Nanobiosensors for Point-of-Care Medical Diagnostics

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Preface

With rapid urbanization, increase in human life expectancy and environmental pollution, people are more susceptible to various diseases. So, the cost of healthcare is increasing at an alarming rate. Early diagnosis plays an important role in curing and managing many life-threatening diseases. For instance, chances of curing cancer increase when detected at an early stage. In the case of diabetic patient, detection of glucose level at regular intervals is important for its proper management.

Conventional detection methods or techniques and equipment are limited to established hospitals or sophisticated laboratory setup. These facilities are expensive and need properly trained manpower for operation. These facilities are hardly available in the rural areas putting limitations on diagnostic confirmations. Emergent of situation, like Covid-19 pandemic where healthcare infrastructures are overburdened, manpower are overstressed, the use of point-of-care (POC) biosensors is of great help. Biosensors draw huge attention due to ease of application, high sensitivity, high selectivity, low cost, and low turnaround time. They help in non-invasive or minimally invasive, diagnosis of diseases. Point-of-care biosensors are the translated version of biosensor knowledge base. Even though the field of biosensors has been increasing rapidly, there is a huge gap between the existing knowledge base and their translation into working POCs. Understanding the reasons behind this gap will motivate more people to work towards filling this existing gap.

Chapter 1 focusses on the application of POC biosensors for healthcare applications and how they are helping in curbing disease transmission/treatment and management of different diseases. It also gives an overview of existing commercial biosensors available in the market.

Chapter 2 reviews the application of different biomolecules used in POC biosensors. It also highlights the design and applications of various biomolecules with the aim of addressing the existing challenges in POC biosensors in healthcare diagnostics.

Chapter 3 highlights different nanomaterials used for the development of POC biosensors along with their challenges and future prospects.

Chapter 4 discusses new generation molecular techniques used in POC biosensors for the detection of infectious. This chapter elaborates various advantages and pitfalls of molecular methods in colorimetric, electrochemical, fluorescence, magnetic, chemiluminescence, surface plasmon resonance (SPR), and surface-enhanced Raman scattering (SERS)-based POC biosensors that have led to the development of miniaturized lab-on-a-chip (LOC) devices are discussed.

Chapter 5 elaborates on POC biosensors for glucose sensing. This chapter also highlights the challenges and future prospects in glucose sensing biosensors.

Chapter 6 focusses on several combinations of affordable POC equivalents that are alternative to cumbersome and traditional diagnostic tests that have been proven to be effective in emergency healthcare. Analysis of the time-saving benefit to cost ratio is crucial to improve the overall processing of a cancer patient culminating in decreased turnaround times as well as quicker diagnosis leading to rapid patient disposition.

Chapter 7 elucidates a wide range of POC diagnostic platforms used for diagnosis of the cardiovascular diseases using different biochemical biomarkers. These POC platforms have a promising impact on the early prediction and diagnosis of the future of cardiac care.

Chapter 8 discusses different POC nanobiosensors for determining vitamin deficiency which is affecting people of all ages. The chapter also explains how these devices can help primary care practitioners and general physicians to detect vitamin deficiencies and optimize vitamin supplementation doses.

Chapter 9 presents recent POC biosensors for the diagnosis of neurological disorders and how nanoparticles can be used for their efficient diagnosis and progressions.

Chapter 10 provides update about POC biosensors for sexually transmitted diseases and how the advancement of POC biosensors improves the recovery rate, decreases the complications of these diseases, and benefits the public health of societies by breaking the chain of disease transmission.

Chapter 11 elucidates about recent advancement of microfluids-based nanobiosensors and their potential as well as challenges in healthcare sector.

Chapter 12 presents an overview about recent advancement on nanobiosensors in disease diagnosis and their potential in early diagnosis and treatments.

In nutshell, this book depicts the current status and future prospects of POC biosensors for healthcare applications. The chapters deal with the basics of POC biosensors, nanomaterials used for the biosensor, and applications of the biosensors for the detection of different diseases. The target group of the book is post-graduate, PhD students, and advanced researchers. It will be helpful for both novices and the experts working in the field of biosensors.

Shillong, Meghalaya, India — Manashjit Gogoi
Guwahati, Assam, India — Sanjukta Patra
Kharagpur, West Bengal, India — Debasree Kundu

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Dr. Debasree Kundu expresses her sincere gratitude to her husband and family members for their constant support and motivation.

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Manashjit Gogoi is working as an Assistant Professor in the Department of Biomedical Engineering, North-Eastern Hill University, Shillong, since last 10 years. He did his PhD in Biomedical Engineering from Indian Institute of Technology, Bombay, Mumbai. His research area focusses on Bio-nanotechnology and its application in healthcare diagnostic and therapeutics. He is an erudite researcher and is presently undertaking 04 research projects from various funding agencies. He has to his credit 19 research articles, 20 book chapters, and 01 patent (filed). He has actively supervised PhD (02 completed and 03 undergoing) students. He served as Section Editor of Current Pathobiology Reports (2020–2021) published by Springer for a special issue on 'Nano drug delivery' and is a member of several scientific associations. His commendable efforts in exploiting nanocarriers for cancer therapy help to realize his true potential for serving the scientific interests in the field of healthcare.

Sanjukta Patra is working in the Department of Biosciences and Bioengineering at the Indian Institute of Technology Guwahati, Assam, India, since last 13 years and is also heading the School of Agro and Rural Technology of the Institute. She has earned her PhD in Biotechnology from Central Food Technological Research Institute, Mysuru. Her PhD thesis encompassed study of microbial and enzymatic biotransformation of coffee and tea processing wastes. She has expertise in enzyme engineering, enzymes in biosensors, enzymatic remediation, applied microbiology, and environmental microbiology. Presently, she has a dedicated research group of 'Enzyme and Microbial Technology', where her team is working towards diverse aspects of enzymes, the challenges in enzymatic process and its applications. She is also the course instructor for graduate and post-graduate students. She has actively supervised several PhD (07 completed and 12 undergoing) and various MTech (14) students towards different aspects of enzymes and its applications. Her undaunted enthusiasm and research intellect enabled her to have patents (03 granted),

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Debasree Kundu is working as Women Scientist in the Department of Biotechnology at the Indian Institute of Technology Kharagpur, West Bengal, India. Prior to this, she was associated with the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Assam, India, where she was pursuing her post-doctoral research and worked towards unravelling the mechanistic insights into pesticides-induced adaptive response in microbes using proteomic approaches. She has co-supervised several post-graduate dissertations pertaining to enzyme responsive nanoparticles for drug delivery and therapeutic applications. She has nurtured her research instincts that prompted her to have 31 (21 research articles and 10 review articles in edited books) peer-reviewed research publications. She has also granted patent to her credit. She has made sincere efforts and maintained consistency towards her research endeavour of enzymatic biotechnology and interdisciplinary research interests.

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Chapter 1
Point-of-Care Biosensors for Healthcare Applications

Bethuel Daurai and Manashjit Gogoi

1.1 Introduction

Biosensor is a device that detects chemical molecules via biochemical processes mediated by enzymes, immunosystems, tissues, organelles, or entire cells, generally using electrical, thermal, or optical signals (Monošík et al. 2012; Turner 2013, 2014; Jurado-Sánchez 2018). Biosensors are composed of bioreceptors, transducers, and an electronic processing unit (containing a signal processing unit and a display system). Bioreceptors or recognition elements are immobilized biological molecules like enzyme, substrate, complementary DNA, and antigen which detect or sense target analytes based on their affinity (Davis et al. 1995; Abd El-Hamid et al. 2015; Senbua et al. 2020). The transducer converts biochemical signal into an electrical signal as a consequence of the analyte's interactions with the bioreceptor. The strength of the signal produced by the biological reaction is proportional to the concentration of the analyte (Arkin and Ross 1994; Nikhil et al. 2016). The electrical signal is subsequently translated into a readable value for the analyte concentration by the signal processing unit, which consists of a microcontroller. A display system will then be integrated to display useful information of the analyte. The use of a signal processing unit allows the use of mathematical models for further analysis, storage, and transfer of the results and data. The schematic representation is given in Fig. 1.1.

Point-of-care (POC) biosensors are scaled down to compact device or equipment that quantifies or qualifies certain analytes for the determination of diseases and ailment conditions (Gouvêa 2011; Soler et al. 2019) near the patient. POC biosensor helps a person to analyse samples without a sophisticated instrument or an expert (Luppa et al. 2011). A blood glucose system is one of the greatest examples of a POC

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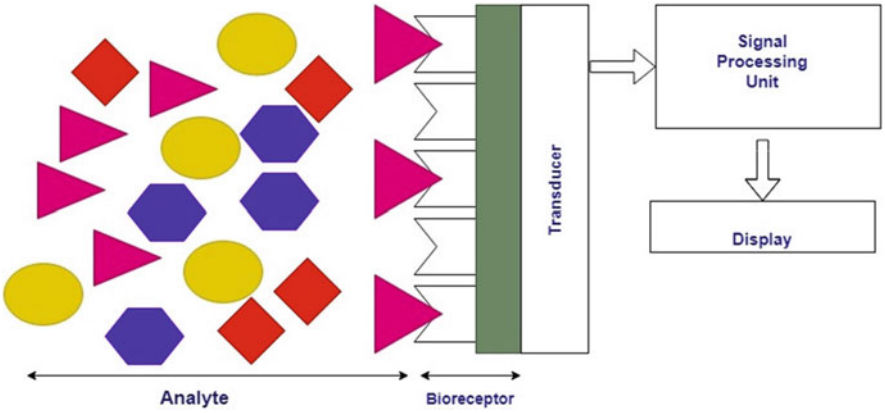

Fig. 1.1 Schematic diagram of a biosensor where an analyte binds to a bioreceptor which is then translated into a readable signal by a transducer

gadget that uses an electrochemical detection process. It is now widely used in household for a diabetic patient (Newman and Turner 2005). POC testing shortens the therapeutic turnaround time (TTAT), i.e. the time between placing an order for a test and receiving an order for therapy (Fermann and Suyama 2002). POC test depends on several factors like regulatory standard, the cost-effectiveness and ease of using the device. POC testing needs proper and regular validation by regulatory boards with strict criteria to meet the standard of a laboratory test (Lippi et al. 2013). There is also a requirement of integrating the results of POC testing with laboratory information system and hospital information system. POC testing requires less quantity of sample to give accurate and rapid results which improves the quality and efficiency of care in an emergency service, operation room, critical care, and in outpatient setting (Kost et al. 1999; Larsson et al. 2015).

Rapid diagnostic tests (RDT) are also useful in the healthcare perspective as they can give qualitative results in a matter of seconds which are important in times of an epidemic (Gavin and Thomson Jr 2004). POC devices could be characterized for clinical investigation at or close to the area of patient consideration. The main objective of POC device is to give quick and simple result from an analytic test that shortens the time taken when compared with a test done in laboratory equipments and highly trained professionals (Arefin et al. 2017; Brazaca et al. 2017; Harris et al. 2017). POC biosensors have a number of unique requirements. The device should give quick result in an emergency clinic or primary healthcare centres where sophisticated facilities are not available readily (Tiwari 2010). Storage should be possible at room temperature or in an ordinary refrigerator. Miniaturization is also an important part in a POC biosensor (Liu et al. 2020). Smaller devices are easier to carry and helpful for POC applications.

Wearable biosensors (WB) are non-invasive natural sensors incorporated into different wearables, for example, watches, garments, gauzes, glasses, contact focal points, rings, etc. (Ghafar-Zadeh 2015). WB can also be a POC testing element as

they can often be used to determine the physical parameters like blood oxygen saturation, heart rate and even electrocardiogram data can now be collected on the move using non-invasive mode (Arefin et al. 2017).

1.2 Biosensors for POC Testing

One of the most significant utilizations of biosensors is the POC testing. POC testing is the act of playing out a demonstrative or prognostic test close to the patient to give fast outcomes, which means the test itself must be fast and effortlessly performed without the utilization of costly and complicated instrumentation (Baryeh et al. 2017).

Microfluidic-based biosensor provides platform for cell growth and analysis of the samples. Most platforms used for microfluidics are based on PDMS, paper, silicone, and polymer (Pandey et al. 2018). Filter paper and nitrocellulose membrane are cost-effective and have been widely used in laminar flow type of biosensor for mainly qualitative analysis. Lab-on-chip (LOC), a miniaturized version of a lab made from PDMS, paper, and paraffin pellets are one of the platforms of affordable POC biosensor (Kumar et al. 2013; Patel et al. 2016). They are specifically designed for resource-limited settings where only the biological samples are sufficient to do the tests (Zarei 2017). LOC biosensors are labelled because the bioreceptors and other transducers are used to get the desired signal. The label-free type of biosensors are more advantageous for POC testing as the analyte themselves acts like a transducer to get the desired signal (Cooper 2003; Baryeh et al. 2017).

Several nanomaterials including gold nanoparticle (AuNP), quantum dots (QD), carbon nanotube (CNT), silica nanoparticles (SiNP), and graphene are used in POC biosensors. These nanoparticles are employed in biosensors to increase the biosensors' sensitivity, reliability, and responsiveness (Brazaca et al. 2017). Nanomaterials have a high surface area to volume ratio; however, they have drawbacks such as toxicity, loading capacity limitations, and particle aggregation. Moreover, multi-layered structures of paper are also used as a microfluidic channel by restricting areas of paper with hydrophobic material. Biomarkers determine the accuracy and resolution of the analyte detection technique.

Based on the design and usage, POC biosensors can be categorised as a handheld device (which may consist of an electronic component) and a tabletop/benchtop device which can be as big as a portable autoanalyser. Handheld POC biosensors can be as simple as a cassette-based test strip, or it can have an electronic component with display of results. Rapid diagnostic tests (RDT) provide results in as little as a few seconds to a few hours, depending on the test. Figure 1.2 is a typical cassette for rapid tests that gives qualitative analysis of the analyte. It works on the principle of lateral flow immunochromatographic assay (LFIA). Some common examples of RDT/LFIAs done are pregnancy test, malarial test, test for coronavirus disease 2019, and some other viral diseases like influenza. Most RDTs are qualitative in

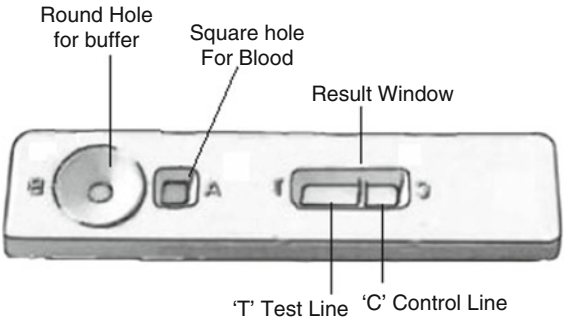

Fig. 1.2 A typical RDT/LFIA cassette where the change in colour of the test line indicates the presence of the specific analyte of specific quantity

determining the disease. There are some diseases that require the quantification of the analyte.

RDT of certain diseases and conditions are already available in the market for end users. Pregnancy test kit, a POC (RDT) device in India, is available in a variety of brands most of them working with the same principle of immunochromatography method with minor modifications to detect hCG hormone in the urine. Some of them are Prega News by Mankind Pharmaceutical Ltd., i-can by Piramal Healthcare, Velocit by Dr. Reddy's, etc.

There are many other rapid test kits using biosensor and chromatography like rheumatoid factor, Widal (salmonella typhi and paratyphi), dengue NS1 antigen, dengue IgG/IgM, C-reactive protein, and even HIV. Although these POC tests provide rapid and precise findings, laboratory procedure and validation with a polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA) will always remain the gold standard. Figure 1.3 shows a typical rapid diagnostic device with a cassette. Rapid test biosensors can also have complicated electronic systems to detect analytes using an electrochemical sensing process. For example, a handheld POC biosensor determines the amylase level in saliva to determine the stress level in patients (Yamaguchi et al. 2006). This kind of kit does not require any expert professional to test.

Blood glucose analysers are widely used in handheld POC rapid test systems that work using an electrochemical sensing process. OneTouch, Accu chek, TrueTrack, and Omnitest are some lines of devices that are used in testing blood glucose.

Benchtop POC biosensor for troponin I by Pathfast (Mitsubishi Chemica) is equivalently effective as the FDA-approved laboratory test (Peacock et al. 2016). Benchtop devices are bigger in size than a handheld device. A better option is available in order to make it portable and POC. Augustine et al. reported using an autoanalyser as a point-of-care haemoglobin test, although the cost of the autoanalyzer is very high (Augustine et al. 2020).

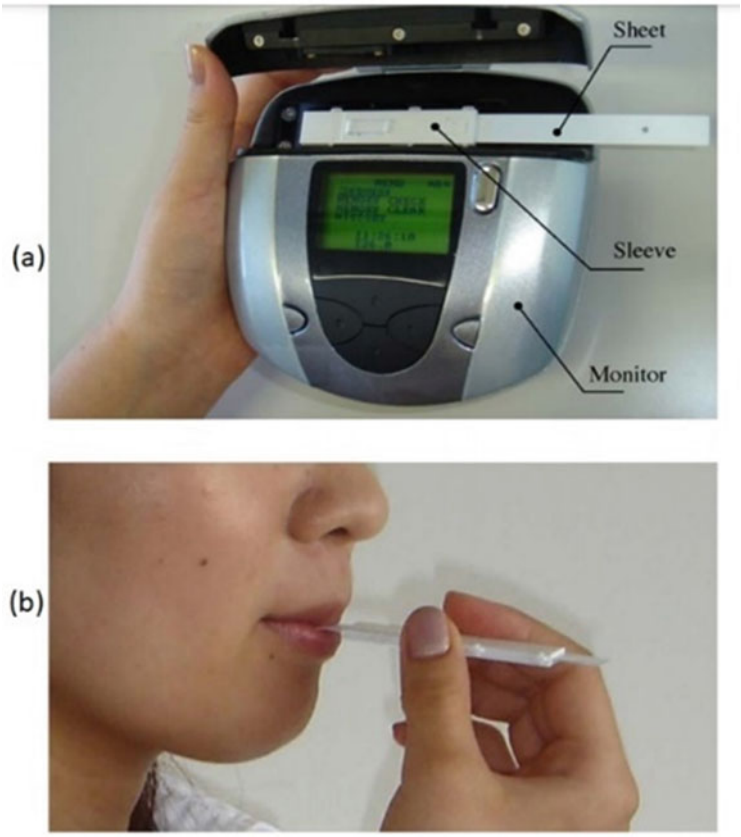

Fig. 1.3 (**a**) Handheld α-amylase tester from saliva. (**b**) Sample collection from saliva using paper (Yamaguchi et al. 2006)

1.3 Application of POC Biosensors in Healthcare

POC biosensor in the healthcare system reduces the TTAT. All diseases have biomarkers in one or more biological fluids or materials (Griffiths et al. 2002; Strimbu and Tavel 2010). A qualitative or quantitative analysis of these biomarkers will identify the disease. Based on this, biosensors for POC testing are designed to get a better TTAT (Mayeux 2004). Many POC biosensors are already available for various diseases like cardiovascular diseases, pancreatic disease, kidney disease, neurological problem, diabetes, urinary tract infection, etc. We discuss some of the diseases, their biomarkers, and the POC biosensors available for the biomarkers.

A survey conducted by Satyanarayana et al. from various primary healthcare providers in India showed that the most used POC tests are pregnancy test followed by malaria, glucose, HIV, typhoid, tuberculosis, syphilis, hepatitis (A, B, or C), influenza, and dengue (Satyanarayana et al. 2014). Although the number of glucose

tests may be more than malaria because the home tests are unaccounted for and the widespread of the disease in India and all over the world (Ramachandran et al. 2001). The test for COVID-19 disease may now be the most tests being done using a biosensor.

1.3.1 POC Biosensors for Detection of Cardiovascular Diseases

Cardiovascular diseases are related to the heart and the vascular system. Myocardial infarction, heart failure, and acute coronary syndrome are the most common cardiovascular illnesses. By 2035, the American Heart Association (AHA) predicts that 45.1% of the population in the United States will have a cardiovascular disease. In 2016, the number of global death from cardiovascular disease was estimated to be 17.6 million (Benjamin et al. 2017; Ouyang et al. 2020). Biomarkers recommended for myocardial infarction and acute coronary syndrome are cardiac troponin I (cTnI), cardiac troponin T (cTnT), and creatine kinase myocardial band (CK-MB) (O'Gara et al. 2013; Jneid et al. 2017; Thygesen et al. 2018). The level of these biomarkers is elevated from the onset of the symptom till several days. Biomarkers for the diagnosis of heart failure include highly sensitive c-reactive protein, B-type natriuretic peptide (BNP), and N-type propeptide BNP (NT-proBNP) (Greenland et al. 2010). Other emerging biomarkers like copeptin, myoglobin, and myeloperoxidase can indicate myocardial infarction (Melanson et al. 2004; Mythili and Malathi 2015; Roffi et al. 2016). POC devices for cardiovascular diseases are either handheld or benchtop ones. The RDT biosensors can be categorized under the handheld biosensor system.

1.3.1.1 Handheld POC Biosensor Devices

There are three commercially available handheld POC devices: Roche's Cobas h 232, Abbott's i-STAT, and Philips' Minicare I-20. Cobas h 232 is based on the principle of chromatography using gold nanoparticles. It can detect cTnT (0.04–2 ng/mL), myoglobin (30–700 ng/mL), NT-proBNP (0.06–9 ng/mL), and CK-MB (1–40 ng/mL) (Hex et al. 2018; Ouyang et al. 2020).

The i-STAT uses the principle of electrochemical sensing where there are two sites for enzyme-linked immunosorbent assay (ELISA) using microchannels. It can detect ctnI (0.02–50 ng/mL), BNP (0.015–5 ng/mL), and CK-MB (0.6–150 ng/mL) (Martin 2010; Loewenstein et al. 2013; Ouyang et al. 2020). And, the Minicare I-20 is a handheld device by Philips that utilizes magnetic particles. The device can detect only cTnI for a range of 0.018–6.1 ng/mL (Kemper et al. 2017; Ouyang et al. 2020). All the assays need whole blood or plasma as sample with a volume in the range of 17–150 μL and generate results within a response time of maximum 12 min.

Zhang et al. studied a label-free optical biosensor with photonic crystal to detect cTnI. The biosensor was able to detect cTnI with level as low as 0.1 ng/mL (Zhang et al. 2014). In another study, Shanmugam et al. created an electrochemical biosensor to detect cTnT using a ZnO nanoparticle with a detection limit of 0.1 pg/mL (Shanmugam et al. 2016). For the characterization of the biosensor's response to cTnT, DC and AC-based electrochemical impedence spectroscopy was used. Another ZnO nanostructure-based label-free, multiplexed electrochemical biosensor was developed (Shanmugam et al. 2018). Human blood serum was used to detect cTnT, cTnI, and BNP, with a detection limit of 1 pg/mL.

