrumented SHERLOCK (miSHERLOCK) that uses biodegradable polylactic acid for 3D printing the device, thus minimizing cost and reducing the environmental footprint. It is also linked to an automated mobile app.

Table 10.1 List of electrochemical biosensors developed to detect COVID-19

S. No.	Type of electrochemical biosensor	Substrate	Immobilized material/analyte	Monitored compound	Sample	Limit of detection (LOD)	Time duration	References
1	Amperometry	Cobalt-functionalized TiO2 nanotubes (TNTs)	SARS-CoV-2 S-RBD protein	Label-free	nasal secretions and saliva samples	~0.7 nM	30 s	[52]
2	Paper-based amperometry	Graphene film with Au electrode on filter paper	RNA (N-gene)	Antisense oligonucleotide (ssDNA)-capped gold nanoparticles	Nasopharyngeal swab, saliva	6.9 copies/μL	<5 min	[1]
3	Differential pulse voltammetry (ultrasensitive super-sandwich-type)	Au@SCX8-RGO-TB	RNA (ORF1ab)	Labeled signal probe	Sputum, urine, serum, and saliva	3 aM	3 h	[59]
4	Differential pulse voltammetry (DPV)	Gold-padded printed circuit board (PCB) electrodes	SARS-CoV-2 amplicons	Methylene Blue	Simulated spiked wastewater samples	–	–	[31]
5	Electrochemical impedance-based sensing (EIS)(capacitive immunosensing assay)	16-well-plate with sensing electrodes	CR3022 antibody	Label-free detection	Serum specimens from COVID-19 and non-COVID-19 patients	Clinically relevant antibody concentrations	5 min	[45]

(continued)

Table 10.1 (continued)

S. No.	Type of electrochemical biosensor	Substrate	Immobilized material/analyte	Monitored compound	Sample	Limit of detection (LOD)	Time duration	References
6	Impedance	Reduced graphene oxide (rGO) nanoflake-functionalized AuNP micropillar array	Antibodies to SARS-CoV-2 S1 protein receptor-binding domain (RBD)	Label-free	Serum (antibodies to SARS-CoV-2 spike S1 protein) Serum (antibodies to SARS-CoV-2 receptor-binding domain (RBD))	2.8×10^{-15} M 16.9×10^{-15} M	10 s	[3]
7	Semiconductor analyzer	Graphene FET	SARS-CoV 2 spike antibody/ SARS-CoV 2 spike protein	Label-free detection	PBS buffer Cells culture	1.00 fg.mL^{-1} 1.6×10^{1} PFU.mL^{-1}	~400 s	[48]
8	Liquid-gated FET transfer characteristics	SWCNT FET	SARS-CoV 2 spike protein SARS-CoV 2 nucleocapsid protein	Label-free detection	PCR positive nasopharyngeal swab samples and negative nasopharyngeal swab samples	0.55 fg/mL 0.016 fg/mL	<5 min	[49]
9	Square wave voltammetry (SWV)	Array of carbon electrodes	MERS-CoV and HumanCoV proteins/antibody for each virus	Reduction peak current of ferro/ferricyanide redox couple	Spiked nasal samples (HumanCoV) Spiked nasal samples (MERS-CoV)	0.400 pg.mL^{-1} 1.00 pg.mL^{-1}	~20 min	[35]

(continued)

Table 10.1 (continued)

S. No.	Type of electrochemical biosensor	Substrate	Immobilized material/analyte	Monitored compound	Sample	Limit of detection (LOD)	Time duration	References
10	Magnetic force-assisted immunoassay	Gold on magnetic nanoprobes	Receptor-binding domains (RBDs) of SARS-CoV-2 spike protein antigens	Magnetic nanoprobes (MNPs)	Nasopharyngeal swab specimen in either a reagent solution or viral transport media	3.0×10^{1} TCID50/mL	<10 min	[7]

Asymptomatic cases of COVID-19 pose a threat to containing the spread of the virus since it does not show any typical symptoms and is hence difficult to recognize or detect if a person has SARS-CoV-2 infection. Also, during RT-PCR tests for COVID-19 [46], it was found that it failed to detect some cases of SARS-CoV-2 infection. All of these, coupled with the reckless behavior of the public, led to the subsequent waves of COVID-19 that several countries have faced till now. Chest X-ray proved to be a reliable method to detect the virus that infects the lungs early, even before significant symptoms are seen in the patients. AI-Based Intelligent COVID-19 detector Technology for Medical Assistance (ATMAN) is web-based software developed by CAIR, DRDO, and built using deep convolution neural network. This application can automatically pre-process the images from the X-ray test and classify the patient as normal, COVID-19, or pneumonia class. ATMAN is a tested and validated software approved for use. Thus, the use of AI is also proving itself as a valuable tool to enhance diagnostics [13].

Hence, we can see much potential for developments in the field of diagnostics in this world, where new diseases and organisms get reported while new technologies are also found to contain them. From this paper, one can get a rough idea of the developments in the field of electrochemical biosensors that have been developed to detect the SARS-CoV-2 virus. Some future aspects and advantages of these electrochemical biosensors are that the efficiency of the current systems can be increased through new materials and technologies that are being introduced. In addition to this, most of these biosensor designs mentioned before can also aid in the detection of other pathogens if they are modified accordingly.

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