1.3.1.2 RDT POC Biosensor Devices

Some qualitative readers do not need reader device and the result can directly be determined by viewing it with the naked eye. RapiCard InstaTest by Cortez Diagnostics, Nano-Check ND-CD301S and ND-CD402 by Nano-Ditech, and LifeSign by Princeton BioMeditech, CardioDetect by Renesa are some of the LFIA to detect cTnI, CK-MB, and Myo within the of 1.5–100 ng/mL (Ip 2010; Zrari and Mohammed 2016). They all work on the principle of lateral flow chromatographic assay based (LFIA) by use of specific bioreceptor for cTnI, CK-MB, and Myo. Most of the samples were whole blood/plasma sample with maximum response time or TTAT of 15–20 min (Pezzuto et al. 2019; Ouyang et al. 2020).

LifeSign by Princeton BioMeditech detects cTnI (minimum 1.5 ng/mL), CK-MB (minimum 5 ng/mL), and Myo (minimum 50 ng/mL) (Audet et al. 2015; Ouyang et al. 2020). Figure 1.4 shows a LifeSign which is a cassette-based. If the analyte is present in the sample the line appears indicating its presence.

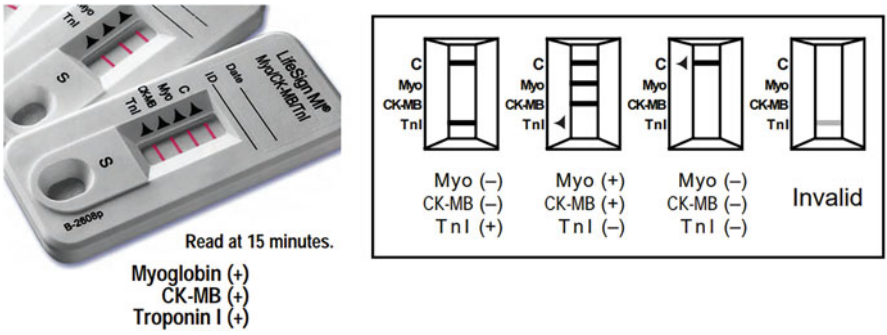

Fig. 1.4 An LFIA cassette of LifeSign for detection of cTnI, CK-MB, and Myo. The control proofs migration and performs test of dye conjugates. If no control the test is invalid (Biomeditech n.d.)

1.3.1.3 Benchtop POC Biosensor Devices

Benchtop-based devices that can detect multiple biomarkers are comparatively smaller than the laboratory system with a smaller TTAT but still bigger than a handheld device and can sometime require an expert to operate the system. Most devices with multiparameter capabilities for cardiovascular diseases, that are available commercially, are based on immunoassays with antibodies as their biomarkers (Ouyang et al. 2020). The transduction unit is optical based, either fluorescence, chemiluminescence, or colorimetric transducer. The use of chromatographic assay is most common for antibody capture and gold nanoparticle to give optical results. A test line on a paper strip containing functionalized colloidal gold nanoparticles captures antibody and causes a colour shift to indicate the presence of an antibody. Multiple lines on the same strip or multiple strips on the same cartridge techniques have been used for multiple biomarker detection. Multiple fluorescent materials were used in the Fluoro-Check AMI 3 in 1 to detect different biomarkers (Juntunen 2018).

The Triage Cardiac Panel based on microcapillary is used to test multiple biomarkers with different zones, where different reagents target biomarkers to produce fluorescent output (Lingervelder et al. 2019; Boeddinghaus et al. 2020). The Triage Cardiac Panel series has a better lower detection limit than the Nano-Check AMI series (Mojibi et al. 2018; Çelik 2019; Ouyang et al. 2020). This is because fluorescence assays are more sensitive as transduction element as compared to colorimetry. These devices have a TTAT of 15–20 min and need a sample volume of 75–800 μL.

1.3.2 POC Biosensor for Cholesterol Detection

Cholesterol level is also an important factor in the human body. It can be used to determine neurological as well as cardiological diseases. Higher level of cholesterol indicates cardiological illness and lower level can indicate anaemia and hyperthyroidism. The main bioreceptor for cholesterol is cholesterol oxidase. The use of an electrochemical biosensor to measure cholesterol levels in the blood was studied by Kaur et al. (2018). Platinum electrodes coated with thin layer of nickel oxide on the working electrode and bare platinum on the counter electrode were employed in microfluidic channels which were made of polydimethylsiloxane. Cholesterol oxidase was immobilized on the nickel electrode. With a range of 0.12–10.23 mM and a sensitivity of 45 A/mM/cm^2, a limit of detection of 0.10 mM was achieved (Fig. 1.5).

Kaur et al. (2017) developed another microfluidic channel using paper for the analysis of cholesterol. SU 8 photoresist was used to make the microfluidic channels (Kaur et al. 2017). Amperometric measurement is done when the cholesterol comes

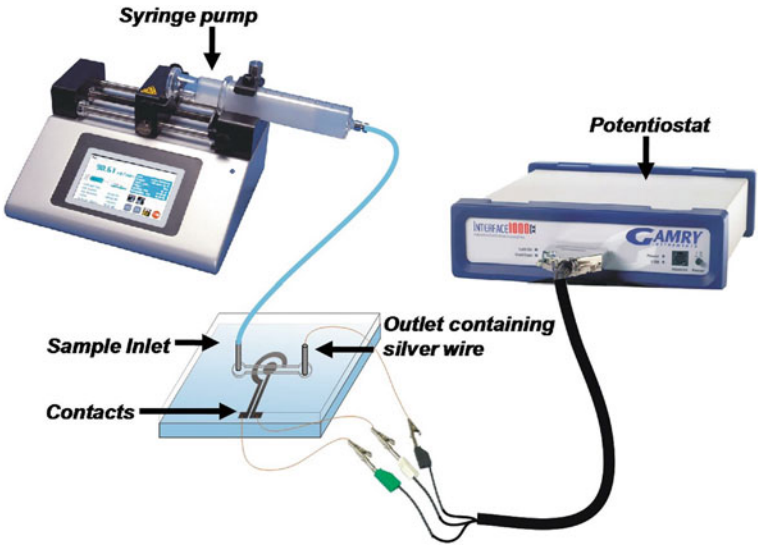

Fig. 1.5 Amperometric biosensor made of PDMS for the microfluidic channel to quantify cholesterol (Kaur et al. 2018)

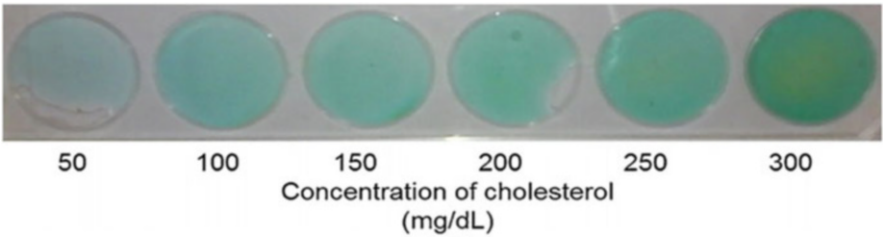

Fig. 1.6 Colour change of the nanofiber mat with increased concentrations of cholesterol (Dhawane et al. 2019)

in contact with the cholesterol oxidase. The working and counter electrodes were made of graphite, while the reference electrode was comprised of silver and was treated with nickel oxide nanoparticles. The sensitivity achieved was 26 μA/mM/cm^2 with a range of 0.12–10.23 mM.

Dhawane et al. reported a cholesterol-detecting optical biosensor utilizing chitosan nanofiber (Dhawane et al. 2019). Chitosan nanofiber mat was prepared using chitosan and polyvinyl alcohol (PVA) in a 0.7:1 (w/w) ratio. Cholesterol oxidase was immobilized on the chitosan nanofiber mat as a bioreceptor for the cholesterol. UV-Visible spectrophotometer is used for the colorimetric detection of the cholesterol level by determining the resultant H_2O_2 with a mixture of chromogenic substance (3,3′,5,5′-tetramethylbenzidine hydrochloride). Different concentration ranging from 50 to 300 mg/dL was measured using the chitosan nanofiber mat (Fig. 1.6).

Yang et al. investigated an electrochemical non-enzymatic biosensor for cholesterol detection (Yang et al. 2012). On a glass substrate, platinum nanoparticles and carbon nanotubes are alternatively deposited layer by layer, resulting in a total of 24 bilayers. A linear range of 0.005–10 mM was achieved with a very low detection limit.

1.3.3 POC Biosensors for Detection of Diabetes

Diabetes is a condition when the blood sugar is more than 3.5–5.5 mmol/L (Bunescu et al. 2013; Güemes et al. 2016). Biosensor for diabetes is one of the most widely used POC biosensors by patients themselves. The inability to produce insulin increases the blood sugar level. There are three generations of glucose biosensors. Amperometric enzymatic biosensing was the first generation of glucose biosensor with advantages like simplicity, miniaturization, and cheap (Karyakin et al. 1995; Wang 2001; Freeman et al. 2013). The resultant H_2O_2 solution from the reaction of glucose and glucose oxidase (bioreceptor) was measured electrochemically. The first-generation glucose biosensor had disadvantages. The first was the interaction of electroactive chemicals such as ascorbic acid, uric acid, and acetaminophen in the blood (Kulkarni and Slaughter 2016; Scholten and Meng 2018). This drawback was addressed by adding catalyst to H_2O_2, like Prussian blue or noble metal. The second drawback was oxygen deficit caused due to oxygen insolubility in the biological fluid. A diffusion limiting membrane was used to address this drawback by adjusting the flux of oxygen and glucose to the surface of the electrode (Abdel-Hamid et al. 1995; Matsumoto et al. 2001; Zhu et al. 2012). Another way to address this issue is to use oxygen-rich carbon past electrode (Wang and Lu 1998). In the first generation, the current of the reduction in O_2 is measured amperometrically. The first-generation glucose biosensors are POC because of their miniature structure and simplicity to make the reading devices.

In the second generation, the oxygen transfer is addressed by the use of mediators between the glucose oxidase and the electrode with materials. These materials make the transfer of electron faster and also solve the oxygen deficit problem in the first generation (Zhang et al. 2020).

The third generation of glucose biosensor incorporates glucose oxidase directly with the electrode thereby eliminating the need of mediators for the transfer of electron (Koopal et al. 1994; Mehmeti et al. 2017). New development of electrodes based on nanomaterials like graphene, carbon nanotubes, etc., has improved the sensitivities and also overcomes the drawbacks of the first generation and second generation (Tang et al. 2000; Alwarappan et al. 2010; Fu et al. 2011).

A non-enzymatic biosensor is mainly focused on an artificial enzyme that is made from nanomaterials (Gooding 2019). Teymourian et al. discussed three drawbacks of non-enzymatic biosensor, i.e. lack of specificity because of not using selective recognition element, alkaline pH of the solution and emphasis of research on the

synthesis of nanomaterial and its fabrication instead targeting the glucose itself (Juska and Pemble 2020; Teymourian et al. 2020).

Atanasov and Wilkins discussed about implantable glucose sensors with insulin pump for continuous monitoring of blood glucose using an electrochemical biosensor (Atanasov and Wilkins 1994). The device was implanted subcutaneously. The use of third-generation glucose biosensor made it easy to implant the device which could measure up to 500 mg/dL. Elsherif et al. also studied a photonic microstructure made using glucose selective hydrogel functionalized with phenylboronic acid (Elsherif et al. 2018). It was integrated with a contact lens. On contact with glucose the microstructure swells up and modulated Bragg's diffraction is measured between zero order and first order using a smartphone camera. The sensitivity achieved was 12 nm/mM with a response time of maximum of 30 min. When associated with telemedicine frameworks, wireless systems and smartphone-based glucometer allows medicinal service suppliers to screen the physiological attributes of patients after therapeutics or medications (Weymouth et al. 2018).

One of the most trending research studies is on non-invasive and wireless sensors. Glucose POC biosensors using infrared and Raman spectroscopy have now been developed which showed promising in contrast to the result from an invasive method (Buford et al. 2008; Sivaraman and Shankar 2016). A tattoo-based amperometric biosensor for glucose was used to monitor the blood glucose during and after eating food (Bandodkar et al. 2015). The limit of detection of the biosensor was 10 μM. The on-body test also showed specific spike in glucose as compared to the Accu-Chek Aviva Plus.

The BeatO Glucometer is India's first POC glucose metre that uses a smartphone to measure blood glucose levels (George et al. 2019; Mondal et al. 2020; Sabharwal et al. 2021). Although the ease of use in BeatO glucometer, the study by Mondal et al. found that the accuracy is 74% of the required clinical use regulation. Some other handheld POC glucometer includes Gluco One by Dr. Morepen, Accu-Chek, OneTouch, Dr. Trust, etc.

1.3.4 POC Biosensors for Detection of Kidney Diseases

Kidney is one of the most important organs to filter out toxins and waste materials from the human body. Kidney diseases may be acute or chronic with many biomarkers (Chawla and Kimmel 2012). Although serum creatinine and blood urea nitrogen are often used as biomarkers for detecting acute kidney illness, they are not sensitive enough and they may indicate other non-renal functions (Tesch 2010; Edelstein 2016, 2017). There are more sensitive and specific biomarkers like cystatin-c, beta trace protein, urine albumin, uric acid, neutrophil gelatinase-associated lipocalin (NGAL), etc. (Nickolas et al. 2008; Fassett et al. 2011). The Nova Stat sensor POC and the ABL800 flex analyser are two well-known POC devices for measuring creatinine and estimating glomerular filtration rate (eGFR).

The normal level of creatinine in the blood is 45–90 μM (male) and 60–110 μM (female) (Killard and Smyth 2000; Tseng et al. 2018). It is also present in urine (4.4–18 mM) (Ruedas-Rama and Hall 2010; Li et al. 2015), saliva (4.4–17 μM) (Lloyd et al. 1996; Venkatapathy et al. 2014), and sweat (9.4–18 μM) (Bass and Dobalian 1953; Al-Tamer and Hadi 1997). There are more studies of optical-based readout than electrochemical biosensing in previous studies (Cánovas et al. 2019). Dasgupta et al. studied the usage of ferric chloride as a creatinine receptor. With the increase in the creatinine, ferric chloride concentration decreases thereby reducing the current signal (Randviir and Banks 2013; Dasgupta et al. 2018). It has also been stated that a carbon paste electrode can be used on a sodium chloride solution. Singh et al. investigated the utilization of reduced graphene oxide, coated on a 3D printed silver electrode, with stabilized binary copper and iron oxide (Singh et al. 2021). It was a non-enzymatic sensor which was able to eliminate the interference of urea, glutathione, etc. It was however not a commercialized sensor but has a scope of POC biosensing. Snaith et al. compared Nova StatSensor, Abbott i-STAT, and Radiometer ABL800 FLEX with the Roche Cobas 8000 series as a reference and found that ABL800 FLEX had the best concordance with the reference, followed by Abbott i-STAT, and finally Nova StatSensor (Snaith et al. 2018).

Progression of mild to moderate kidney disease can be predicted with Cystatin C level in blood serum (Onopiuk et al. 2015). It is also said to be more reliable than creatinine (Bleher et al. 2012). Devi et al. discussed the fabrication of electrochemical sensor with the modification of the carbon working electrode with graphene oxide-chitosan conjugated with anti-cystatin C antibody (Devi and Krishnan 2020). This can have application in POC because of the miniature size of the system. A disposable immunosensor was also developed which was made of gold nanoparticles conjugated with anti-cystatin C antibody which was fabricated using screen printing technology (Lopes et al. 2019). Bleher et al. reported the use of anti-cystatin antibody as bioreceptor in glass substrate to have a label-free optical biosensor to diagnose kidney diseases (Bleher et al. 2012). But, creatine is still the preferred biomarker over Cystatin C (Shlipak et al. 2013). Cystatin C has also been known to be used as a biomarker in diabetes retinopathy (Wong et al. 2015).

Albumin in the urine is also an indication of renal failure. Huang et al. reported a biosensor made of microfluidic channels made of polydimethylsiloxane (Huang et al. 2007). The electrode materials were coated on a glass substrate. The limit of detection was found to be 5 ppm which was lower and better than the commercially available strips (Kouri et al. 2000; Huang et al. 2007). Smith et al. conducted an experiment on a commercially available Mission Urinalysis Reagent Strip (manufactured by Acon laboratories) (Smith et al. 2016). The strip provides colour change which could be analysed using simple software and a smartphone as demonstrated by Smith et al. The *Mission*® U120 Urine Analyzer and Ultra Urine Analyser are POC devices manufactured by Acon. Liu and Ma designed a new portable urine analyser which can meet the standard of The *Mission*® U120 Urine Analyzer and it can detect analytes like creatinine, microalbumin, ascorbic acid, etc. (Liu and Ma 2018).

1.3.5 POC Biosensors for Detection of Cancer

Cancer is unwanted growth and uncontrolled rapid division of cells that can be benign as well as malignant. Some biomarkers for solid tumours are carcinoembryonic antigen (CEA) for colon, CA125 for ovarian, protein-specific antigen (PSA) for prostate, CA15-3 or CA27.29 for breast cancer (Soper et al. 2006). Antibodies are commonly used as recognition elements but there are other approaches like aptamers, peptides, and molecular imprinting of polymers (DeRoock et al. 2001; Cerchia et al. 2002; Chambers and Johnston 2003). Polyclonal and monoclonal antibodies are most common approaches for targeting the antigen. To target cells peptides have also been used as they are smaller in size and have high selective bond affinity to the binding sites of the cells. Aptamers are often created using the exponential enrichment process, which involves the systemic development of ligands. Optical, piezoelectric, and electrochemical transduction are often studied with these biorecognition elements.

Mavrikou et al. developed a electrochemical POC-based biosensor for the determination of PSA in the blood serum (Mavrikou et al. 2018). Anti-PSA antibody was used as a biorecognition element by electroinserting it into vero cell. An 8-channel potentiostat was use used where all the three electrodes were screen printed with carbon, silver/silver chloride and the rest were ceramic substrate. Gold nanoparticles were used for amplification of the carbon working electrode. Khan et al. developed a biosensor to quantitatively determine the presence of PSA in saliva electrochemically (Khan et al. 2018). They used paper-based graphene nanoplatelets with diblock copolymer and gold electrode which showed a detection range of 0.1–100 ng/mL and the device gave results within 3–5 min.

1.3.6 POC Biosensors for Detection of COVID-19

The pandemic SARS-Cov2 has led to rapid production of antibody-based biosensors. With no effective medicine rapid and mass testing was the only solution, which could only be achieved by POC biosensor (Bahl et al. 2020). With the affinity of an antigen to the antibody, the SARS-Cov2 can be detected using the specific antibody (Samson et al. 2020).

In 2020, Cepheid received emergency use authorization for the Xpert® Xpress SARS-CoV-2 test, which provided findings in 45 min. The principle of reverse transcriptase polymerase chain reaction (RT-PCR) was used and was therefore a quantitative determination of the pathogen (Vashist 2020). Abbott ID Now™ developed an isothermal nucleic acid amplification technology base detection unit that can quantify SARS-Cov-2. The result was given within 5 min time and moreover, due to its lightweight, it could be used as a POC device.

A rapid LFIA biosensors-based sensing of IgG and IgM antibodies from blood serum/plasma was widely used in suspected patients. The test developed by

BioMedomics, USA, could give the result in 10 min (Jamshaid et al. 2020). LFIA-based antigen test is one of the most carried out tests.

1.3.7 POC Biosensor for Detection of Other Diseases

Many other POC biosensors are available and commercially manufactured by different brands for various purposes. RDT LFIA for malaria is available in all primary healthcare centres in India (Singh et al. 2010, 2013). Vyas et al. compared the SD Bioline Malaria antigens test kit for the two types of malaria (plasmodium vivax and falciparum) with standard blood smear microscopy (Vyas et al. 2014). The sensitivity found for *Plasmodium falciparum* was 100% and for *Plasmodium vivax* it was 98.6%. The RDT was based on the principle of detection of antigens with the immunochromatography method in the blood from finger prick. Some detect only plasmodium falciparum, and some can detect all *Plasmodium vivax*, malariae, and ovale. Another kit available in the Indian market for malaria *Plasmodium falciparum* and *Plasmodium vivax* are Alere Trueline by Alere Medical, etc.

Pardee et al. developed a biosensor for Zika virus made from inexpensive paper and claimed to be effective for early detection (Meagher et al. 2016; Pardee et al. 2016). Cluster regularly interspaced short palindromic repeats-associated protein 9 nuclease (CRISPR-Cas9) technology was incorporated with the paper to detect the ribonucleic acid (RNA) genome of the virus. The RNA of Zika virus is isothermally amplified and the virus was differentiated from the Dengue virus using the colorimetric method. CRISPR-Cas9 is then used to differentiate the American and African strain. Song et al. developed a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) disposable cassette for the detection of zika virus from oral swab sample (Song et al. 2016). Symptomatic patients who have acquired Zika virus have 10^3–10^6 plaque-forming units (PFU). The sensitivity of this POC-based RT-LAMP was compared with a benchtop RT-LAMP where the device could detect 5 PFU as compared to 3 PFU in a benchtop device. Siemens' Rapidlab 1200 is a portable blood gas analyser that may be used in the field as a POC device (Ali et al. 2013; Kaushik et al. 2014).

POC biosensors are already used widely for various diseases. Qualitative POC tests are easier to achieve than quantitative POC test. Paper-based sensors for distance-based measurement have also seen applications in the medical field. Haematocrit measurement of the red blood cells (RBC) in the blood was done by Berry et al. using a simple distance-based calculation with a paper (Berry et al. 2016; Frantz et al. 2020). Although the sensitivity of the assay is at its prime stage as the assay was semi-quantitative. The distance travelled by the RBC cannot directly correlate to the haematocrit and also coagulants affect the flow of RBC through the channel.

1.4 Challenges and Future Prospects

For a POC biosensor parameters like high specificity, high sensitivity, accuracy, and long storage time are highly desirable. With the use of specific bioreceptors to biomarkers, the specificity can be achieved. Sensitivity requirements directly relate to the LOD of a biosensor. Since biomarkers in the biological samples are of trace quantity in some diseases, achieving the required sensitivity is still a challenge. Label-free electrochemical biosensors with semiconductor technology are considered to be highly reproducible, accurate, and can be used for real-time analysis (Chen et al. 2020). But there is an issue of lower specificity in label-free. Labelled biosensors have more specificity and sensitivity and can be more reliable for clinical analysis (Sin et al. 2014).

Climatic conditions like temperature, pressure, and humidity may also affect the parameter along with the stability of the POC biosensor. An example of the effects temperature in a paper-based micro-calorimetric biosensor for glucose estimation was studied by Davaji and Lee (2014). The heat loss to the surrounding (based on the thermal conductivity) affects the output of the sensor because in the calorimetric method heat generated is measured. Therefore, with time the output will reduce due to quasi-adiabatic conditions. Proteins and other biological products are also affected by temperature which are used as bioreceptor in POC biosensors. These climatic conditions are there for directly linked to the decline of the required parameters like loss of sensitivity.

Another challenge of a POC biosensor is clinical analysis. If the result is not interpreted by a certified physician, treatment cannot be given. One solution to this is, if a POC platform (the transducer) is connected to a smartphone through various non-wired modalities like a Bluetooth, zigbee, and near field connection (NFC), the results can be communicated to a doctor or a certified physician (Kassal et al. 2018).

There is also a lack of regulatory boards in most developing nations which leads to the use of sub-standard POC test devices which were mainly seen for malaria test kit (Peeling and Mabey 2010; Drain et al. 2014). The requirement of a specific regulatory board of POC devices will help advocate the use of tests and treatment. Engel et al. found pricing and the relation between the patient and doctor also is a barrier for POC testing in India (Engel et al. 2015).

Miniaturization is the way forward for POC biosensor devices in healthcare. Microelectromechanical systems (MEMs) are one method of miniaturization of a biosensor. But for MEMs the fabrication cost can be very high making the biosensor expensive (Derkus 2016). The main concern is the cost of fabrication and achieving an environmental friendly material (Mejía-Salazar et al. 2020). Paper-based biosensor reduces the cost of fabrication and is used in both optical and electrochemical type of biosensor (Shergujri et al. 2019). Paper is biodegradable therefore making it environmentally friendly. There are many ways of fabricating microfluidic channels in a paper further reducing the cost of the biosensor. Application of paper base microfluidic biosensors is already used for the detection of various diseases like the

qualitative analysis using LFIA. But for quantitative readouts, a transducer is needed for colorimetric, luminescence, and electrochemical detection.

1.5 Conclusions

In the healthcare sector, biosensors are required for diagnosis and monitoring of different diseases and ailments with enhanced sensitivity and accuracy near the patients. POC-based biosensors have been used to determine various analytes, like pathogens, proteins, nucleic acids, and glucose, for the identification of a host of ailments or sicknesses, as well as malignant growth of tumours (Inan et al. 2017; Syedmoradi et al. 2017). Most diseases can be determined with either the presence of a biomarker or elevation in biomarker contained in the biological materials of the human body.

POC biosensor devices are advantageous where there is no sophisticated laboratory equipments and experts to operate them. POC biosensor also gives a significant TTAT which reduces the time the patient gets the actual treatment. With the increase in diabetes worldwide, the need of POC device was urgent in the 1970–1980s which gave birth to POC biosensing for dipstick urine-based colorimeter and blood-based glucometer (Mazzaferri et al. 1970; Sönksen et al. 1978). LFIA (RDT) is also a common application of POC biosensor where qualitative results of diseases are quickly determined. Pregnancy test, malaria test, and even the coronavirus disease 2019 current used LFIA RDT. Paper-based LFIA and non-LFIA biosensors are more widely used because of the cost-effectiveness. Cost-effectiveness of a POC biosensor comes as a result of low-cost material, simple fabrication, and ease of functionalization (Choi 2020). Sensitivity and selectivity of biomarkers are very specific to the bioreceptors making the margin of error very less probable with ideally no false result.

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Chapter 2
An Overview of Biomolecules Used in the Development of Point-of-Care Sensor

Girish Chandra Mohanta and Satish Kumar Pandey

2.1 Introduction

The field of biosensor was first introduced in 1962 by Clark and Lyons with their invention of "glucose biosensor" by immobilizing the glucose oxidase enzyme on the surface of oxygen. The biosensor provided a specific and selective response to glucose concentrations sensed by the immobilized enzyme and transduced into a measurable signal by oxygen electrode (Clark Jr and Lyons 1962). Since then, the biosensors fields have grown at an exponential pace. According to IUPAC, a biosensor is a self-integrated, compact system, capable of providing quantitative or semi-quantitative information about its target analyte in a highly sensitive and specific manner (Thévenot et al. 1999, 2001). The biosensor consists of different components such as biorecognition element which is in direct spatial connection with a transducing element. The transducing element is capable of sensing minute physico-chemical changes occurring during biorecognition molecule-analyte interaction and relays it as a measurable output signal. The information of the output signal is then processed and reported as readable data in the form of digital display and print-out of color/spectral change. The overall goal of a biosensor is to combine and utilize the high specificity of biomolecules with high sensitivity of different transducing elements, so different analytical purposes in various fields (Carpenter et al. 2018). In the past few decades, biosensors have already revolutionized various fields such as medical diagnostics, process control, agriculture and environmental monitoring, quality control, defense, pathogen screening, drug discovery, mining,

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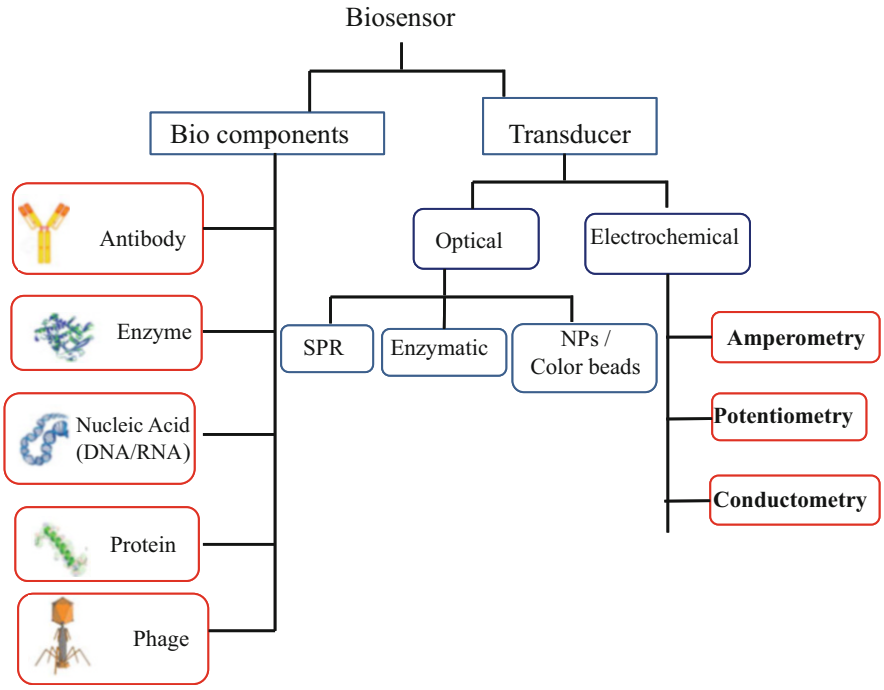

Fig. 2.1 Components of a sensor comprising biological receptors, transducers, and detectors

and several more. A schematic of a complete biosensor system is illustrated in Fig. 2.1.

In recent times, significant developments in the field of miniaturized electronic and optical components, rechargeable and potable power sources, and low-cost fabrication techniques have fueled the next phase in the field of biosensor development of so-called "point-of-care (POC)" biosensors (Kaushik and Mujawar 2018). The POC biosensors have tremendous potential in disease prognosis and diagnosis, status monitoring in hospital emergency setups, and facilitating penetration of healthcare services to remote regions and resource-constraint countries. As compared with traditional laboratory-based setups, POC biosensors require very small amount of sample and reagents, have rapid turn-around times, and offer significant improvement in potability and ease-of-operations (Patel et al. 2016). Furthermore, emerging technological innovations have now enabled the next generation of POC biosensors which offers self-testing capabilities for the patient without any requirement of hospital visit. The result could also be shared wirelessly with the healthcare provider (e.g., doctor) through internet. Going one step ahead, efforts are now being made in realizing mobile-based and wearable POC biosensors. These biosensors are being developed as standalone internet-of-things (IoT) system or smartphone-based platform which is capable of measuring target analyte in collected samples and directly communicate the results to clinician and patient through wireless

communication (Noah and Ndangili 2019). Efforts are also being made toward development of wearable biosensors capable of sampling and measuring analyte directly on the body (Kim et al. 2019b). These smartphones and wearable POC biosensor systems typically require combination and integration of highly complex network of various components such as sampling platform, biorecognition element, enzymes, oligonucleotide, transducing elements, integrated power source, signal processing and read out, and various potable or wearable form factors (Shrivastava et al. 2020).

One of the critical components of biosensors is its biorecognition element which is usually a biomolecule responsible for providing sensor specificity. It does so by facilitating highly specific binding to the target analytes under optimized conditions. Various types of biomolecules have been used as the recognition element in POC biosensors which can be broadly categorized according to their chemical nature such as peptides, proteins, nucleotides, glycans, and whole cells. This chapter is aimed at providing a brief overview of various biomolecules utilized in POC biosensors.

2.2 Proteins

Proteins are perhaps the most versatile and abundantly utilized class of biomolecules for the recognition of target analytes. Several different types of proteinaceous molecules ranging from simple peptides to more complex antibodies, enzymes, multienzyme complexes, and lectins are utilized in biosensors.

2.2.1 Peptides

Peptides are constituted from short repeats of natural or synthetic amino acids joined together by peptide bonds similar to that of a natural protein. Thus, with appropriate sequence, peptides are able to work as a functional substitute for more complex and bulky proteins. For example, as a functional substitute of enzymes itself, peptides provide a continent tool for screening of enzyme inhibitors (Zozulia et al. 2018). Furthermore, by tuning the physico-chemical properties of peptides, supramolecular assemblies like tubes, strips, bilayers, micelles, and fibers can be easily obtained. This supramolecular assembly of peptides is driven by the non-covalent interactions such as electrostatic, hydrophobic, and van der Waals interactions (Ekiz et al. 2016).

In biosensor applications, peptides are utilized as biorecognition elements dues to their excellent stability against denaturation, high specificity, low cost and ease of preparation, facile modification, tunable and versatile chemical properties, and high affinity toward target analyte. Till date, peptide-based biosensors have been developed for various analytes ranging from simple metal ions, volatile organic compounds, pollutants to more complex proteins, enzymes, antibodies nucleotides, and whole cells (Karimzadeh et al. 2018). For example, Kim et al. have reported a

peptide-based POC electrochemical biosensor for sensitive detection of dengue virus (DENV) (Kim et al. 2019c). The peptide was synthetically developed against dengue virus non-structural (NS1) protein antigen and immobilized on the gold sensor surface. The specific interaction between the peptide and antigen was probed by square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) techniques. The sensor exhibited high stability and sensitivity with a limit of detection (LOD) of 1.49 μg/mL.

Recently, antimicrobial peptides (AMPs) have gained tremendous research attention due to their potential applications in broad-spectrum antimicrobial activity, immunomodulatory effects, and therapeutic value (Mahlapuu et al. 2016). These natural peptides are generally short, positively charged, and exhibit broad-spectrum antimicrobial activity either through directly disrupting the bacterial membrane or via modulating the host immune system. Furthermore, their excellent binding affinity makes them potential biorecognition element in biosensors (Hoyos-Nogués et al. 2018). For example, Wilson et al. described a POC electrochemical biosensor for single step, low cost, and sensitive detection of several pathogenic microorganisms including *E. coli*, *S. aureus*, and *S. typhi* in potable water and fruit juice using antimicrobial peptide melittin (MLT) (Wilson et al. 2019).

Another potential application of peptides is their application in fabricating "Bioelectronic Nose" for the detection of various volatile organic compounds (VOCs) (Barbosa et al. 2018). The composition and quantitative detection of various VOCs emanating from livestock and fish could be easily correlated with their freshness. For example, the quantitative information of trimethylamine (TMA) emission during microbial spoilage of fish and other seafoods can be considered as its freshness indicator. Accordingly, Lee et al. have reported a single-walled carbon nanotube (SWCNT)-based field effect transistor (FET), an electrochemical biosensor for ultrasensitive detection of TMA to ascertain the freshness quality of oysters (Lee et al. 2015). They utilized olfactory receptor-derived peptides (ORPs) as biorecognition probes which enabled parts per trillion (ppt) level sensing in real time.

2.2.2 Enzymes

The use of enzymes as biorecognition element is perhaps the most representative and most studied among the various classes of biosensors. Particularly, oxidoreductase class of enzymes has been extensively utilized for fabricating POC electrochemical biosensors against various clinical and environmental analytes (Dzyadevych et al. 2008; Bollella and Gorton 2018). Several of these electrochemical biosensors have been already marketed as successful POC sensor devices (Kucherenko et al. 2019). The most successful among them is the glucose biosensor whose market value is exponentially increasing due to the rise in the global burden of diabetes (Yoo and Lee 2010).

The oxidoreductase class of enzymes catalyzes the transfer of electrons from a reductant to the substrate bringing about an oxidation-reduction reaction. Consequently, these enzymes are highly suitable for integration into an electronic device which could sensitively detect the electron flow (amperometric) or potentially generated (potentiometric) during the enzyme-analyte interaction (Karunakaran et al. 2015; Bollella and Gorton 2018). Traditionally this has been achieved by fabricating an enzyme electrode by directly immobilizing the enzyme over the electrode surface. For example, a glucose-specific enzyme electrode was first reported by Clarke and Lyon in 1962 (Clark Jr and Lyons 1962). They immobilized a glucose oxidase enzyme layer over the oxygen electrode and monitored the consumption of oxygen during the enzyme-catalyzed reaction.

$$\mathrm{GOD_{(ox)}} + \mathrm{Glucose} \rightarrow \mathrm{Gluconolactone} + \mathrm{GOD_{(red)}}$$

$$\mathrm{GOD_{(red)}} + \mathrm{O_2\ (dissolved)} \rightarrow \mathrm{GOD_{(ox)}} + \mathrm{H_2O_2}$$

The peroxide generated during the regeneration step was measured amperometrically at the anodic potential of +0.6 V (vs. Ag/AgCl). However, the sensitivity of these early generation glucose biosensors was always dependent on the amount of dissolved oxygen. Furthermore, electroactive components in the blood such as uric acid were a frequent interferent in these studies. To overcome these issues, the second generation of enzyme electrodes utilized a secondary electron mediator for efficient transfer of electron to the electrodes. This also enhanced the sensitivity of the biosensors. For example, Cess and co-workers utilized such as ferrocene as the electron mediator which significantly improved the sensitivity and stability of the biosensor (Cass et al. 1984). Furthermore, by utilizing graphite as electrode material, Cess and co-workers reported the first printable enzyme electrode which was commercialized by the US MediSense company in 1987.

The sensing reaction in presence of mediator modifies to:

$$\mathrm{GOD_{(ox)}} + \mathrm{Glucose} \rightarrow \mathrm{Gluconolactone} + \mathrm{GOD_{(red)}}$$

$$\mathrm{GOD_{(red)}} + \mathrm{M_{(ox)}} \rightarrow \mathrm{GOD_{(ox)}} + \mathrm{M_{(red)}}$$

$$\mathrm{M_{(red)}} \rightarrow \mathrm{M_{(ox)}} + \mathrm{nH^+} + \mathrm{ne^-}$$

The electron released during regeneration of the mediator is detected amperometrically.

The next stage of enzymatic biosensor development was fueled by the application of conducting polymers as the electron transfer medium (Lakard 2020). Pioneering work of Shirakawa, MacDiarmid, and Heeger in this field paved the development of several different conducting polymers, which earned them the Nobel Prize in Chemistry in 2000 (Shirakawa et al. 1977). These polymers are constituted from alternating single (σ) and double (π) bonds and with delocalized π electrons across their entire structure by the virtue of which, polymer can easily undergo reversible oxidation-reduction reactions. Conducting polymer combines the high degree of

flexibility of polymers amenable for device fabrication while maintaining the excellent conductivity of conventional semiconductor materials. Biosensor devices fabricated using conduction polymers exhibited improved sensitivity, low cost, higher processability, and longer functional stability (Aydemir et al. 2016). For instance, Dong et al. utilized Nafion (a sulfonated tetrafluoroethylene-based fluoropolymer-copolymer) for immobilizing ferrocene and glucose oxide on the electrode surface. Because of the hydrophilic and hydrophobic nature of the Nafion, the enzyme and ferrocene mediator are retained within the polymer, thus increasing the stability and shelf-life of the biosensor (Dong et al. 1992).

The incorporation of nanomaterials in the sensing layer is another promising trend which is recently emerging (Kucherenko et al. 2019). The physico-chemical properties of nanomaterials are vastly different from their bulk counterpart because of the quantum confinement effect at such a scale (<100 nm). The very high surface-to-volume ratio of these nanomaterials effectively increases the sensor surface area and facilitates better enzyme immobilization. Furthermore, they impart additional transduction properties to the sensor device such as magnetic properties, optical (fluorescence, plasmonic), and higher electrical conductivity (Guisbiers et al. 2012). In effect, these properties of nanomaterials significantly enhance the overall sensitivity of the sensors. It also improves the LOD, reproducibility, stability, and sensor response time (Malhotra and Ali 2018). For instance, the inclusion of gold nanoparticles (AuNPs) in glucose oxidase-polypyrrole electrochemical biosensor not only increased the linear range of detection but also enhanced the sensitivity of the biosensor depending on the size of the AuNPs (German et al. 2012). Gold nanoparticles have been extensively utilized for fabricating enzyme biosensors because of their easy and tunable synthesis, excellent optical and plasmonic properties, catalytic activity, and facile biofunctionalization through gold-thiol chemistry (Hutter and Maysinger 2013). Several different types of nanomaterials are used in modern-day enzyme biosensors which include metal oxide nanoparticles, metallic nanoparticles, fluorescent semiconductor nanoparticles (or quantum dots), magnetic nanoparticles, and various carbon nanostructures such as carbon nanotubes (CNTs), graphene, graphene oxides, fullerenes, and fluorescent carbon dots (Holzinger et al. 2014).

Another important class of electrochemical biosensors is the potentiometric biosensors (Ding and Qin 2020). The amperometric biosensors are largely limited in utilizing only oxidoreductase class of enzymes which brings about a change in the oxidation-reduction status of the substrate or the mediator. On the other hand, potentiometric biosensors measure the change in the electrochemical potential caused by small ions (e.g., NH_4^+, H^+, etc.) generated during the enzyme-catalyzed reaction. Thus, potentiometric biosensors utilize an ion-selective electrode (e.g., pH electrode) for elucidating quantitative information. This implied that, other classes of enzymes such as transferases and hydrolases could also be used for the fabrication of POC biosensors. For example, potentiometric urea biosensors have been extensively studied due to their clinical relevance. Urea is an important by-product, filtered and excreted in urine by the kidneys. Its clinical levels thus can be utilized to evaluate renal dysfunctions. The potentiometric biosensing of urea by immobilized urease

enzyme has been therefore extensively explored (Singh et al. 2008). Urease catalyzes the break-down of urea into constituent ammonium and bicarbonate ions which could be electrochemically determined by using pH or ammonium ion selective electrodes. Similarly, ammonia released during the conversion of creatinine into methylhydantoin catalyzed by enzyme creatinine iminohydrolase has been potentiometrically determined (Koncki 2007).

Interestingly, inhibition of enzyme activity can also be utilized as an effective biosensing technique. An exemplary example of this includes inhibition of acetylcholine esterase (AChE) enzyme activity by the organophosphate (OP) pesticides for the detection of the later (Dhull et al. 2013). Naturally, AChE is found in the neuromuscular junctions and cholinergic neuronal synapses, where it catalysis the breakdown of acetylcholine neurotransmitter to terminate the synaptic transmission. The OP pesticides are strong inhibitors of the AChE enzyme by irreversibly blocking the active site. This causes hyperactive neuronal transmission leading to paralytic conditions and eventually fatal. The extent of enzyme inhibition is directly proportional to the amount of OP pesticide present and therefore constitutes sensing principle.

Remarkably, some nanomaterials also mimic the catalytic activity of natural enzymes and are therefore increasingly employed in sensor fabrication. Copper oxide nanoclusters are known to exhibit peroxidase-like activity and therefore are utilized in the construction of electrochemical and chemiluminescent biosensors (Hu et al. 2013; Shi et al. 2014). For instance, a chemiluminescent biosensor utilizing cholesterol oxidase and Cu nanocluster has been reported by Xu et al. (2016). The Cu nanocluster catalyzes the reaction between hydrogen peroxide (H_2O_2) generated by oxidase activity and luminol, producing a chemiluminescent signal. The sensor exhibited improves stability, wide working range (0.05–10 mM), and high sensitivity (LOD 1.5 μM).

2.2.3 Antibodies

Antibodies are the protective glycoproteins produced by lymphocyte cells in response to foreign antigens. Antibodies are an important part of host immune system and are characterized by their high solubility, high antigen binding affinity, and exceptional specificity (Sharma et al. 2016). The basic structure (Fig. 2.2) of an antibody can be divided into two major fragments: an antigen-binding fragment (Fab) and a constant region fragment (Fc fragment). The Fab fragment contains the complementarity-determining region (CDR) which is responsible for the antigen specificity, while the Fc region is host-specific and mediates several host-immune responses such as antibody-mediated cytotoxicity (ADCC), phagocytosis, antigen presentation, complement-mediated lysis, and regulation of immune cell activation and proliferation (Neurath 2008; Chaplin 2010).

Because of their excellent specificity, high stability, and easy and scalable production, antibodies have emerged as versatile and important biorecognition

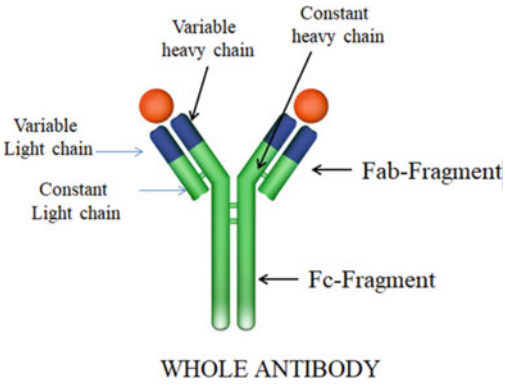

Fig. 2.2 Schematic illustration of antibody structure

molecules in modern-day biosensors. In contrast to enzymes which are available against very few numbers of substrates, antibodies can be generated against virtually any type of analytes using host immune system. Consequently, antibodies have expanded the number of analytes that could be sensed in POC-type biosensors. The use of antibodies as biorecognition probes offers several advantages such as label-free detection, high-throughput screening, limited hands-on time, high sensitivity, and smaller form-factor (Sharma et al. 2016). There are different forms of antibodies which are currently utilized in biosensors. Since antibodies are produced by the host immune system, these biosensors are also called as immunosensors.

2.2.3.1 Polyclonal Antibodies

Naturally, antibodies are produced by the host immune response against the invading foreign antigen. These antibodies are produced by plasma B-cells after their activation and differentiation through antigen presentation. However, a single antibody identifies only a specific region of the antigen called epitope. Naturally derived antibodies therefore consist of heterogeneous populations capable of interacting with different epitopes of the antigen with varying degree of affinity and selectivity. These antibodies are suitable as biorecognition elements against large analytes such as protein antigens and whole cell pathogens (Borrebaeck 2000; Ascoli and Aggeler 2018). A major advantage of polyclonal antibodies is their economical and rapid production capacity. However, due to their heterogeneous binding affinity and selectivity, standardization of assay for biosensing becomes challenging.

2.2.3.2 Monoclonal Antibodies

The field of antibody biosensors was revolutionized by Kohler and Milstein with their invention of hybridoma technology for in-vitro production of monoclonal antibodies (Liu 2014). In hybridoma technology, the antibody-producing B-cells

are fused with drug-sensitive transformed myeloma cells. The successfully fused cells called hybridoma cells are then screened for antibodies capable of recognizing antigen using selective media. In the process, a single clone of cell secreting specific antigen can be continuously propagated further. These monoclonal antibodies offer several advantages viz., very high specificity and convenient and scalable production, and facile standardization of immunoassays (Appleby and Reischl 1998; Gao et al. 2018). Because of their exceptional specificity, monoclonal antibodies are suitable for the detection of cancerous cells, small molecule analytes, and construction of immunotherapy drugs.

2.2.3.3 Recombinant Antibodies

The basic structure of antibody consists of four polypeptide chains: two heavy and two light chains, joined together by disulfide linkages. Each polypeptides chain is further divided into variable region, which contains the antigen binding domain and a constant region. Proteolytic degradation studies have demonstrated that the antigen binding domain resides in the variable fragment (Fv) of the antibody and can also be utilized alone for recognition of the antigen without losing the sensitivity and specificity of natural antibodies. Thus several studies in the past have utilized these antibody fragments as biorecognition elements in biosensors (Saerens et al. 2008; Sharma and Mutharasan 2013).

Progress in recombinant DNA technology has revolutionized the field of antibody production as well (Frenzel et al. 2013). It allowed the production of small antibody fragments using DNA vectors in a bacterial expression system called single-chain antibodies (scAb) (Zeng et al. 2012). These scabs consist of a single variable region from each light and heavy polypeptide chains joined together by a small amino acid peptide linker, while retaining the antigen binding capability. They can be genetically engineered to contain different affinity tags like His-tags or incorporated with chemical functional groups which would further facilitate their high-density immobilization over the sensor surface. Furthermore, their small size allows multimers (e.g., dimer, trimer) to be formed which has shown to increase the assay sensitivity by improving the avidity and stability of antigen interaction (Kang and Seong 2020).

2.2.4 Nanobodies

The antigen binding domain of mammalian antibodies consists of two variable regions: one each from heavy chain (VH) and light chain (VL) (Yang and Shah 2020). This VH-VL fragment of antibody is called variable fragment (Fv), and the antibody structure is conserved across all the mammals. One notable exception to this is the presence of IgG immunoglobulins called heavy-chain antibodies (HCAbs) in members of Camelidae family. The HCAbs lack any L-chain polypeptide and also one constant (CH1) region of heavy chain, thereby have lower molecular of 90 kDa

as against 150 kDa of conventional antibodies and therefore also regarded as nanobodies. Another critical difference among the two is the presence of N-terminal VHH region which constitutes antigen binding domains of the HCAbs. Thus, it is the functional equivalent of Fab domain of normal antibodies. The VHH region of HCAbs consists of longer CDR regions arranged as finger-like projections. This allows greater interaction affinity and accessibility to cavity-like epitopes of antigens. Combined with the antigen binding affinity of CDR1 and CDR2, nanobodies exhibit higher affinity and specificity. Several key properties of nanobodies make them more suitable as biorecognition element in comparison with traditional antibodies (Hassanzadeh-Ghassabeh et al. 2013). This includes small size, higher affinity and robust stability, higher resistance to thermal, chemical and proteolytic degradation, and easy and scalable preparation by recombinant DNA technology.

2.2.5 Lateral Flow Assay (LFA) Immunosensors

In the past few decades, the various innovations and low cost in the development of antibodies have enabled the fabrication of several paper-based POC biosensors some of which have been successfully commercialized. Lateral flow assay (LFA) is one such paper-based immunoassay which enables direct detection and quantification of analyte even in complex samples such as urine, saliva, whole blood, food extract, and several others (Sajid et al. 2015; Koczula Katarzyna and Gallotta 2016). The basic design of the lateral flow assay is represented in Fig. 2.3. The LFA consists of several components (e.g., sample pad, conjugate release pad, membrane, adsorbent pad, and back plate) which are arranged together so as to allow a uniform and continuous liquid flow through capillary action. The liquid (sample containing analyte) is applied at the sample pad which is pre-charged with buffer components and surfactants to ensure proper conditions for binding interactions between the sample analyte and antibodies immobilized in the next conjugate release pad. These antibodies are labeled with colorimetric or fluorescent reagents for subsequent detection and quantification at the test line. The test line and control line are created on a porous membrane such as nitrocellulose and are immobilized with primary antibodies against the analyte and secondary antibodies against the labeled antibodies, respectively. Positive signal at the test line indicates positive detection which can be subsequently quantified through colorimetric or fluorescent signals, while the control line is used to ensure proper liquid flow. Alternatively, several test lines can be created, wherein the extent of positive test lines can be correlated with the amount of analyte present or multiplexing (Kim et al. 2019a). To ensure the proper migration of the liquid along the test strip and prevent any back-flow, an adsorbent pad is attached at the end. In case of smaller analytes which does not have multiple antigenic determinants to bind with antibodies on the test line, standard labeled analytes are immobilized in conjugate release pads. The analytes present in the sample thus act as blocking agents for labeled analytes and thus a positive test is

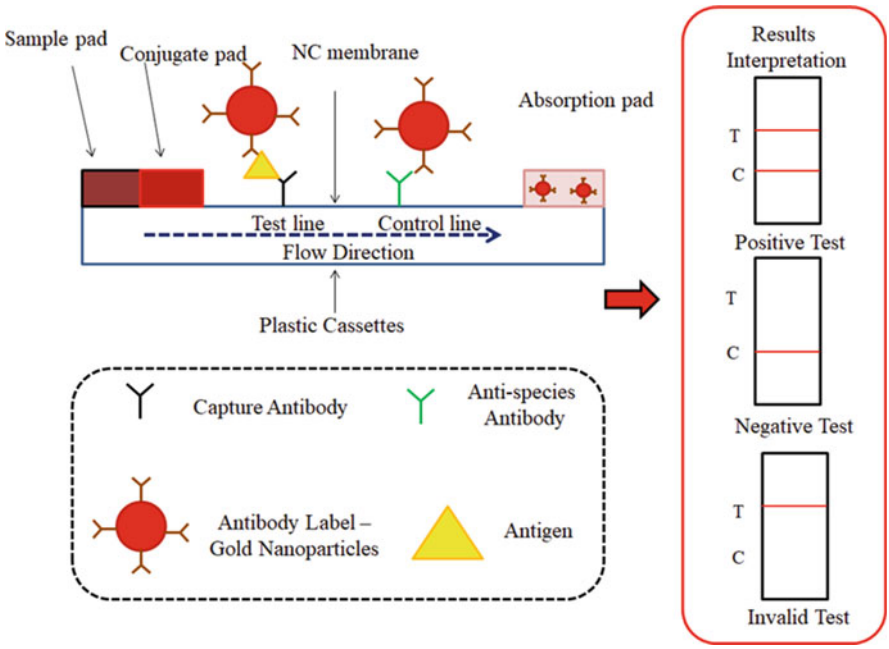

Fig. 2.3 Schematic presentation of typical lateral flow assay (LFA)

indicated by a negative test line, whereas the control line is not affected. Another remarkable property of the LFA biosensor is their rapid response. A typical LFA biosensor requires between 5 and 30 min to complete without additional assistance, which is why it is also called a rapid test (Hesterberg and Crosby 1996). Consequently, LFA biosensor has been utilized as screening tools in large populations. For example, LFA biosensors are an indispensable tool for screening antibodies (serosurvey) against SARS-CoV-2 virus in various countries (Houston et al. 2021).

2.2.6 Lectins

Lectins are a class of glycoproteins which selectively binds to free sugar (glycans) or glycoconjugates such as glycoproteins, glycosylated bacterial cells, or viral particles. Lectins exhibit complex specificity such that they are not only able to recognize different monosaccharides within a glycan chain but also the linkages between them or branching. For instance, the influenza virus which can infect humans can recognize the sialic acid residues linked to galactose by α-2,6-glycosidic linkages while avian influenza virus recognized only α-2,3 linkages. Consequently, avian flu viruses cannot infect humans and vice versa (Belický et al. 2016).

Recently, the application of lectins as passive immobilization tool as well as active biorecognition element in biosensors has witnessed an upward shift. The binding ability of lectins to glycosylated proteins has been utilized as a convenient immobilization tool. Concanavalin A (Con A) is a prototypical lectin which recognizes D-glucose and D-mannose sugar moieties. It has been extensively utilized for immobilizing glycoprotein enzymes such as glucose oxidase (GOx) and horse radish peroxidase (HRP) on the transducer surface of the biosensor (Monzo et al. 2007). Conversely, it has been also utilized as an active biorecognition element for the detection of glucose, bacterial toxins, pathogenic bacterial cells as well as cancer cells (Wang and Anzai 2015; Mi et al. 2021). An interesting application of lectins has emerged in glycoproteomic studies, wherein different lectins are immobilized in a well-defined microarray format to identify and quantify different glycans and glycoproteins present in human sera (Hu and Wong 2009; Yue and Haab 2009).

2.2.7 Receptors Proteins

Interestingly, several biological receptor proteins have been also utilized as biorecognition elements for the construction of POC biosensors. Some of these include G-protein-coupled receptors (GPCRs) (Leifert et al. 2010), acetylcholine receptors (Eldefrawi et al. 1988), taste receptors (Wu et al. 2014), and olfactory receptor (Du et al. 2013). For example, Chen et al. fabricated a electrochemical biosensor sensitive to monosodium glutamate (MSG) and sodium inosinate (IMP) using umami receptor proteins (Chen et al. 2020). Similarly, dopamine, a neurotransmitter, sensitive SPR biosensor, was reported by Miura and co-worker using the D_3-dopamine receptor (Sunita et al. 2006). However, due to complex preparation methods of functional receptors and their subsequent instability, fabrication of receptor-based POC biosensors remains a challenging task.

2.3 Nucleic Acid

In the past some decades, the research in nucleic acid-based technologies has witnessed an exponential rise, particularly after the invention of sequencing and amplification techniques (Du and Dong 2017). The principle of complementary base pairing of DNA and the exclusive specificity it provides has been extensively explored in different DNA-hybridization-based molecular techniques such as fluorescence in-situ hybridization (FISH), DNA microarray analysis, and polymerase chain reaction (PCR)-based amplification techniques. Recent innovations and advances in technology have enabled the fabrication of POC biosensors using nucleic acids (DNA or sometimes RNA) as biorecognition elements.

2.3.1 Nucleic Acid Amplification Tests (NAATs)

The invention of polymerase chain reaction (PCR) by Kary B. Mullis was perhaps the single most significant breakthrough technology which significantly boosted the entire field of nucleic acid research (Dove 2018). The PCR technique enabled researchers to make millions of copies of otherwise scarce DNA in a very short duration and in a continental manner. Since then, nucleic acid amplification techniques have occupied the center stage for DNA-based molecular diagnostics.

In the recent past, the discovery of several novel DNA polymerases has led to the development of many isothermal nucleic acid amplification techniques (Bodulev and Sakharov 2020). Unlike conventional PCR technique which requires thermal denaturation and annealing steps, isothermal amplifications are carried out at a single temperature and therefore can be used under a POC setup. Emerging isothermal amplification techniques are briefly discussed.

2.3.1.1 Loop-Mediated Isothermal Amplification (LAMP)

The LAMP technique was first proposed by Notomi and co-workers in 2000, and since then has been extensively explored for point-of-care diagnostic application (Notomi et al. 2000). The LAMP technique employs 4–6 sets of primers which recognizes 4–6 distinct sites in the target DNA and therefore provides unparalleled specificity. The basic working design of LAMP is illustrated in Fig. 2.4. A single DNA polymerase is used in LAMP which amplifies the target DNA in two stages: the first structure-producing stage and the second cyclic amplification stage. The amplification is initiated by one of the outer primers (F3), which produces a single-strand DNA (ssDNA) template by the strand displacement activity (SDA) of DNA polymerase. This template DNA is then primed by another outer primer B3 and one inner primer BIP (backward inner primer), the extension of which produces a stem-loop DNA structure. In the next stage, the loop regions are primed by inner primers (FIP and BIP) and extended alternatively. This cyclic amplification stage produces more than a billion copies of target DNA under a very short period of time (usually <1 h). The amplified products are detected either optically or electrochemical in real-time or via end-point methods. The exceptional specificity and simplicity of LAMP have encouraged their application in the detection of viral or bacterial pathogens and single nucleotide polymorphisms (Wong et al. 2018; Huang et al. 2020).

Recently, the integration of LAMP assay in microfluidics devices has enabled the fabrication of highly advanced nucleic acid POC biosensors for disease diagnosis (Zhang et al. 2019). An excellent example of this is demonstrated by Ganguli et al., with the detection of the SARS-CoV-2 virus on a smartphone integrated POC LAMP assay. The amplified LAMP products were detected in real-time by fluorescent intercalating dye monitored by a smartphone system. The device exhibited a remarkable limit of detection (LOD) of just 50 RNA copies per μL, whereas

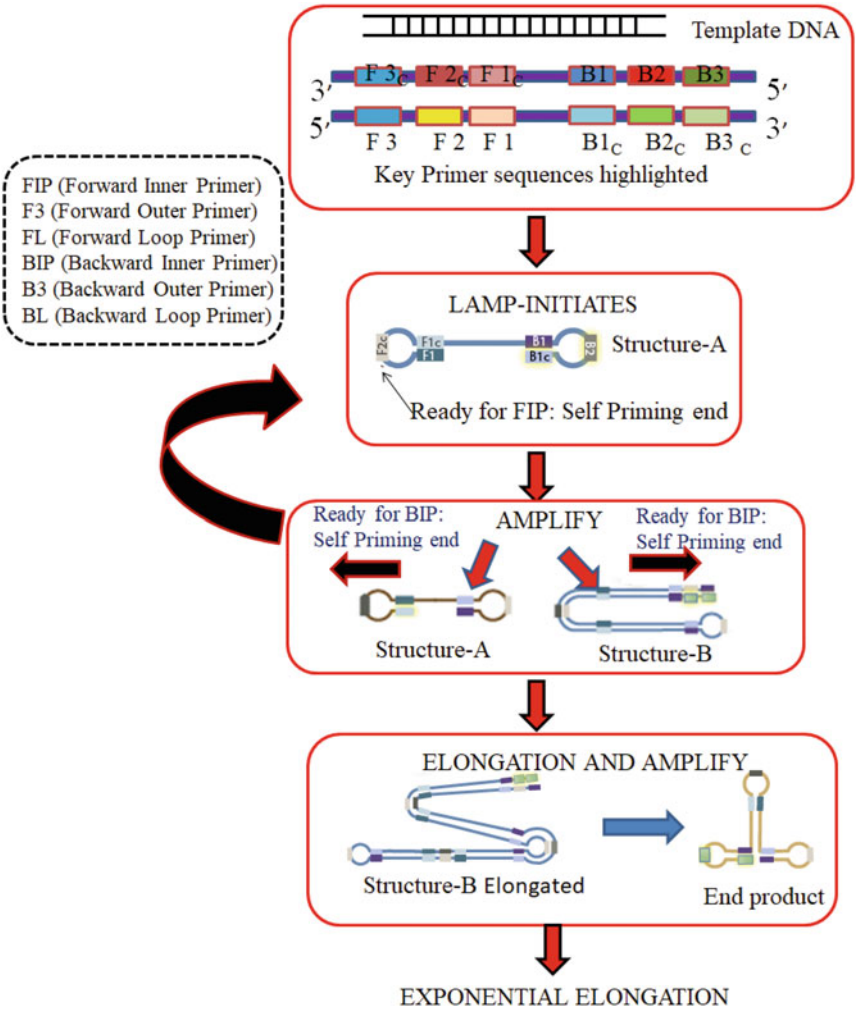

Fig. 2.4 Basic steps involved in the loop-mediated isothermal amplification technique

identification of positive or negative samples took just 30 min from the sample collection point (Ganguli et al. 2020). Alternatively, LAMP is also being integrated with lateral flow assay (LFA) devices for end-point detection with improved sensitivity (Zhu et al. 2020).

2.3.1.2 Nucleic Acid Sequence-Based Amplification (NASBA)

The NASBA amplification technique was first introduced in 1991 for specifically detecting RNA targets (Deiman et al. 2002). The technique utilizes three enzymes:

reverse transcriptase, RNase H, and DNA-dependent RNA polymerase, working in tandem to amplify and detect ssRNA targets. In brief, the amplification cycle starts by the conversion of ssRNA into complementary cDNA by reverse transcriptase and RNaseH. Next, several copies of original target RNA are transcribed from this template cDNA by using a second primer having a promoter binding site for T7 DNA-dependent RNA polymerase. This target in-turn becomes a template for further cDNA formation and hence the amplification is continued. In general, NASBA can produce 10^9 copies of target RNA within 1–2 h at 41 °C which is comparable to the real-time PCRs (RT-PCR). One major advantage of NASBA is that the amplified products (cDNA and RNA) are single-stranded and therefore end-point hybridization detection can be accomplished directly without any denaturation step.

2.3.1.3 Rolling Circle Amplification (RCA)

The RCA amplification technique is a simple, convenient, and highly sensitive amplification technique, which utilizes a circular DNA template (padlock template) and a single enzyme ϕ DNA polymerase with high-strand displacement activity. In case of pathogen or genomic DNA strand detection, an additional DNA ligase enzyme is required to seal the circular template using the target DNA as a template (Xu et al. 2021). Because of the circular DNA template and strand displacement activity, the amplified product of RCA consists of hundreds to thousands of repetitive DNA sequences complementary to template DNA. Thus, by modifying the template DNA, several functional DNA sequences such as aptamer, DNAzymes, or sites for restriction enzymes can be amplified (Gu et al. 2018). More than billion-fold amplified product could be obtained within 90 min at 60 °C. Due to its simplicity, RCA has been optimized to detect several different types of analytes including DNA, SNPs, DNA methylation status, pathogens, and microRNA.

2.3.1.4 Molecular Beacon-Assisted Detection-Amplification (BAD AMP)

In a unique approach, simultaneous DNA detection and amplification was achieved by using a molecular beacon (MB, Sect. 2.3.2) probe for identification of the target sequence as well as DNA polymerase and nicking-endonuclease for amplification. In presence of the target sequence (trigger DNA), the molecular beacon adopts an open configuration indicated by fluorescence activation. Another primer complementary to the 3′ end of the molecular beacon then binds and extends the MB sequence. This constitutes the linear amplification stage of the process. Next, the nicking-endonuclease creates a single strand nick into the newly synthesized strand creating a 3′-end for extension by the DNA polymerase. In the process, it creates further trigger DNA strands which initiate the exponential amplification stage (Connolly and Trau 2010, 2011).

2.3.1.5 Hybridization Chain Reaction (HCR)

Almost all the DNA amplification strategy utilizes DNA polymerases which in turn brings complexity of their own such as repetitive standardization of the assay, enzyme inhibition, critical temperature and pH dependence and such. In contrast, an HCR amplification does not utilize any enzyme but instead relies on two pairs of stable DNA hairpins for target identification and amplification (Dirks and Pierce 2004). The working mechanism of HCR is illustrated in Fig. 2.5. In absence of target DNA, the two pairs of DNA hairpins remain stable in a closed conformation until a target sequence complementary to one of the hairpin structures is recognized. The successful recognition of the target DNA triggers a cascade of hybridization events between the two hairpins to produce long chains of nicked double-stranded DNA. Apparently, the average molecular weight of the amplified product would be inversely proportional to the amount of trigger DNA. One basic difference between HCR and other amplification strategies is that the amplification takes place in a linear fashion in contrast to exponential amplification. However, recently branched HCR has been reported which could potentially increase the amplified products as well as bring multiplexing capability (Xu et al. 2019).

2.3.2 Molecular Beacons (MB)

The hybridization specificity of DNA and RNA has been extensively explored as fluorescent detection probes. Molecular beacons are such single-stranded, stem-loop DNA molecular probes used in biosensor technology (Stobiecka and Chałupa 2015). The working mechanism of MB arises from the fact that the stem-loop structure of the probes brings the 5′-end and 3′-ends of DNA in the close proximity. Thus, a pair of fluorophore-quencher can be dynamically controlled to either "turn-on" or "turn-off" based on the "open" or "closed" configuration of the MB, respectively. The

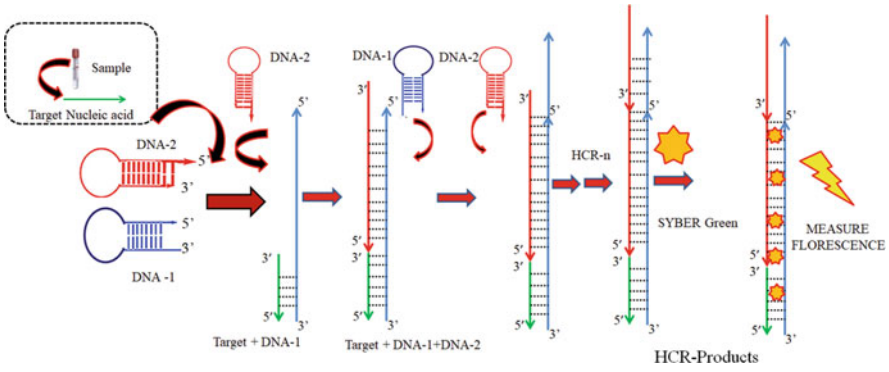

Fig. 2.5 Schematic illustration of working mechanism of hybridization chain reaction HCR

sequence complementary to the target DNA is usually kept in the loop region of the probe so that a stable open configuration is achieved by hybridization with the target DNA.

Recently, several nanomaterials have emerged as excellent alternatives for traditional fluorophores and quenchers utilized in MB. For instance, quantum dots (or semiconductor nanocrystals), known to exhibit higher quantum yield and narrow emission spectra, has emerged as an excellent alternative to a conventional organic fluorophore for MB. Higher quantum yield and narrow emission spectra allowed for the fabrication of biosensors with higher sensitivity and with multiplexing capability (Adegoke and Park 2016). Likewise, gold nanoparticles are used as excellent quenchers in MB, which significantly reduces the background (non-specific fluorescence) signal and increases the sensitivity (Uddayasankar and Krull 2013). Interestingly, surface-immobilized MBs have been reported as biorecognition elements in DNA-hybridization-based POC biosensors, further expanding their versatile application (Du et al. 2005).

2.3.3 Aptamers

Aptamers were first introduced in 1990 and since then have become an invaluable tool in biosensor development and therapeutics. Aptamers are single-stranded DNA (usually 30–100 bp) which can fold into a three-dimensional structure and bind to the target analyte with exceptional specificity and affinity. In contrast to amplification and molecular beacon strategy which relies on complementary hybridization, aptamer can selectively bind to analyte through structural complementary and non-covalent interactions. As a result, wide variety of analytes including proteins, peptide, ions, and even whole cell organisms could also be detected by aptamer. This significantly expands the application area and assay formats for aptamer biosensors in contrast to conventional DNA probe biosensors which are limited to only hybridization-based assays. The selectivity and affinity displayed by aptamer rivals even to that of natural antibodies (Thiviyanathan and Gorenstein 2012). However, unlike antibodies, aptamers are selected in vitro through a process called "systemic evolution of ligands by exponential enrichment or SELEX". This offers a great advantage over traditional antibodies in terms of, lower cost and development time, uniform homogeneity, higher stability, and freedom of customized modifications. Thus biosensors developed from aptamers as bio-recognizing elements tend to possess better thermal and chemical stability, reproducible assay design, and high transducer surface coverage allowing better sensitivity (Song et al. 2008; Zhou et al. 2014).

Different assay formats for aptamer-based biosensors have been reported till date. These assay formats can be categorized from simple analyte-binding to complex structure-switching aptamer formats.

2.3.3.1 Simple Analyte-Binding Assay

In this format, aptamers are simply utilized as affinity probes instead of conventional antibodies. The assay design is simply based on traditional formats replacing antibodies with aptamers such as enzyme-linked immunosorbent assay (ELISA), refractive index (RI) change-based assays such as SPR biosensors, colorimetric assays, or aptamer-linked precipitation assays (Kirby et al. 2004). One major disadvantage of this assay format is an extensive amount of washing steps and assay standardization for different sample types.

2.3.3.2 Aptamer Folding Assay

Aptamers are known to undergo large conformational change on analyte binding. Such adaptive changes in their conformation are also responsible for the high analyte affinity displayed by aptamers. This large conformational shift is what is utilized by sensors employing labeled aptamers. The conformational change brings a significant change in the local environment of the fluorophore label causing detectable alterations in its signal (Dickey et al. 2016). The sensitivity of the aptamer is further improved by incorporation of fluorophore-quencher pairs (similar to MB), wherein conformation change analyte binding produces "turn on" fluorescence. Biosensors against potassium ions, ATP/adenine, mycotoxins, and various protein analytes have been reported based on this format (Urata et al. 2007; Guo et al. 2020).

2.3.3.3 Aptamer-Switching Sensors

One of the unique advantage aptamers possess is that they can be utilized in both DNA-based sensors through their hybridization capability as well as non-nucleic acid targets through conformational binding. Consequently, several competitive assay-based biosensors have been designed wherein the fluorophore-aptamer conjugate is initially blocked by a complementary DNA linked with quencher, which is then released on target analyte binding, resulting in a sensitive and specific "turn-on" fluorescence signal. Such structure-switching aptamer assays provide higher sensitivity as compared with aptamer-folding assays (Feagin et al. 2018). Recently, two-dimensional nanomaterials such as graphene and molybdenum disulfide (MoS_2) have been utilized as blocking substrates for aptamers due to their natural affinity for single-stranded DNAs as well as fluorescence quenching ability (Zhu et al. 2015; Kalantar-zadeh and Ou 2016).

2.3.3.4 Split-Aptamer Sensors

Interestingly, the analyte-binding sequence of the aptamer can be split into two parts, which can then be self-assembled onto the analyte to bring a change in fluorescence signal. Such split aptamers have been utilized for the detection of HIV Tat protein, cocaine, and adenosine (Hashim et al. 2017; Yu et al. 2017; Zhou et al. 2019). However, one major disadvantage of such approach is the reduced affinity of the aptamers due to splitting.

2.3.4 Catalytic Nucleic Acids

The discovery of ribozyme (RNA enzymes) in the 1980s broke the early understanding that all enzymes are proteins and derived an entirely new field of catalytic nucleic acids (Kruger et al. 1982; Guerrier-Takada et al. 1983). However, significant developments in the field were only noticed after the report of the first catalytic DNA (DNAzymes) by Breaker and Joyce in 1994 (Breaker and Joyce 1994). The DNAzyme (GR5) was able to cleave RNA using lead (Pb^{2+}) ions as a co-factor. Unlike RNAzymes, DNAzyme has not been found in nature and have to be selected in-vitro. The long-term stability, cost-effectiveness, programmable structure and chemical modification, and easy immobilization of DNAzymes make them an attractive candidate as biorecognition elements in biosensors (Safdar et al. 2020). The dependence of DNAzymes on metal ions as co-factor for its catalytic activity has been extensively explored in fabricating metal ion biosensors (Lake et al. 2019). The metal ion co-factor transiently binds to the phosphate group of the DNAzyme to carry out the catalytic activity. The signal can be obtained either optically by using fluorophore-quencher pair, colorimetrically by employing gold nanoparticle aggregation-dispersion, or electrochemically using methylene blue label. DnAzymes for sensing small molecules like histidine and proteins have been also reported (Zhao et al. 2013).

Nearly, all the early reported DNAzymes utilized RNA as the catalytic substrate and metal ions as co-factors. In 1996, Li and Sen reported another class of DNAzymes called "G-quadruplex containing DNAzymes" (Li and Sen 1996). These DNAzymes have G-quadruplex (G4) structure and upon binding to hemin (Iron (III) protoporphyrin IX) exhibit peroxidase-like activity. It can be also used for amplifying detection signal. For example, on combining with RCA, multiple G-quadruplex DNAzyme/hemin complexes could be generated for highly sensitive detection assay (Peng et al. 2018).

2.3.5 *Peptide Nucleic Acids (PNA)*

The peptide nucleic acid is a synthetic analog of nucleic acids (DNA and RNA) having a peptide backbone instead of phosphodiester bonds (Saarbach et al. 2019). Remarkably, PNAs form more stable duplexes with RNA or DNA than RNA or DNA homoduplexes (Egholm et al. 1993). PNAs are also able to form homoduplexes (PNA-PNA), hetetroduplexes (DNA-PNA), and even triplex helix structures with DNA duplexes (Englund et al. 2006). Furthermore, PNAs are resistant to enzymatic degradation by peptidases, nucleases, or proteases, have high thermal and chemical stability and higher affinity for complementary DNA due to its neutral and flexible backbone. Interestingly, PNA can be also synthesized from artificial nucleobases such pseudoisocytosine, 2-aminopurine, 2,6-diaminopurine, thiazole, hypoxanthine, thiouracil, and others (Singh et al. 2020). These properties of PNA have prompted huge research interest for their use in diagnostic and therapeutic applications. One major drawback of PNA is its poor membrane permeability and cellular uptake (Nielsen 2005). However, modification of the backbone such as introduction of chirality, cationic charge, or modification of linkers/nucleobases has been reported to alleviate some of these problems. Because of their excellent hybridization capability, PNA has been utilized as an alternative to DNA probes in PCR amplifications, reactions, microarray assay, chromosomal analysis, and fluorescence in-situ hybridization (FISH) (Pellestor and Paulasova 2004).

In biosensors, PNA has been utilized as a biorecognition probe for the detection of nucleic acid disease biomarkers such as microRNA (miRNA), cell-free DNA, and point mutations of cancer cells (Demidov 2013; D'Agata et al. 2017).

2.4 Whole-Cell Biosensors

In the past few decades, whole-cell biosensors have attracted significant attention in sensing of environmental pollution and biomedical diagnostics (Gu et al. 2004; Gui et al. 2017). Although, whole-cell-based biosensors are not as sensitive as other biomolecular sensors, but they provide significant advantages in terms of tunability and in-situ pharmacological studies. For example, the cells can be tuned to sense a variety of analytes by simple genetic engineering such as heavy metals, pesticides, nutrients, and hydrocarbons. Furthermore, the pharmacological effects of the analyte (e.g., pollutants, drugs, etc.) on cell physiology and toxicology could be simultaneously obtained. Unlike biomolecular sensors which recognize binding interaction of the analyte directly on the transducer surface, whole-cell biosensors utilize the protein expression system of the cells to report a positive signal. Several reporter genes are used as expression systems including β-galactosidase, green-fluorescent protein, and prokaryotic and eukaryotic luciferases. These expression systems of reporter genes are kept under either inducible expression or constitutive expression

control. Another critical component of whole-cell biosensors is the regulatory proteins. These regulatory proteins are responsible for providing specificity to the biosensor. For example, GoIS protein is a regulatory protein of MerR family, which possesses high selectivity for gold ions (Pontel et al. 2007). Site-directed mutagenesis at the 77th amino acid position has enabled it to detect other heavy metal ions as well like mercury, lead, and cadmium (Cerminati et al. 2015). Similarly, successful detection of chlorpyrifos (CPF) pesticide was achieved by introducing a chpA promoter (from *Sinorhizobium meliloti*) in control of a reporter expression system in *E. coli* (Whangsuk et al. 2016). Finally, the selection of an appropriate host cell for sensor construction is another crucial component. The choice of host cell is often driven by the environmental conditions of the end application. For example, *Alcanivorax borkumensis* SK2 is known to exhibit high salinity tolerance and therefore well-suited for the detection of pollutants in seawater (Sevilla et al. 2015). Similarly, biosensor based on *Pseudomonas putida* DOT-T1E has been explored in heavily contaminated water due to their high tolerance (Espinosa-Urgel et al. 2015). The *Acinetobacter baylyi* ADP1 is known to grow on oil-water interface and emulsify oil droplets of 10–80 μm size. This makes them highly suitable for detecting long-chain hydrocarbons (C_7–C_{36}) in a variety of environments such as sea, soil, and other aquatic bodies (Ratajczak et al. 1998).

2.4.1 Bacteriophage-Based Biosensors

Recently, the extraordinary host-specificity of bacteriophages has been extensively explored for sensing pathogenic and drug-resistant bacteria. In comparison to other molecular probes (e.g., antibodies, DNA, etc.), whole-cell bacteriophages or their receptor binding proteins (RBPs) or more recently phage-display peptides (PDPs) exhibit several advantages such as host-specificity, mass scalability, higher tolerance to thermal or pH variations, and facile immobilization on transducer surface (Singh et al. 2012; Aliakbar Ahovan et al. 2020). Another unique opportunity of phage-based detection is the opportunity of genetically modifying the phage with reporter genes. This genetically modified phage is also called as "reporter phage" and is able to display positive results directly by infecting the host bacteria (Meile et al. 2020). For example, a phage-containing luciferase expression system was able to sensitively detect (LOD ~ 5 CFU/mL) the pathogenic *E. coli* O157:H7 strain by bioluminescence directly on the food samples (Kim et al. 2017). Since bacteriophages are capable of infecting only viable bacterial cells, these sensors can directly provide information on bacterial load in complex matrices such as food without any purification or extraction steps. The only downside to these biosensors is the time required for the accumulation of detectable signal.

2.5 Future Aspects

The miniaturized point-of-care (POC) biosensors have huge potential for revolutionizing the field of disease diagnostics, environmental pollutant monitoring, food safety and security, and screening of pathogenic organism. The impact of POC glucose biosensors on the management and treatment of diabetes is one such example (Yoo and Lee 2010). Perhaps, no other situation could have highlighted the importance of POC biosensor much better than the current COVID-19 disease pandemic caused by the SARS-CoV-2 coronavirus (Hu et al. 2021). Since no vaccine or treatment regime was available, the only response available to mankind was to prevent the spreading of the disease. In the process, worldwide lockdowns were called upon and people suffered huge economic losses. Unfortunately, apart from the loss of livelihoods, more than 2.5 million people have also loosed their life so far, till the time of writing this chapter. To prevent the further spread of the virus, it was paramount to screen the general population for viral infection accurately and as quickly as possible (Tré-Hardy et al. 2020). Point-of-care (POC) biosensors such as lateral flow (LFAs) for sensing the antibodies or viral proteins in patients have played a key role in such large-scale screening (Wu et al. 2020). Novel formats of nucleic acid testing like LAMP assay have also significantly reduced the analysis time in suspected cases (Ganguli et al. 2020).

The discovery of novel biological pathways can be further translated into highly specific and sensitive biosensors. For example, the discovery of CRISPR (Clustered regularly interspaced short palindromic repeats) as a natural defense mechanism adopted by bacteria against bacteriophages has led to the development of novel CRISPR-based biosensing assays (Bonini et al. 2021). These novel biosensors have further pushed the limit of detection to femtomolar (10^{-15}) and attomolar (10^{-18}) range while retaining the capability of being miniaturized biosensors (Aman et al. 2020).

The next revolution in POC biosensors is perhaps the incorporation of technological advances like internet of things (IOT) and cloud-based services (Ibrahim et al. 2020). Smartphone-assisted hand-held and wearable biosensors are also novel innovations currently being incorporated in biosensors (Purohit et al. 2020). These technologies will not only benefit users with real-time and remote monitoring of the analytes but also in rapid and effective decision monitoring. For instance, large data of population surveys can be remotely updated at rural peripheral centers and analyzed at a far-located central console in real-time. Penetration of smartphone technology and increased mobile connectivity will further strengthen such studies.

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Chapter 3
Nanomaterials for Point-of-Care Biosensors

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3.1 Introduction

In recent years, tremendous demand for biosensors has been perceived in the field of healthcare diagnostics as they are easy to use, economical, robust, quick, and sensitive. POC biosensors are drawing huge attention because most of the people who do not have access to the high-end sophisticated laboratories for specific diagnosis can easily perform their diagnosis with the help of these biosensors. In addition to these, tests in laboratories require skilled manpower and patients might have to wait for long to get their samples evaluated. The usage of POC biosensors has a massive impact on the healthcare sector as these types of tests could be easily performed by the patient himself which will reduce overcrowding in hospitals and other healthcare facilities.

Nanomaterials are those materials whose dimensions range from 1 to 100 nm (Lines 2008). Nanomaterials have different properties which enable greater levels of application on the sensing platform (Gogotsi 2006). As a result of their very small sizes, nanomaterials exhibit quantum effects, which result in the variation of physicochemical properties when compared to the bulk materials, e.g., higher electrical conductivity, melting point, absorption, and emission range (Asha and Narain 2020; Roduner 2006). Nanomaterials have high electron mobility (the ability to move quickly through a metal or semiconductor when an electric field is applied); higher surface-to-volume ratios make these materials very much suitable for development of biosensors (Brazaca et al. 2017).

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3.2 Components of Biosensors

A biosensor comprises four main components: (1) analyte, (2) a bio-receptor, (3) a transducer, and (4) an electronic detector system. Analyte is the substance whose chemical constituents are required to be determined. The biological element or bio-receptor used for detection can be an antigen/antibody, enzyme, protein, cells, tissues, nucleic acids, aptamers, etc. The transducer used in the biosensor is selected based on the detection method. Various transducers are used viz. electrochemical, optical, piezoelectric, mass spectrometer, and MEMS-based transduction systems (Karunakaran et al. 2015). Each of the transducers has its own unique functionalities. The detector system consists of a signal amplifier, processor, and display. The amplifier has the ability to enhance small signal input to a much higher signal output. The signal processor used in the biosensor helps in reducing the signal-to-noise ratio, converting signals from analog to digital mode which are displayed on the screen (Huang et al. 2007). The principle of the operation of a biosensor is shown in Fig. 3.1.

3.2.1 Analytes

Analyte is the component or substance that a biosensor wants to detect and quantify (Nikhil et al. 2016). It requires identifying appropriate biomarkers which are to be detected in different body fluids. Some of the frequently determined analytes from our body fluids are glucose, alcohol, triglycerides, cholesterol, HDL (high-density lipoprotein) cholesterol, glycerol, lactose, bilirubin, creatinine, calcium, phosphorus, etc. (Irjala and Grönroos 1998; Madira et al. 1993).

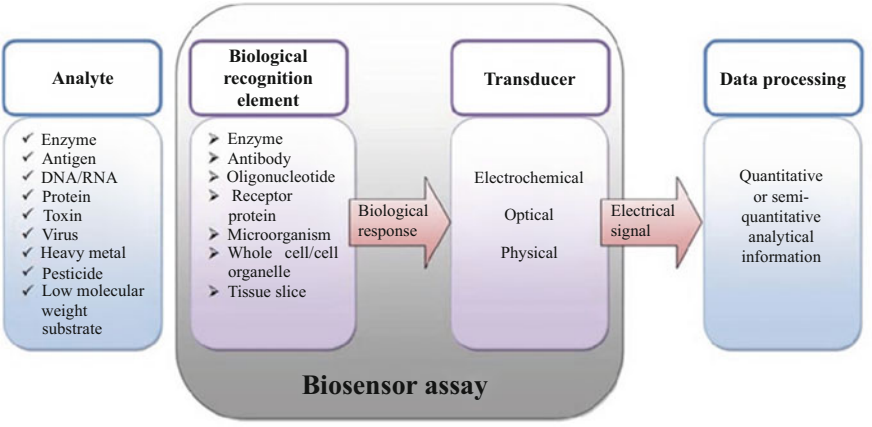

Fig. 3.1 The working principle of biosensors and their main components (Shavanova et al. 2016)

3.2.2 Biological Recognition Elements/Bioreceptors

Bioreceptors are biological components/molecules which specifically and selectively bind with a biomarker/analyte to generate a signal which is captured and converted to a measurable signal by a transducer. The bioreceptor should have high specificity to bind with an analyte. Biosensors can be classified according to the bioreceptors used such as antibody/antigen, enzymes, ligands, nucleic acids, DNA/RNA based, etc. (Kang and Kim 2020; Vo-Dinh and Cullum 2000; Gupta et al. 2018). In a biosensor, the binding reaction between biomarker/analytes and bioreceptor produces a physicochemical change and generates a detectable/measurable signal. Enzymes have the exceptional potentiality of selectively binding to their substrates (Marazuela and Moreno-Bondi 2002). Biosensors which use nucleic acids as receptors are based on base-pair interactions or aptamers (Du and Dong 2017). The principle of base-pairing is used such as adenine-thymine and cytosine-guanine in DNA. The integral sequences of the nucleic acids are synthesized and immobilized on the surface biosensor. Aptamers can be conjugated with fluorescent nanoparticles for optical detection to target various antigens or complex cells (Herr et al. 2006; Wang et al. 2008).

3.2.3 Transducers

Transducer helps in transformation of one form of energy to another suitable form of energy, which is in general a readable electrical signal. Electrical signals are converted from other forms of physical quantities such as light, mass, sound, force, acceleration, torque, etc. The output from transducers is always proportional to the input energy provided to the device. Biosensors can also be categorized according to the different transducers they use, i.e., electrochemical, optical, piezoelectric, MEMS-based, mass, etc. Electrochemical biosensors use electrodes as their transducer. Electrochemical biosensors convert the chemical reaction taking place between the analyte and bioreceptor to a measurable electrical signal. Electrochemical biosensors are used for both quantitative and qualitative analysis (Simões and Xavier 2017; Cao et al. 2019; Miyake 2003). In an optical biosensor, an optical transducer detects optical energy from a light source and converts it to electrical energy. Optical biosensors use interferometry, optical fibers, photonic crystals, quantum dot detection, etc., for conversion of signals (Parmin et al. 2019). Piezoelectric transducer uses the piezoelectric effect to measure any form of energy and converts it to electrical energy. When mechanical stress is induced in the piezoelectric material (generally a quartz crystal or ceramic such as barium titanate), electrical charges are produced on the surface of the material (Redwood 1961). Microelectromechanical systems (MEMS) are used to fabricate micro-sized IC (integrated circuit) chips whose size ranges from micrometers to millimeters. As the name electromechanical suggests, the input is in the mode of a motion, displacement, or

mechanical stress which is converted to electrical signals (Adams Thomas and Layton 2010).

3.2.4 Signal Processing Unit

General biosensing devices mostly work on the principle of a single input (analyte), while multi-signal logic gates rely on collectively marked analytes (inputs) to produce positive/negative responses. Different biomarkers are used as inputs for enzymes regulating the physiological information, such as biochemical logic systems which can determine fast and decisive evaluation of the extensive physiological condition and would introduce favorable appropriate healing interventions. Biocatalytic reactions are imitated by computer networks using this signal-processing circuitry with these input/output signals which are expressed by biochemical means. For the determination of various physiological conditions, the established enzyme logic circuitry was tested for the fabrication of novel digital multi-analyte biosensors. For example, three enzymes namely lactate oxidase, horseradish peroxidase, and glucose dehydrogenase were detected by a biocomputing system composed of a sequence of AND–IDENTITY logic gates to process biochemical information related to pathophysiological conditions originating from traumatic brain injury and hemorrhagic shock. Glucose, lactate, and norepinephrine are the three biochemical substances preferred as physiological biomarkers to target the damage occurring in the brain and were used to explain the idea of biochemical signal processing and its responses. The type of damage to the brain can be analyzed with the information specified by the concentration arrangement of these markers. An unusual increase in the concentration of glucose might derive from hemorrhagic shock while irregular physiological lactate concentration could be caused by hemorrhagic shock or/and traumatic damage to the brain. Higher concentration of norepinephrine can be an indication of any sudden brain shock. Thus, glucose, lactate, and norepinephrine were used as chemical data indications for the enzyme logic systems (Manesh et al. 2009).

3.3 Nanomaterials Used in POC Biosensors

Nanomaterials have lately fascinated much significance among the research community due to their increased need for control of appropriate molecules which exist in the human body and environment. The well-regulated synthesis process and tuning properties of nanomaterials demand proficiency in different disciplines such as physics, chemistry, electronics, computer science, biology, engineering, agriculture, etc., which leads to the development of novel and multifunctional nanotechnologies. In such circumstances, the appealing properties of nanomaterials have attracted the world scientific community toward its utilization in diverse sectors

such as health, food, security, transport, and information technology, etc. The detection limits and sensitivities of biosensors can also be boosted with the help of nanomaterials.

3.3.1 Biological Recognition Elements/Receptors

3.3.1.1 Enzymes

Enzymes are biological catalysts which stimulate chemical reactions by minimizing the activation energy. It is a type of protein which consists of one or more polypeptide chains and amino acids. The reactions are catalyzed in different ways after the binding of enzymes to the substrates: leading substrates along in a desirable adjustment by settling the bond structures of substrates so it can be effortlessly broken, laying out perfect environmental conditions for a reaction to happen, or engage directly in their chemical reaction by formation of temporary covalent bonds with the substrates. Enzymes can be controlled by inhibition and activation with the help of other molecules which would help in the chemical reaction. It reduces the activation energy required to reach the transition state. Some of the reactions may require a single substrate to be fragmented to multiple particles whereas in other reactions, more substrates might be required for the reaction. The enzyme and its substrate binds at the enzyme's active site. The process of binding takes place in the active site. Enzymes have an exceptional blend of amino acid leftovers or side chains within their active sites. The size of an enzyme depends on its need to have adequate surface area to contain specific binding sites for its unification into metabolic complexes. A biocatalyst that integrates with the conductive electrodes is required for the manufacture of an enzyme-based biosensor so that the enzyme catalytic conversion information can be communicated electrically (Willner and Katz 2000). As a result, electrical changes at the conductive supporter, such as reactant reduction or product composition in the biocatalytic process, give useful electronic transduction information on the biological recognition event taking place at the electrode. Furthermore, the decrease in the enzymatic product is linked to the absorption of the target biomolecule (Wang et al. 2014). The most extensively used enzyme biosensors in the healthcare sector are those designed for glucose, lactate, urea, and cholesterol. Glucose is by far the most comprehensively studied analyte. Glucose biosensors are mostly used for controlling and managing diabetes (Wilson and Hu 2000; Heller and Feldman 2008).

3.3.1.2 Nucleic Acids

Nucleic acid is a large molecule or polymer made up of a string of repeating units or monomers called nucleotides. Nucleic acids are very stable in nature. DNA and RNA are the two forms of nucleic acids found in human bodies. DNA molecules

never exit the nucleus, instead communicating with the rest of the cell through an RNA intermediary. In the form of chromosomes, DNA carries the genetic code of the cell and is passed down from parents to kids. Its a double-helical structure with two strands that travel in opposing directions, are joined by hydrogen bonds, and are complementary to one another. RNA is made up of pentose sugar (ribose), a nitrogenous base, and a phosphate group and is single-stranded. Protein production and regulation are both aided by RNA. Hybridization of DNA or RNA is the biorecognition mechanism. The specific biorecognition in DNA biosensors is based on the correspondence of adenine-thymine (A-T) and cytosine-guanine (C-G) pairings in DNA. DNA biosensors are used to track the interactions between DNA and ligands (Piehler et al. 1997). Surface plasmon resonance (SPR) was used to track the real-time binding of low molecular weight ligands to DNA fragments that were irreversibly bound to the sensor surface via coulombic interactions. Based on liquid-crystalline dispersions produced from DNA-polycation complexes, sandwich-type biosensors are used to detect chemicals and physical conditions that alter the ability of specific DNA crosslinkers to bind between neighboring DNA molecules (Skuridin et al. 1996). A SPR-based biosensor was fabricated which used peptide nucleic acid as the biorecognition element (Sawata et al. 1999). The peptide nucleic acid has a strong binding affinity for oligonucleotide sequences, allowing for direct detection of double-stranded DNA that has been extended by a polymerase chain reaction.

3.3.1.3 Antibodies

Antibodies are glycoproteins naturally produced in the body when it comes to the contact of an invading foreign particle such as microorganisms or viruses. Antibodies are important components of the immune system's defense against infection and disease. The immune system of a human body generally produces a large group of antibodies when it is attacked by a particular type of antigen. Each antibody is secreted by a B-cell, which is an antibody-producing plasma cell. B-cells are a type of white blood cell that aids the immune system in combating germs and viruses by creating antibodies against them. Antibody-based biosensors use the mechanism of detection of analyte concentration by imparting changes in the output of the transducer which takes place due to the binding event or through the displacement reactions which occur in the biosensor identical to that of immunoassays (North 1985). A dual-electrode antibody-based biosensor was reported in which one of the electrodes was coated with a membrane containing the antibody. The other electrode was coated only with a membrane specially for binding purposes (Taylor et al. 1991). Carbon nanotubes (CNTs) were used to create a multienzyme immunosensor that targets prostate-specific antigen (PSA), increasing sensitivity by reducing particle size and increasing the number of labels in the system (Jensen et al. 2009). An amperometric reversible immunosensor with rabbit IgG antibodies in an electrodeposited polypyrrole layer on an electrode has been constructed (Gooding et al. 2004). Antibody-bound super-paramagnetic nanoparticles and two

independently operated electromagnets on either side of a plastic case are used in an optomagnetic immunosensor for detection of cardiac markers. The replaced nanoparticles are pulled through the media to the optical sensor surface by one electromagnet, which is then switched on to remove loosely bound or unbound particles from the sensor surface by the higher electromagnet. It is possible to do an optical determination of binding. After the sample fluid has been introduced, there are no additional wash phases, resulting in a simple one-step measurement (Bruls et al. 2009).

3.3.2 Nanomaterials or Nanostructured Materials

Nanomaterials as well as nanostructured materials possess unique characteristics which can alter the physical, chemical, electrical, and optical properties of a substance. Nanomaterials such as ZnO, ZnS, TiO_2, Gd_2O_3, and SiO_2 having characteristics such as large bandgap, high binding energy, piezoelectricity, and biocompatibility are highly recommendable for innovation of sensors, anodes, lithium-ion batteries, nanogenerators for generation, and storage of electricity (Sharma et al. 2021; Araneo et al. 2016; Ossai and Raghavan 2018; Li et al. 2016; Chaudhari and Srinivasan 2012). Nanostructured materials come in many forms. Nanowires, nanotubes, nanobelts, nanosprings, nanoribbons, nanorings, and nanosheets are some of the examples of nanostructured materials (Zhang et al. 2016; Liu et al. 2013). Nanostructured materials have unique properties due to the minor adjustments in the morphology, size, and shape which can be done during their synthesis by varying the temperature, pressure, pH balance, and addition of different chemicals such as various enzymes or catalysts (Cho and Lee 2008; Xu and Wang 2011). Molecular beam epitaxy, sputtering, chemical vapor deposition, wet chemical approach, laser ablation, reverse micelle emulsification, sol-gel process, electric-arc deposition, electrospinning, and other techniques can all be used for synthesis of nanomaterials (Barczak et al. 2016). Though nanostructured materials are very useful in research purposes and other industrial sectors, they has their own limitations as well. These nanostructures contain defects in their lattices due to heteroepitaxy during synthesis. Heteroepitaxy is the growth of one crystal on the surface of another. Heteroepitaxial growth also had the consequences of surface restoration, step bunching, faceting, and lattice defects (Teichert 2002). This results in non-uniformity in the nanostructures. This problem with the synthesis has been addressed by varying the time of synthesis to reduce the lattice defects but the growth defect problems are still to be solved. Organic nanomaterials and inorganic nanomaterials are the two types of nanoparticles.

3.3.2.1 Organic Nanoparticles

Proteins, carbohydrates, lipids, and other organic substances are used to synthesize organic nanoparticles. Organic nanoparticles are fabricated from proteins, carbohydrates, lipids, and other organic compounds. Organic nanoparticles mostly contain carbon-based nanoparticles such as carbon nanotubes (CNTs), graphene, nanofibers, fullerenes, dendrimers, liposomes, etc. Some of the organic nanoparticles are described below.

Carbon-Based Nanoparticles

Carbon-based nanoparticles are a family of nanoparticles which has unique properties such as high mechanical strength, high conductivity, chemical versatility, etc. Carbon-based nanoparticles include carbon nanotubes (CNTs), fullerenes (C60), graphene, carbon quantum dots, and nanodiamonds.

Carbon nanotubes (CNTs) are smooth cylinder rolls of graphene sheets with rare intrinsic properties. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNNTs) are two types of carbon nanotubes (MWCNTs). The classification is done on the basis of the number of graphene layers it contains. The diameter of the CNTs varies from as low as 3 to 100 nm. The MWCNTs contain layers which are networked with each other by van der Waals forces (Li and Chou 2003; He et al. 2010) and various crystal combinations having different mechanical, electrical, and optical properties. Some of the examples of CNT biosensor are as follows: (1) a galactose biosensor was designed to detect the levels of galactose in blood plasma by using SWCNT dispersed in chitosan while retaining high selectivity and sensitivity (Oliveira et al. 2015), (2) MWCNT was grown on a platinum substrate to create an amperometric biosensor for measuring glucose oxidase (Gao et al. 2003), and (3) another biosensor was designed by coating conducting polymers onto MWCNTs for detection of glucose (Demirci Uzun et al. 2014).

Fullerenes molecules are purely made up of one type of carbon atom which can form different shapes such as cubes, spheres, tubes, etc. The most distinguished feature of fullerenes is that it has a hollow core or an empty space inside the molecule. Fullerenes also act as sensitizers for the production of singlet oxygen. Hence, it has found uses in cancer therapy (Jiang et al. 2015; Chen et al. 2012; Huang et al. 2012; Jacobs et al. 2010). But it has the same disadvantages as the CNTs. Its poor solubility has been a big issue against its use in nanomedicine. To overcome this problem, a few methods have been developed so that fullerenes can be functionalized with hydrophilic groups to improve their water solubility (Chen et al. 2009). A glucose biosensor is developed by immobilization of fullerene upon a porous carbon electrode for detection of glucose oxidase in the human body (Balla et al. 2019). For the detection of the urease enzyme, another potentiometric biosensor was created. For the creation of the biosensor, fullerene is used as a matrix for

immobilizing urease (Saeedfar et al. 2013). Fullerenes also act as electrochemical mediators and enzyme stabilizers (Sotiropoulou et al. 2003).

Dendrimers

Dendrimers are mostly branched, star-shaped molecules. These are molecules with a radially symmetric structure that is well-defined, homogeneous, and monodisperse (Sampathkumar and Yarema 2007). A dendrimer has a central core that is made up of a collection of atoms. Different chemical interactions cause the branches of other atoms (also known as "dendrons") to develop from the central core. The outer area is covered by different functional end groups which help it in performing different types of chemical reactions. The synthesis of dendrimers is generally based on one of the two approaches known as the convergent and divergent approach. The synthesis of the dendrimer in the divergent approach begins with the core of the dendrimer, to which the branches are linked in a stepwise way by building pieces. In the convergent technique, the synthesis begins with the outside area, starting with the molecular structure and ending with the dendrimer's outermost area (Grayson and Frechet 2001). Polyvalency, electrostatic interactions, and pharmacokinetic features are only a few of the benefits of dendrimers. Polyvalency is one of the most important properties as it has a wide range of functionalization and also causes multiple interactions with different biological receptor sites. Dendrimer's surfaces include a variety of end-groups, many of which are identical, and when these groups are charged, the surface functions like a polyelectrolyte, attracting molecules with opposite charges (Abbasi et al. 2014). One of the most important things to consider for successful biological applications of dendrimers such as drug delivery, imaging, photodynamic therapy, and neutron capture therapy is their pharmacokinetic properties. Dendrimers have several medical and practical applications. It is used in the production of anticancer drugs, drug delivery, gene delivery, imaging, and sensors. Dendrimers exhibit globular geometry, high surface functionality, high mechanical and chemical stability which makes them perfect for immobilization of biomolecules. The use of dendrimers in biosensors increases the sensibility, specificity, and linearity of the biosensors (Zeng and Zimmerman 1997). Using a zirconia-polypropylene imine dendrimer, an electrochemical biosensor for urea detection has been developed. The dendrimer was deposited on screen-printed carbon electrodes. The biosensor had high selectivity and reactivity (Shukla et al. 2014). An amperometric biosensor for glucose determination has been developed. Glucose oxidase was immobilized on a co-polymer electrochemically assembled from polyamidoamine (PAMAM) dendrimers (Şenel and Nergiz 2012).

3.3.2.2 Inorganic Nanoparticles

Inorganic nanoparticles have a central core which possess inorganic materials that define their fluorescent, magnetic, thermal, and electronic properties. Metal

nanoparticles such as gold, silver, and others, as well as their oxides/sulfides found naturally, are examples of inorganic nanoparticles. Other types of inorganic nanoparticles include magnetic nanoparticles, polymeric nanoparticles, semiconductor nanoparticles, quantum dots, etc.

Metal Nanoparticles

Metal nanoparticles have piqued the interest of researchers all over the world due to their enormous potential in the field of nanotechnology. These particles can be synthesized and conjugated with different chemicals to activate different functional groups so that these nanoparticles can be immobilized with different antibodies, ligands, or drugs of interest. Metal nanoparticles are mostly gold nanoparticles, silver nanoparticles, nanocages, and nanoshells.

Gold nanoparticles are suspensions of particles in the nanometer size range. Gold nanoparticles are either of deep reddish color (particles smaller than 100 nm) or yellowish color (large particles) (Tong et al. 2009). These nanoparticles have intriguing optical properties and exhibit the phenomenon of LSPR. These nanoparticles have interesting optical properties and follow localized surface plasmon resonance (LSPR) phenomenon. In the presence of an electromagnetic field of light, the free electrons in the nanoparticles oscillate with respect to the lattice structure (Murphy et al. 2008). After absorption, the surface plasmon begins to decay over time, and the absorbed light is converted to heat due to light scattering. The properties of gold nanoparticles are determined by their shape and size. Gold nanoparticles have distinctive features which have been the focus of substantial research with a wide range of applications in biological imaging, electronics, and material sciences (Jain et al. 2008). The electrodes of electrochemical biosensors have been modified with gold nanoparticles to enable high-efficiency enzyme immobilization (Mody et al. 2010). They also aid in the transport of electrons from proteins that have been immobilized to electrode surfaces.

Silver nanoparticles have found their use in different sectors such as healthcare, food, medical instrumentation, and industrial purposes due to their unique optical, thermal, electrical, and biological properties (Mukherjee et al. 2001; Barkat et al. 2018; Yaqoob et al. 2020). Silver nanoparticles are synthesized using physical, chemical, and green chemistry methods. The chemical route for synthesis makes the use of three substances namely, (1) a precursor, (2) a reducing agent, and (3) a capping agent. These nanoparticles can be synthesized by two processes generally known as the "top-down" and "bottom-up" approaches (de Oliveira et al. 2020). The "top-down" approach consists of mechanically grinding the bulk particles of the material along with special stabilizing agents (Usman et al. 2020; Richards and Bönnemann 2005). Chemical reduction and electrochemical methods are commonly used in the "bottom-up" approach. The physical synthesis methods include spark discharging, pyrolysis, and milling techniques (Rane et al. 2018; Pluym et al. 1993; Singh et al. 2019; Gautam et al. 2021; Malekzadeh and Swihart 2021). Both the physical and chemical methods have their own advantages and disadvantages. The

advantages of chemical methods are that they have higher yield than the physical methods, lesser or no consumption of energy, and uniform distribution of the particles. The advantages of physical methods are that they do not use any hazardous chemicals for synthesizing nanoparticles, no reducing agent is used, and the process is very fast (Ghorbani et al. 2011; Shameli et al. 2010; Iravani et al. 2014; Shanmuganathan et al. 2019).

Magnetic Nanoparticles

The properties of magnetic nanoparticles can be changed to suit their functioning as per requirement by changing the magnetic fields. These nanoparticles have superparamagnetism, high saturation field, high field irreversibility, and other features. These qualities are the result of nanoparticle's magnetic behavior, which is caused by their finite size and surface effects (Batlle and Labarta 2002). At low temperatures, the thermal energy decreases and the magnetic moments become blocked. This is referred to as blocking temperature. The effects of magnetization of these particles above the blocking temperature are almost the same as that of superparamagnetism except that of immensely large moments (Bean and Livingston 1959).

Magnetic nanoparticles can be synthesized using a variety of techniques. Some of the methods are co-precipitation, microemulsions, polyols, etc. In the co-precipitation method, stoichiometric preparation of ferric and ferrous hydroxides yields spherical magnetite homogenous particles in the aqueous media (Massart and Cabuil 1987). The size of the particles can be changed by altering the aqueous media's pH and ionic strength. With the increase in pH and ionic strength, the size of the particle reduces (Jolivet et al. 2003). These parameters also affect the chemical composition of the material. Microemulsions are transparent and thermodynamically stable media. In these methods, fine microdroplets of the aqueous medium are collected in the assemblies of the surfactant molecules dispersed in the oil phase. Microemulsions have shown to be a very simple and decent method to prepare magnetic nanoparticles (Zhang et al. 1999) and are useful for many applications. Polyols are organic compounds which contain multiple hydroxyl groups. In this process, the liquid polyol acts as the solvent media, the precursors are the metallic particles, and a reducing agent is used. The metallic precursor is dissolved in the polyol and then the solution is heated to the boiling temperature of the metallic particles by stirring it. A specific size and shape can be obtained by controlling the kinetic of precipitation. The average size of the particles can be obtained by heterogeneous nucleation where the growth of the particles is completely separated which results in the formation of uniform particles. Magnetic nanoparticles are induced in the transducer of the biosensor or dispersed into the sample to be used on the biosensor which is followed by an external magnetic field on the detection surface of the biosensor (Rocha-Santos 2014).

Semiconductor Nanoparticles

Nanoparticles that have an electrical conductivity between a conductor and an insulator are known as semiconductor nanoparticles. Semiconductor nanoparticles consist of different compounds. It is based on the groups of the periodic table into which these elements are formed. For example, nanoparticles like zinc oxide (ZnO), zinc sulfide (ZnS), cadmium sulfide (CdS), and cadmium selenide (CdSe) come under the category of class II–VI semiconductors. Silicon (Si) and germanium (Gr) in group IV and GaP (gallium phosphide), GaAs (gallium arsenide), and InAs (indium arsenide) are in group III–V semiconductors (Nosaka and Nosaka 2019). This class of nanoparticles (generally quantum dots) are exceptionally fluorescent because of the confined bandgaps which result in unique optical properties. This confinement allows these nanoparticles to emit different wavelengths of visible light spectrum. Sol-gel procedure, solvothermal/hydrothermal method, co-precipitation method, and other methods can all be used to synthesize semiconductor nanoparticles. In sol-gel processes, the alkoxide of the metal is first hydrolyzed and then condensation of the reaction takes place. The "sol" is a dispersed system which consists of solids in the form of chains in the dispersion liquid medium. The "gel" is a stiff mass of the substance which is like a viscous form of liquid. The sol is formed by controlled hydrolysis followed by a condensation reaction of metal alkoxides, which in later stages forms a network of resultant glasses. The advantages of the sol-gel method include its simplicity, cost-effectiveness, and ease of synthesizing pure materials. Solvothermal processes are used to synthesize nanoparticles with different morphologies. This process is described as a chemical reaction that occurs in a closed system in the presence of a solvent at a temperature greater than the solvent's boiling point. In comparison to bulk materials, semiconductor nanoparticles exhibit a variety of beneficial physical and chemical properties as well as interesting capabilities. Some of the most eminent properties of these nanoparticles are narrow emission spectra, high surface functionality, and continuous absorption band. Semiconductor nanoparticles are coupled with biorecognition elements such as enzymes or DNA to help develop photoelectrochemical reactions in the biosensors (Curri et al. 2002).

Polymeric Nanoparticles

Biocompatible and biodegradable polymers are used to make polymeric nanoparticles (PNPs). PNPs are generally used in the production and delivery of drugs. Nanospheres or nanocapsules can be procured depending on the method of nanoparticle preparation. The medication is stuffed into a chamber comprising a polymer structure which are vesicular systems and termed as nanocapsules (Tolve et al. 2016). Nanospheres are matrix particles, which means that the entire mass is solid and the active ingredients are absorbed on the sphere's surface walls or remain contained within the sphere's structure (Fang and Bhandari 2010). In the preparation of PNPs, mostly two types of polymers are used: (1) natural polymers such as

chitosan, gelatin, albumin, etc., and (2) synthetic polymers such as polylactides, polyglycolides, polyanhydrides, etc. PNPs have been synthesized using a variety of techniques. Some of them are solvent evaporation, nanoprecipitation, emulsification, etc. In the solvent evaporation method, emulsions and polymer solutions are made in volatile solvents. The emulsions are converted into nanoparticle suspensions when the solvent for the polymer is evaporated. For the preparation of single emulsion, oil-in-water (o/w) method is used and for double emulsions, water-in-oil-in-water (w/o/w) method is used. In these methods, ultrasonication is used followed by evaporation of the solvent under vacuum. The nanoparticles are then washed and freeze-dried (Bilati et al. 2005). The solvent displacement method involves precipitation of a polymer from an organic solution and diffusion of the solvent in an aqueous medium (Reis et al. 2006). The polymer (generally polylactides) is dissolved in the solvent (mostly water). Rapid diffusion of the solvent into the non-solvent phases results in a decrease of surface tension in between the two phases, forming droplets.

PNPs find their uses in different theranostic applications such as drug delivery, imaging, and sensing. PNPs have electrochemical properties similar to transducers and hence they can be used for manufacturing biosensors. Wearable devices such as smartwatches are based on PNP biosensors which can detect different biological fluids such as blood and sweat (Rao and Geckeler 2011).

Hydrogels

Hydrogels are three-dimensional network structures formed by the physical and chemical cross-linking of hydrophilic monomers (Lima-Tenorio et al. 2015). It is made up of an enclosed polymer network, the material absorbs water and takes on the fluidic properties of the water. The surface of the hydrogels becomes wet and malleable when dipped in water, which significantly boosts biocompatibility. Hydrogels have no effect on the metabolic processes of living organisms, and metabolites can freely travel through them. Small changes in the environment, such as temperature, pH, ionic strength, and electric and magnetic fields are particularly sensitive to hydrogels (Rodkate et al. 2015; Samchenko et al. 2011; Wang et al. 2019; Andrade et al. 2021). There are different types of hydrogels. Some of them are conductive hydrogels, injectable hydrogels, sliding hydrogel, nanocomposite hydrogels, etc. Each type of hydrogel has properties of its own. Conductive hydrogels have very good electrical conductivity (Kloxin et al. 2010; Dai et al. 2009). Conducting hydrogels can be further categorized into two types based on the additives used, (1) conducting hydrogels-based conducting polymers and (2) conducting hydrogels-based metallic nanoparticles. Injectable hydrogels have inherent flowability and can be adhered via injection. Injectable hydrogels exhibit sol-gel phase transitions when subjected to different environmental conditions (such as changes in temperature or pressure) (Park et al. 2013). These hydrogels can also be divided into two different types: (1) light irradiation hydrogels and (2) self-assembling hydrogels. The formation of unbreakable covalent bonds with the introduction of visible or ultraviolet light radiation occurs in light irradiation

hydrogels, while self-assembling hydrogels form quickly (Koutsopoulos and Zhang 2012). Interconnected non-covalent bonds in sliding hydrogels allow them to slide over a threaded polymeric matrix (Peak et al. 2013) and nanocomposite hydrogels are created through the chemical covalent bonding of a polymeric matrix and raw materials such as carbon-based nanoparticles, polymer nanoparticles, and metal oxides (Merino et al. 2015; Tavakoli and Tang 2017). Polyvinyl chloride, polyethylene glycol, polyacrylate, and electroconductive hydrogels are some of the most regularly used hydrogels. Alginate, chitin, chitosan, agarose, cellulose, dextran, and hyaluronic acids are examples of biological hydrogels. A biosensor has been developed for detection of cholic acid present in hepatocytes with the help of molecularly imprinted photonic hydrogels. These hydrogels help in rapid and ultrasensitive detection of cholic acid (Wu et al. 2008). Another photonic hydrogel biosensor for detection of biologically significant cholesterol concentration in the human body (Maurer et al. 2008).

Molecular Machines

Molecular machines are extremely small devices that can transform one form of energy into another at the molecular level. Molecular machines occur naturally and can also be synthetically produced. These machines generally rely on intermolecular and intramolecular forces for their functioning (Amendola et al. 2001). Ionic forces and van der Waals forces are some of the forces which act upon the function of the geometry of the distinctive molecule. Different types of family of molecular machines such as kinesin, myosin, and dynein all depend on ATP for initial energy source. DNA tweezers and flagella motors are also examples of protein-based molecular machines. Apart from that, the synthetically prepared chemical molecular machines have also found their uses in different scientific utilities. A few of these machines resemble commonly used macroscale equipment such as gears, propellers, etc. Synthetic molecular machines contain organic compounds such as hydrogen, nitrogen, carbon, etc. These molecular machines can operate in a number of ways such as chemically, electrostatically, and photochemically. These machines are much more robust than their natural counterparts. Rotaxanes and catenanes are examples of synthetically produced molecular machines (Ashton et al. 1996). The entire goal of molecular machines is the utilization of numerous biological factors, the function of which creates a motion, force, or signal at the cellular level, much like the components of a machine react to the reaction of a biological activity. A DNA walker is a type of molecular machine which has found its uses in biosensors for signal amplification. An aptasensor was fabricated for detection of C-reactive protein (CRP) based on a DNA walker sandwich assay. The biosensor was found to be highly reactive and sensitive (Wang et al. 2018). Another biosensor based on a 3D-DNA walking machine was designed to identify tenascin-C and lead ion. It improved nanomachine sensitivity and loading efficiencies (Xu et al. 2018).

Thin Films

A thin film is the product of a set of layers of materials which are placed one upon another ranging in the size of nanometers to a several micrometers in thickness. The film coatings are deposited on a substrate material. Thin films have various characteristics which can be altered to improve the performance of the substrate. Thin films can be prepared by chemical deposition, physical deposition, and sputtering techniques. The thin films which are prepared using the CVD procedure have a high rate of deposition and conformal growth, good uniformity, and compatibility (Davis and Higson 2005). In physical vapor deposition (PVD), deposition of the required material is released from the source to the substrate by evaporation. The metal to be deposited on the substrate is heated and its vapor is created in the high vacuum to control the scattering of the molecules in gaseous form. After that, the metal vapor is spread and condensed on the substrate's surface. The advantage of this process is that it has lower risk in case of metal deposition but the thin films produced in this process are of lower quality than the ones prepared by the CVD technique. Thin films are used to enhance the surface properties of the solids. Some of the properties of solids which can have an increased effect using thin films are hardness, corrosion resistance, abrasion resistance, transmission, absorption, and reflection. Thin films are also used as protective coatings so that they can be protected from wear, scratches, corrosion, and fingerprints. It is also used as an anti-reflective coating which reduces the reflection of optical surfaces. The use of thin films on the electrodes of an electrochemical biosensor enables it to control and stabilize different enzymes and proteins. It increases the sensitivity and stability of the biosensors (Gong et al. 2010). A biosensor has been developed using a composite thin film containing CNTs and Tin(IV) oxide (SnO_2). The application of thin film in the biosensor significantly enhanced the sensing surface area (Verma et al. 2014). Another biosensor has been fabricated for detection of uric acid using copper oxide thin film which was grown on a platinum-coated glass substrate. The biosensor had a wide linear range and had an enhanced affinity toward its analyte (uric acid) (Jindal et al. 2012).

3.4 Challenges and Future Prospects

In the recent few years, many researchers have tried to come up with new techniques for fabrication of POC biosensors for both academic purposes and commercialization in the healthcare sector. POC devices have revolutionized the healthcare system because it has made it available for the patients rapid and accurate analysis of their health situation without visiting a hospital or a doctor's clinic. It has optimized the diagnosis process, improves treatment management, is available for everybody, and is cost-effective.

One major challenge for these biosensors is that it has been only developed in laboratory environments. For successful commercial applications, such a technology

should be developed that allows mass production of biosensors to high-quality specifications and relatively inexpensively. Nanomaterial toxicity is a severe concern to human wellbeing. Nanomaterials can easily enter the human skin due to their tiny size. Nanomaterials, according to scientists, can easily enter human tissues and disrupt the normal metabolic environment of cells (Vishwakarma et al. 2010). It has been found in human and animal studies that nanomaterials after penetrating the human body through the nasal or oral route penetrate easily to different organs of the body (liver, spleen, brain, lungs, and the gastrointestinal tract) (Hagens et al. 2007; Nemmar et al. 2002; Kundu et al. 2021). Nanomaterials have unexpected clinical outcomes due to their unique physicochemical properties in various biological systems. Hence, tackling the toxicity issues of nanomaterials would be an arduous task. Moreover, the disposal of nanomaterials is also an issue. It should be treated and disposed of as "hazardous waste". Any waste containing nanomaterials should be disposed of with utmost care. The materials which come in contact with nanomaterials such as PPE kits, wipes, bottles, and laboratory materials should be considered as waste and disposed of. Temperature control is another issue in the synthesis of nanomaterials. Nanoparticles may be adjusted to have desirable qualities including porosity and a large surface area, making them good catalysts for biosensors. But temperature should be applied in a controlled manner as its excessive use might alter the properties of the nanomaterials. As special properties are derived from the tiny size of nanomaterials, it is very critical to scientific study and research. Nanomaterials are very hard to produce and take a lot of time and effort. Hence, nanomaterials are very expensive to buy.

Over the recent years, nanomaterials have become increasingly important in commercial space. Advances in nanotechnology are projected to result in several breakthroughs and new opportunities for the global economy. Nanomaterials may be widely used in numerous industries in the future, especially in biosensing, due to their potential applicability. Because of their size, biocompatibility, surface chemistry, relatively good stability, and tunable toxicity in biological systems, nanomaterials can be employed for clinical diagnosis. The use of nanomaterials in biosensors is projected to significantly improve the biosensing sector. Reactivity is one of the most important factors in the biosensing field. Nanomaterials have a greater surface area due to their tiny size which results in increased binding capacity and reactivity.

3.5 Conclusions

Nanomaterials have the potential to exhibit extraordinary and fascinating optical, electromagnetic, and piezoelectric capabilities due to their small size and high surface area. Because of these properties, medical diagnostics, imaging, and healthcare diagnostics have all improved due to the use of nanomaterials. Nanomaterials have been used in most of the biosensing platforms that have been developed recently. Graphene, metallic NPs, and CNTs have been reported to have

increased the selectivity and sensitivity of the biosensors. The development of the healthcare sector is dependent on modern equipment that allows for early diagnosis and treatment of numerous disorders. POC testing is one of the relatively new trends which need to be developed in the coming years. Electrochemical biosensors are one of the most commonly used biosensors for diagnosing a variety of health conditions, but they come with a number of drawbacks. Optical biosensors are the new class of biosensors coming up which can create the whole difference in the field of medical diagnostics as it is very easy to operate. Microfluidics system techniques are employed in a variety of biosensors to detect various biomarkers, assisting in the fabrication of biosensors that provide better and more consistent responses for the diagnosis of various diseases.

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Chapter 4
New-Generation Molecular Techniques in POC Biosensors for Detection of Infectious Diseases

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4.1 Introduction

4.1.1 Infectious Diseases: An Overview of the Challenges and Recent Outbreaks

Infectious diseases are widespread public health problems. This burden has significantly influenced the socioeconomic development of the society. In general, infectious diseases can be broadly categorized into either hospital-acquired (nosocomial) or community-acquired infections. The spread of such diseases occurs through the transmission of disease-causing pathogen either directly (e.g., via physical contact, airborne inhalation) or indirectly (e.g., via contaminated food, water, domestic animals, insects, etc.). Pathogens that originate and circulate through food are generally termed foodborne pathogens, where food plays an important role as a link in the spread of such diseases in the society. Many times, foodborne pathogens can also cause severe infections and food poisoning by releasing microbial toxins. *Salmonella enterica* serovar Typhi, *Escherichia coli* O157:H7, *Shigella dysenteriae*, some strains of *Staphylococcus aureus*, *Vibrio cholerae*, *Streptococcus pyogenes*, *Clostridium botulinum*, *Clostridium perfringens*, *Aeromonas* spp., etc., are some of the major foodborne pathogens that produce toxins and become infectious (Hernández-Cortez et al. 2017). Some of these pathogens can also cause bloodstream

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infections (*Salmonella enterica* serovar Typhi, invasive non-typhoidal *Salmonella enterica* serovar Typhimurium, seroval Enteritidis). Under certain circumstances, infectious disease may turn into a serious complication called sepsis. Sepsis (blood stream infection) is a life-threatening organ dysfunction due to a dysregulated host response to an infection caused by a pathogen (Rudd et al. 2020). Some of the most frequently isolated bacteria in sepsis are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus*, and *Candida albicans* (Mayr et al. 2014). The most challenging factors in addressing the devastating effects of both infectious and foodborne diseases are the evolution of antibiotic-resistant strains, bottlenecks in sample preparations from complex matrices, lack of point-of-care diagnostic systems with acceptable sensitivity levels, and rapid analysis. Among the above, emerging trends in the evolution of antibiotic-resistant strains have become a serious threat. Conventional strategies used for the detection of pathogens include microbial plate counting, molecular techniques such as polymerase chain reaction (PCR), and immunological methods such as ELISA. Apart from these, researchers have recently attempted to develop several alternative methods for the rapid detection of pathogens (Table 4.1).

The time to result in conventional strategies for pathogen detection is often too long. Although highly sensitive and accurate, these technologies are not relevant for on-site testing and POC applications as they require special equipment and assay conditions. The most common challenge is the transport of samples from remote places to reference labs and accredited laboratories. This may have a negative outcome and can significantly impact the quality of diagnostics and preventive measures during outbreaks such as an epidemic or even in a pandemic.

In epidemiology, an epidemic is referred to the disease, disorder, or an infectious agent that clearly exceeds the normal expected level in a community or in a region. Outbreak refers to an epidemic that is limited to a localized increase in the incidence of a disease. An epidemic can affect a large number of people worldwide crossing international boundaries to become a pandemic as COVID-19 pandemic that happened recently. Both epidemics and pandemics can adversely affect human health and the global economy via increased morbidity, mortality, and restriction of trades, disruption of social and geopolitical infrastructures and the global industrial production chains. SARS, AIDS, dengue fever, influenza, smallpox, cholera, plague, and tuberculosis are some examples of major pandemics that humankind has experienced in the past (Jamison 2018). For example, influenza pandemics have occurred about three times since the 1500s including the deadliest Spanish flu that occurred in 1918 that has resulted in more than 20 million deaths worldwide. COVID-19 caused by SARS-CoV-2 was a declared pandemic of the twenty-first century. Therefore, addressing such incidences in the near future outbreaks, epidemics, and pandemics require improved global surveillance and reporting systems and next-generation rapid diagnostic technologies with aimed application at point-of-care settings.

Table 4.1 The most common and current techniques available/reported for the detection of pathogens

Detection technique	Sample concentration	Sensitivity (CFU/mL)	Specificity	Analysis time (in h)
An overview of pathogen detection methods (Mandal et al. 2011)				
Qualitative culture	No	NA	Good	24–72
Qualitative rapid detection	No	NA	Variable	Variable
Most probable number (MPN)	No	<10–100	Good	24–48
Viable counts	No	>10–100	Good	24–72
Impedance	No	100	Moderate	6–24
Bioluminescence	NS	10^4	No	<1–3
Direct epifluorescent filter technique (DEFT), Solid-phase cytometry (SPC)	NS	10^3–10^4	No	<1
Flow cytometry	NS	10^5–10^7	Good	<1
Immunological methods (LFD, ELISA, ELFA)	NS	10^4–10^5	Moderate/ good	<1–3
Nucleic acid-based assays (FISH, PCR)	NS	10^3–10^4	Excellent	<1–3
Advanced techniques for the pathogen detection				
SepsiTest (Molzym, Germany), PCR combined with sequencing, Multiplex	Centrifugation	20–40	Excellent	8–12
SeptiFast (Roche, Germany), Real time-PCR, Multiplex	Yes	3–30	Excellent	6
FAST-ID BSI Panel (QVella), rRNA detection, Multiplex	Automated cartridge	1–2	Good	1
MagicPlex (SeeGene, Korea), Real time-PCR, Multiplex	No	NS	Excellent	3–4
FilmArray Biomerieux, Real time-PCR, Multiplex	No	NA	Good	1
SP-PCR, Multiplex (Vinayaka et al. 2020)	Magnetic bead	10–100	Excellent	2–3
DOT ELISA (Hassan et al. 2021)	No	NS	Good	NS
Direct PCR, Multiplex (Vinayaka et al. 2019)	Magnetic bead	2–4	Excellent	2–3
Direct LAMP in a paper reactor and an immunochromatographic strip (ICS) (Lee et al. 2019)	No	10^3–10^4	Good	1
Real time-PCR, Singleplex (Firoozeh et al. 2019)	QIAamp DNA Mini Kit	NS	Excellent	NS
LAMP (Kaur et al. 2018)	Magnetic bead	5–10	Good	5–6
PCR combined T2 magnetic resonance (T2MR) technology, Multiplex (De Angelis et al. 2018)	No	1	Good	3–5

(continued)

Table 4.1 (continued)

Detection technique	Sample concentration	Sensitivity (CFU/mL)	Specificity	Analysis time (in h)
MALDI-TOF MS, Multiplex (Zhu et al. 2016)	Centrifugation and magnetic bead	10^2–10^3	Excellent	4–6
Real time-PCR, Multiplex (Vutukuru et al. 2016)	Magnetic bead	1	Excellent	2–3
Droplet Digital Detection using 3D particle counter system (Kang et al. 2014)	No	1–10	Good	2–4
SERS, Multiplex (Cheng et al. 2013)	Electrokinetic	10^3	Good	5 min

NS not specified, *NA* not applicable, *SERS* surface-enhanced Raman spectroscopy, *SP-PCR* solid-phase PCR, *LAMP* loop-mediated isothermal amplification, *LOD* limit of detection

4.1.2 Point-of-Care Biosensors for Pathogen Detection

POC devices are characterized specifically by samples-in-results-out configuration, small portable size, ease of use, and cost-efficiency. Biosensors are, in general, developed with such criteria aiming with their application at POC settings or for on-site testing. Biosensor is defined as an analytical device that uses biological components to detect a chemical/bio-chemical reaction. The biosensors typically contain three major parts that include a bio-receptor, a transducer, and an electronic read-out. In principle, the bio-receptor specifically senses and interacts with a targeted analyte resulting in a biochemical reaction that produces a signal. The transducer then reports the signal, proportional to the analyte concentration in the sample, to the electronic read-out that in turn include a signal amplifier, processor, and a display (Bhalla et al. 2016). The biosensors used for pathogen detection can either detect the whole pathogen or detect a pathogen-specific biomolecule such as DNA, RNA, membrane proteins, enzymes, or toxins.

POC devices have emerged and become popular in the last 30 years due to the exploratory growth in the nano and biotechnology sector. Furthermore, the current COVID-19 pandemic has witnessed the tremendous growths and developments of multiple POC devices aiming for rapid detection and screening of the virus (Rezaei et al. 2020). In 2003, World Health Organization (WHO) set the "ASSURED" criteria for the POC (Peeling and Mabey 2010): *A*ffordable, *S*ensitive, *S*pecific, *U*ser-friendly, Rapid, *R*obust, *E*quipment-free, *D*elivered to those who need it. These criteria suit perfect for an ideal POC device to be used in the resource-limited settings. Advancements in the technological science and the material chemistry are the two major forces that drive the microfluidics and the lab-on-a-chip (LOC) fields to the recent advances. In the technological aspect, the intensive development of micro, nano-fabrication technologies and fast prototyping such as 3-D printing, micromachining, laser cutting, milling, and injection molding have

offered the ground tools for the LOC and microfluidics to grow in an unexpected and exciting direction. Especially for industrial mass production, the disposal microfluidic chips (one-time use) made by injection-molding thermoplastic polymers such as cyclic olefin copolymer (COC), cyclo-olefin polymer (COP), polystyrene (PS), etc., are very attractive. Besides, the roles of open-source hardware and software are remarkable in realizing the LOC technology from a lab prototype to a commercialized system. As for the material aspects, the appearance of new materials, for instance, graphene, carbon nanotubes, new type of polymers, and copolymers such as COC, has given rise to sophisticated applications of microfluidics. Typical materials used to fabricate microfluidic chips are glass, silicon, polydimethylsiloxane (PDMS), poly methyl methacrylate (PMMA), and polycarbonate (PC). It is essential to remark that the selection of materials to fabricate a microfluidic chip depends mainly on the requirement of the measurements. For instance, fused silica and cyclic olefin copolymer (COC) are transparent to UV light and hence are suitable for measurements involving laser fluorescence excitation (Izadi et al. 2017). In addition, it is also necessary to draw an attention toward paper-based analytical devices. The basic idea behind paper-based microfluidic analytical devices is to use the micro and nanofluidic channels in the porous cellulose material in its natural form. However, due to the nature of the material (cellulose), there are a lack of mechanical and optical properties such as hardness, transparency, elasticity, and durability when compared to synthetic materials, e.g. glass, silicon, and polymers. On the other hand, paper-based devices have very high hydrophilicity and porosity that are of great advantage for biochemical reactions. These properties facilitate transport of liquid via capillary force, thus external driving forces such as pumping or electrical fields are not required. Attempts have been made to integrate microfluidic systems with advanced analytical techniques such as mass spectrometry (MS), LAMP, polymerase chain reaction (PCR), high-throughput next-generation sequencing (NGS), fluorescence spectrometry, and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (Zhang et al. 2018). However, the major hurdle in these advanced technologies is to have an efficient sample preparation technique integrated with a POC device. It is important to note that biological samples are highly complex, heterogeneous, and most often pathogens are distributed unevenly and at low abundance in these samples. These complexities have become a bottleneck for the development of such POC devices for the rapid and ultra-sensitive detection of pathogens in the actual samples. Therefore, POC devices demand an efficient sample preparation technique that can concentrate the pathogens and simultaneously eliminate the undesired matrix components from the testing samples.

4.2 Sample Concentration: Requirements and Challenges

Sample preparation and the concentration of target are important steps for the accurate identification of pathogens directly from biological samples (blood, food, feed, and feces) in the POC systems. The complexity and heterogeneity of biological matrices and the low abundance of target pathogens (<5–10 CFU/25 g of food/feed/feces and 1 CFU/mL of blood) demand pre-enrichment of pathogens followed by multiple steps for sample preparation. Blood culturing, considered as gold standard technique, has low success rate (30–40%). Specifically for POC applications, the basic intention of the sample preparation is to concentrate the target pathogens and simultaneously eliminate the interfering biological matrices that may obstruct the analytical/bioanalytical performances. The possible advantage of immobilizing a bio-recognition ligand such as an antibody or an aptamer on magnetic beads for immunomagnetic separation has been studied by several researchers (Table 4.1). Magnetic bead-based sample preparation increases the chance of pathogen bio-recognition due to the bead's high surface-to-volume ratio. The strategy is also compatible with various matrix categories (blood, saliva, urine, food, feed, and fecal samples); thus, the techniques are ideally suited for integrating into POC applications. In a previous report, versatility and efficiency of immunomagnetic concentration strategy was demonstrated with *Salmonella enterica* as a model pathogen spiked into different food samples and also in the blood (Vinayaka et al. 2019, 2020). Even in the presence of background microflora, the magnetic bead-based strategy showed a capturing efficiency of >95% for *Salmonella* Typhimurium in food samples and ~80% in the blood. An ideal sample concentration principle should be compatible with the aimed analytical methodology. In this direction, biochemical principles based on the specific interactions between cell surface antigenic biomarkers and the recognition ligands provide higher efficiency and better specificity (Kant et al. 2018).

4.2.1 Aptamers as Next-Generation Affinity Probes

Aptamers have shown their potential as reliable affinity probes in the diagnostic research (Tombelli et al. 2007). Aptamers are short (between 40 and 80 nucleotides) single-stranded oligonucleotides (RNA or DNA) that are capable of binding with high affinity and specificity to various molecules such as proteins, carbohydrates, pesticides, toxicants, allergens, inorganic molecules, small chemical compounds, and even to entire cells (Tombelli et al. 2007). The affinity binding of an aptamer with its target is by adapting a unique three-dimensional structure that fits as a lock and key with the target. Some of the unique properties of aptamers include easier and cheaper production at large scale, flexibility in the structural modification, long-term stability, retention of functionality even after repeated denaturation-renaturation cycles, and easy handling. This makes aptamers an ideal candidate for purification

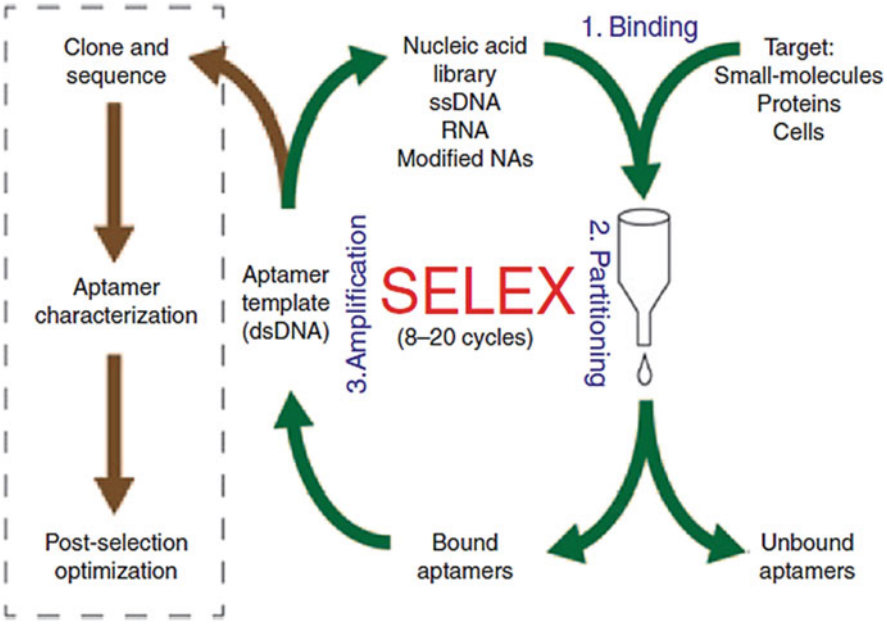

Fig. 4.1 An overview of aptamer selection protocol showing all the four steps of a SELEX technique (Ozer et al. 2014)

of target molecules from complex mixtures, biosensor design, and POC applications. Aptamers are generated or selected in particular from the oligonucleotide pool often called as a "combinatorial library" that contains 10^{14}–10^{15} different oligonucleotides with random sequences (Tombelli et al. 2007). The method of an aptamer selection is an iterative in vitro process called SELEX (systematic evolution of ligands by exponential enrichment). The basic principle of SELEX process is (1) binding (interaction of the target with the oligonucleotide pool under controlled conditions), (2) partitioning (separation of bound sequences from the oligonucleotide pool), and (3) amplification (enrichment of the bound sequences). These entire sequences of steps are repeated in several cycles using enriched aptamer sequences from the previous cycle as a new oligonucleotide pool in the subsequent cycle (Fig. 4.1). The final selection of the aptamer with desired affinity and specificity is decided either by cloning and sequencing or via high-throughput sequencing methods. Identified sequences are further subjected to the scrutiny of downstream tests such as binding affinity, specificity, structural modification, functional inhibition, and stability (Ozer et al. 2014). In recent years, there has been enormous improvement and technological developments in the SELEX process that are both simple, straightforward, and specialized. Ozer et al. (2014) have reviewed some of these most advanced SELEX strategies such as nitrocellulose membrane-based, bead-based, electrophoretic concept, microfluidics, microarrays-based, next-generation sequencing, whole cell SELEX, in vivo SELEX, and small molecule SELEX. In that review,

the pros and cons of each SELEX method were discussed in detail with an emphasis on the quality of the aptamer generated and the technological adaptability (Ozer et al. 2014).

4.2.2 The Role of Aptamers in POC Biosensors

In a biosensor, the biological recognition element is considered the most important component since it will directly influence and determine the sensor performance as well as application prospects of biosensors. Considering molecular recognition as the fundamental role of a biosensor, the primary focus was on antibodies as the bio-recognition elements for several decades. Because of the constraints in antibody generation (an in vivo process), concerns over the batch-to-batch reproducibility and requirement of controlled temperature for storage/stability, biosensor technology started to lean toward aptamers as next-generation biological recognition elements. Incorporation of a biomolecule into a biosensor platform requires chemical modification for the surface immobilization/functionalization, operational flexibility at elevated temperature, and reproducible accuracy in the biological function (Song et al. 2012). In this view, aptamers are the preferred choice as they are ideally allied with the basic requirement of a biosensor and its intended applications at POC sites. Aptamers can be chemically synthesized in different batches with same properties and functionality, they are chemically stable at elevated temperatures, offer more flexibility for chemical modifications than antibodies, and are reusable that allow some of the biosensor platforms to be reused. Aptamers have therefore been integrated and effectively tested in several biosensor applications for the analysis of high-priority targets that include antibiotics (tetracyclines, aminoglycosides), microbial toxins (ochratoxin A, staphylococcal enterotoxins, fumonisin B1, cholera toxin, shiga toxin), pesticides (atrazine, malachite green), pathogens (*Bacillus thuringiensis*, *Bacillus anthracis*, *Campylobacter jejuni*, *Escherichia coli* DH5α strain, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus*, Human Influenza A virus (H3N2), and Rous Sarcoma virus) (McKeague et al. 2011; Torres-Chavolla and Alocilja 2009).

The success of SELEX in generating efficient aptamers depends on various factors such as the combinatorial library, purity of the target pathogen, microbial growth environment, ideal binding, washing, and elution conditions, SELEX monitoring assays, and methods adapted for characterizing selected aptamers (Ozer et al. 2014). However, even with multiple attempts, the technology has not yet become as commercially successful and efficient as initially expected. Unreliable affinity of aptamers in complex biological samples has remained a challenge. One obvious reason is that aptamers require stringent reaction conditions to become functional. The binding strength and specificity of aptamers varies from sample to sample, even among the same type of samples and even for the same target and this has been a challenge in capturing whole cells. These key issues have hindered the widespread application of aptamers in POC biosensors and in multiplexed analysis for pathogen detection. Nevertheless, the unique features of aptamers make them suitable for

developing efficient sample concentration strategies and further integration into POC biosensors based on some of the most advanced molecular detection strategies.

4.3 Advanced Molecular Detection Strategies

The advancement of molecular technology in offering powerful diagnostic tools is unquestionable. Direct PCR (amplification of a target DNA directly from an animal or plant tissue sample without DNA isolation and purification), droplet digital PCR (a method of performing DNA amplification on water-oil emulsion droplets), SP-PCR (amplification of a DNA on a solid support using immobilized primers), and isothermal amplification (nucleic acid amplification at a constant temperature without thermal cycling steps) are some of the advanced molecular techniques. Among these, SP-PCR and LAMP have shown potential for applications in POC biosensors.

4.3.1 Solid-Phase PCR: Principle and Approach

Solid-phase PCR (SP-PCR) is a unique nucleic acid amplification technique in which one or both primers are attached to a solid surface such as silica or polystyrene via the 5′-end while other PCR components are in the liquid phase. The cyclic thermal condition is similar to the regular PCR reaction. In the presence of the DNA target, the immobilized primer binds to the template and amplifies the target region resulting in tethered dense DNA amplicons on the solid support (Fig. 4.2a). This strategy can limit the primers and the amplification product to a well-defined two-dimensional area that facilitates multiplexing PCR assays. This strategy has the potential to overcome current challenges in multiplexed PCR assays, such as primer dimers due to the presence of various primers in high concentration and preferential amplification of one target sequence over another. Conventional nucleic acid amplification requires post-amplification confirmation of amplified products via gel electrophoresis, melting curve analysis, microarray hybridization, or capillary electrophoresis. In contrast, SP-PCR minimizes the interferences between multiplex assays due to spatial separation of the primers and surface-bound amplification. SP-PCR allows parallel DNA amplification on the surface of a solid substrate (Fig. 4.2a). Microbeads (Palanisamy et al. 2010), microtiter plates (Niemeyer et al. 2007), and flat surfaces (Von Nickisch-Rosenegk et al. 2008) are some of the examples used in this technique. With the possibility of immobilization of hundreds of discrete primers on a miniaturized surface, SP-PCR has become an interesting platform for highly multiplexed amplification of multiple target sequences in a single assay.

SP-PCR was initially described in 1994 by Kohsaka and Carson (1994). Later, SP-PCR was further developed and tested for the detection of pathogens by several

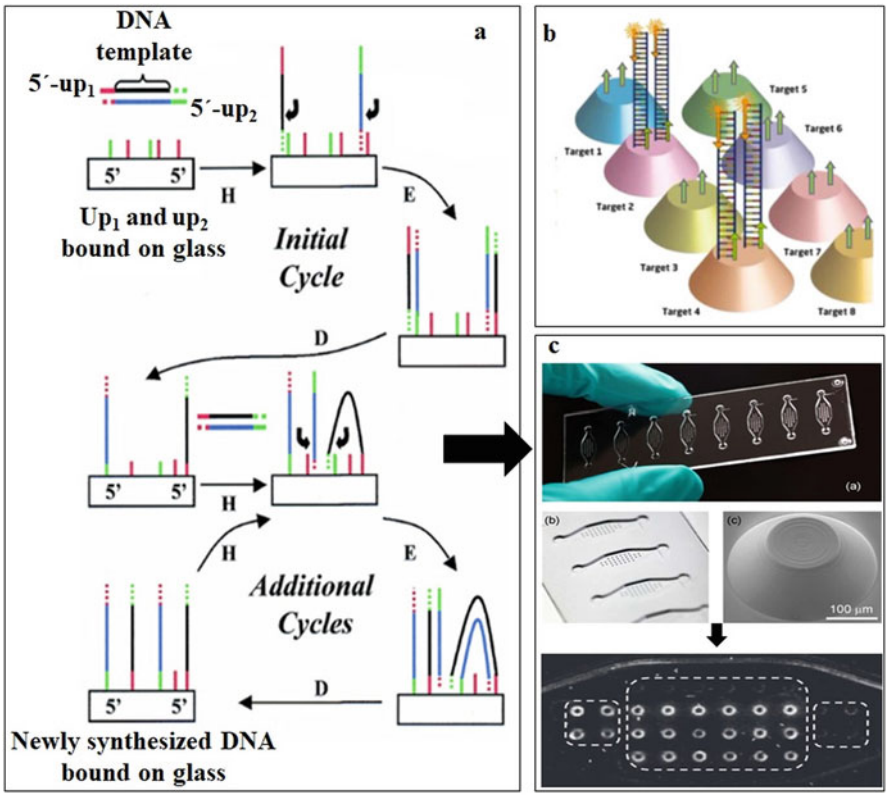

Fig. 4.2 Amplification of DNA by SP-PCR. (**a**) DNA primers (up1 or up2) are covalently attached to the solid support through their 5′-end. Thermo-cycling (*H* hybridization, *E* elongation, *D* denaturation) in the presence of a DNA template, nucleotides, and a thermostable polymerase leads to the amplification on the solid surface. Initial cycle results only in the interfacial amplification as the DNA template is present only in the reaction solution. During the subsequent cycles, in addition to interfacial amplification, surface amplification also takes place with the attached primers in the vicinity. (**b**) Schematic view of a SAF micro-optic structure showing total internal reflection of an incident light toward higher refractive index medium at an angle. (**c**) SAF-embedded polymer micro-chip showing emitted fluorescence on a SAF array. (Reprinted with permission from Adessi et al. 2000; Hung et al. 2015)

researchers (Adessi et al. 2000; Chin et al. 2017; Hung et al. 2017; Vinayaka et al. 2020). However, its widespread application for POC diagnostics is limited due to low amplification efficiency (Khan et al. 2008). Attempts were made by researchers to improve the amplification efficiency by optimizing several parameters that include the length of solid-phase primers, the density of primers as well as the annealing position of the solid-phase primers (Chin et al. 2017). Recently, SP-PCR was reported in combination with magnetic-bead-based pathogens concentration strategy in order to enhance the detection sensitivity and feasibility for POC applications. This combined strategy was reported with *Salmonella enterica* serovar

Typhimurium and serovar Enteridiidis in blood sample with a limit of detection at ~90 CFU/mL (Vinayaka et al. 2020). SP-PCR has the potential as an alternative technique over conventional PCR in multiplex detection. However, lower amplification efficiency, difficulties in differentiating signals from surface-bound amplicons, and unamplified primers in the liquid phase still remain a challenge for this technique.

Biosensors, in general, uses optical, piezoelectric, or electrochemical transducers. In the optical biosensors, fluorescence has been the most used principle of detection. In principle, radiation of light from fluorescent molecules (fluorescence emission) differs substantially between air, water, solid surface, and near the interface of any two mediums. Fluorophores radiate asymmetrically near the interface of two media with different refractive indices and the light radiates more into the higher refractive index medium. Therefore, integration of the SP-PCR into an optical biosensor requires modifications of the solid support. An interesting and advanced technology reported in recent times was supercritical angle fluorescence (SAF, Fig. 4.2b) microscopy that works on the principle of a unique phenomenon called total internal reflection of light (Hung et al. 2015). The SAF technology takes advantage of the principle of asymmetric distribution of fluorescence near the interface. Miniaturization of such SAF structures in an array format is more suited for SP-PCR with increased multiplexed detection capability. Increased fluorescence collection efficiency and superior multiplexing capacity are the two major advantages of SAF micro-lens array technology. This increased fluorescence collection efficiency because of the principle of collimation of the emitted fluorescent light at supercritical angle results in improved assay sensitivity. It was reported that ~1 to 13 fluorophores (per μm^2) can be detected with truncated cone-shaped SAF structure resulting in 36–40-folds increase in the sensitivity (Hung et al. 2015). The only bottleneck lies in the fabrication of such high-quality micro-lens SAF structures that require sophisticated know-how and clean-room fabrication facility. Multiplexed SP-PCR was demonstrated in a truncated cone-shaped SAF structure with high specificity and sensitivity (Hung et al. 2015, 2017; Vinayaka et al. 2020).

4.3.2 Isothermal Amplification: An Overview

Isothermal amplification techniques have drawn immense attention from diagnostic researchers for rapid amplification and identification of nucleic acid (Bodulev and Sakharov 2020). Unlike PCR, isothermal amplification methods are conducted at a constant temperature between 30 and 70 °C. In addition, isothermal amplification methods are reported to be highly sensitive, specific, and tolerant to PCR inhibitors. Several isothermal amplification methods have been developed and reported such as loop-mediated isothermal amplification (LAMP), recombination polymerase amplification (RPA), rolling cycle amplification (RCA), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), etc. (Craw and Balachandran 2012). These methods differ in

Table 4.2 Commercially available devices employing isothermal nucleic acid amplification technology (Craw and Balachandran 2012)

Product	Amplification method	Samples per run	Automated sample preparation	Detection method	Analysis time (min)
Twista (TwistDX, UK)	RPA	8	No	2 channel Fluorescence	10–15
Genie II (OptiGene, UK)	LAMP	16	No	1 channel Fluorescence	Variable
Illumigene Meridian Bioscience	LAMP	10	No	Fluorescence	60
OligoC-TesT CorisBio Concept Belgium	NASBA	1	No	Lateral flow	100
NucliSENS EasyQ Bio Merieux France	NASBA	8–48	Yes	Multichannel Fluorescence	120
APTIMA/ Tigris Gen-Probe USA	TMA	100	Yes	Chemiluminescent DNA probe	180–200
BESt Cassette Type II BioHelix USA	HAD	1	No	Lateral flow	90
ProbeTec Becton Dickinson & Co USA	SAD	46	Yes	Fluorescence	60

the temperature of amplification, the number of primers required, the nature of enzymes, and the template types used for amplification. Several POC platforms have been developed based on isothermal amplification methods and few of them have been commercialized (Table 4.2). Among them, LAMP has proven to be the most promising technique for integrating in POC applications due to its ease of assay design and its compatibility in the device development as a biosensor.

4.3.2.1 Loop-Mediated Isothermal Amplification (LAMP): Principle and Approach

LAMP was described for the first time in 2000 (Notomi 2000) and is currently the most studied method among available isothermal amplification methods. In LAMP, a set of four specific primers that can recognize six distinct regions on the target (Fig. 4.3a) is used. The LAMP primer set includes two inner primers (termed forward inner primer, FIP; and backward inner primer, BIP), and two outer primers (F3 and B3). Each inner primer of LAMP consists of two specific regions (F2 and F1c in FIP, B2 and B1c in BIP) in which, F1c and B1c are complementary to F1 or B1 position on the target. The purpose of using F1c and B1c in the inner primers (FIP or BIP respectively) is to generate a stem-loop structure in the amplified products that will have a self-priming capability (Notomi 2000). LAMP is also unique in terms of the use of a special DNA polymerase with a high-strand displacement activity. This enables synthesizing, displacing, and releasing single-stranded DNA during amplification. In principle, the LAMP process can be separated into two steps: a stem loop generation step (Fig. 4.3b) and the cycling step (Fig. 4.3c). During the stem-loop generation step, FIP anneals to F2c region on the target DNA (Fig. 4.3b-1) and generates amplified complementary strand (Fig. 4.3b-2).

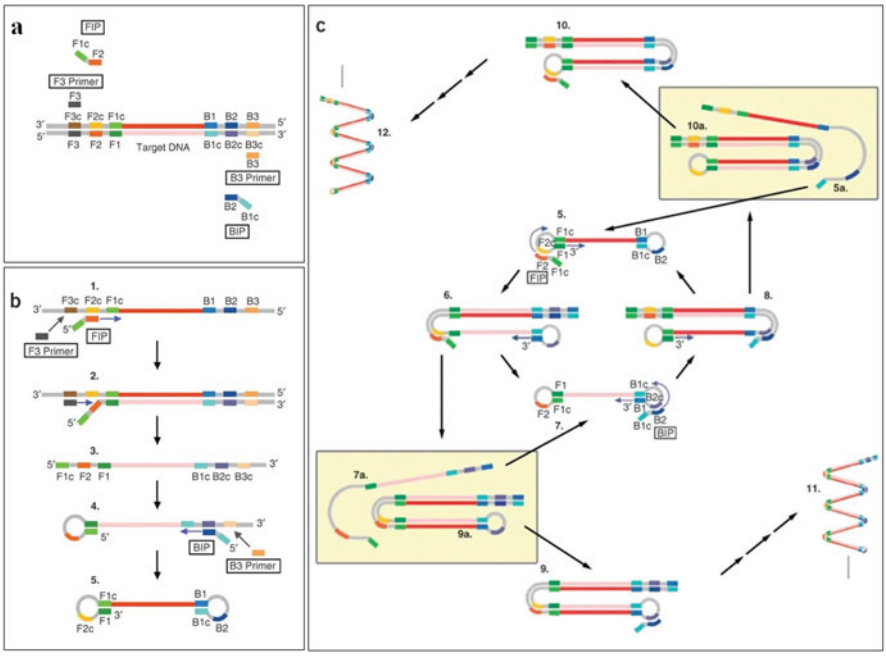

Fig. 4.3 Principle of LAMP method. (**a**) Primer design of the LAMP reaction. (**b**) Starting structure producing step resulting in stem-loop structures. (**c**) Cycling amplification step and self-primed DNA synthesis. (Adapted with permission from Tomita et al. 2008)

Annealing of the outer primer (F3) to the F3c region (Fig. 4.3b-2) on the target DNA initiates strand displacement by the DNA polymerase. This results in the release of the single-stranded FIP-linked complementary strand (Fig. 4.3b-3). This strand forms a stem-loop structure at the 5′ end because of the complementarity between F1c and F1 regions (Fig. 4.3b-4). The single-strand DNA also serves as a template for BIP and B3 primes that extends the new strand (Fig. 4.3b-4) similarly resulting in a structure with stem-loops at both ends that look like a dumbbell structure (Fig. 4.3b-5). The self-prime capability at the 3′-end of this dumbbell structure enables self-priming during cyclic amplification steps and thus are independent of primers (F3/B3 and FIP/BIP) and target DNA strand. This dumbbell structure serves as the starting material in the LAMP cycling steps (Fig. 4.3c-5). In the cycling step, the process continues resulting in the structures of various sizes and secondary conformations consisting of alternately inverted repeats of the target sequence on the same strand. Because of the absence of denaturation steps and self-priming activity at the 3′-ends, LAMP produces amplified products up to 10^9 copies of the target in short time (within an hour). In order to speed up the amplification process, two inner loop primers that include forward loop primer (LF) and backward loop primer (LB) were additionally introduced (Nagamine et al. 2002). These loop primers hybridize to ssDNA on stem-loop regions and prime strand displacement DNA synthesis. The amplified products that form stem-loop structure with a self-priming capability and a DNA polymerase with strand displacement activity are the unique concept and strength of LAMP technology.

4.3.2.2 Strand Displacement DNA Polymerase

The DNA polymerase being used in LAMP technology is unique in its functionality having a high degree of strand displacement activity alongside 5′–3′ polymerase activity. The first generation of strand displacing DNA polymerase used in LAMP was a wild-type *Bst* DNA polymerase. This enzyme is the portion of the *Bacillus stearothermophilus* DNA polymerase protein. The *Bst* DNA polymerase is robust and exhibits greater tolerance to inhibitors found in diagnostic samples compared to the *Taq* polymerase enzymes often used in PCR. However, it has been shown that *Bst* DNA polymerase large fragment does not incorporate dUTP efficiently in LAMP reaction and causes false-positive results at low temperature (25 °C). To overcome these limitations of the wild-type *Bst* DNA polymerase, an in-silico designed *Bst* 2.0 DNA polymerase was engineered and developed by New England Biolabs. The *Bst* 2.0 DNA Polymerase displays improved amplification speed, yield, salt tolerance, and thermo-stability. Further, the enzyme was improved with a WarmStart feature (*Bst* 2.0 warm-start) with a similar performance to *Bst* 2.0 DNA polymerase. This feature enables to set up a LAMP reaction at room temperature that increases robustness and reproducibility of the results. The *Bst* 2.0 WarmStart is designed with a reversibly bound aptamer that prevents polymerase activity at temperatures below 45 °C. There are several strand-displacing DNA polymerases reported that include OmniAmp polymerase (Lucigen), *Bsm* DNA Polymerase (Thermo Scientific), *Gsp*M, *Gsp*SSD DNA Polymerase (OptiGene), Tin DNA Polymerase

(OptiGene), and *Bsu* and *Bst* DNA Polymerase (New England Biolabs). Among them, the *Bst* 2.0 WarmStart has been the most widely used DNA polymerase in LAMP applications.

4.3.2.3 Significance and Challenges in Designing Fold-Back Primers

The capacity of continuous amplification at a constant temperature of LAMP is primarily based on the special strand displacing DNA polymerase and a unique primer with fold-back loop configuration. In contrast to conventional *Taq* polymerase that is widely being used in PCR technique with a strong 5′–3′ exonuclease activity, the *Bst* polymerase has no exonuclease activity. Instead, *Bst* polymerase displaces the non-template strand if it encounters a double-stranded region during DNA polymerization. A unique design of inner primers (FIP and BIP) results in an amplified strand that fold-back at its 3′-end by self-annealed. This hybridization initiates a new DNA strand with self-priming capability enabling primer and template-independent amplification. The fold-back or the so-called "dumbbell structure" also creates a position for the annealing of the loop primers, hence, acting as if a template simultaneously acting as a self-primer at its 3′-end. This unique template mimicking feature increases the possibility of the technique to detect a very low target even at a single copy of genome. This increases the assay sensitivity as well as reduces the reaction time.

4.3.2.4 Detection Principles Adapted in the LAMP Reactions

LAMP can be considered as a potential technique in future generations of POC devices. An ideal POC system is a system where all the steps from sample preparation, amplification, and detection are integrated. The sample preparation step can be minimized in LAMP assays due to the robustness and tolerance of *Bst* DNA polymerase to inhibitors in the samples. The amplification at a constant temperature opens the way for inexpensive instrumentation. Detection of LAMP amplicons is also an important step to be considered while developing POC biosensors. LAMP produces a large amount of amplified product (dsDNA) and magnesium pyrophosphate as a by-product of the reaction (Mori et al. 2004). Both of them have been used to monitor the LAMP reaction either in the real-time amplification or at the end-point amplification by different approaches such as turbidity, end-point color change, or with fluorescence.

Turbidity detection is an early method for monitoring LAMP reactions. Magnesium pyrophosphate ($Mg_2P_2O_7$) formed as a by-product in LAMP reactions is monitored as a measure of turbidity in this method. Magnesium pyrophosphates are formed as a result of reaction between the pyrophosphate ions released in the DNA polymerization with magnesium ion in the reaction buffer yielding a white precipitate (Mori et al. 2004). Turbidity detection does not require any special probes or indicators. However, the method poses some limitations. The method is less

sensitive, and $MgSO_4$ may reduce the amplification efficiency. At low target concentration, it is difficult to distinguish specific and non-specific amplifications.

In LAMP reactions, metal indicators, DNA intercalating dyes, and gold nanoparticles were tested as end-point detectors. The principle of detection is based on the change in color of the reaction mixture if it is positive, from dark yellow to yellow (calcein) (Tomita et al. 2008), purple to blue (hydroxynaphthol blue) (Goto et al. 2009), transparence to light blue (malachite green) (Lucchi et al. 2016), orange to green (SYBR Green I) (Pan et al. 2015), PicoGreen (Tomlinson et al. 2007), or orange to light pink (Propidium Iodide) (Hill et al. 2008). Besides this, metal indicators and DNA dyes, pH-sensitive dyes have also been used and reported. In positive LAMP reactions, high-sensitive pH indicators change color in low concentration buffer from red to yellow (phenol red or cresol red), or from light yellow to red with neutral red pH-sensitive indicators, or from purple to yellow (cresol purple) (Tanner et al. 2015).

Similar to PCR, a simple way to monitor the LAMP reaction is by using a DNA intercalating dye. In negative reactions, the DNA dye is in unbound state and does not fluoresce. While in positive reactions, the dye intercalates to amplified double-stranded DNA and fluoresces strongly. The use of DNA dyes can enhance the sensitivity of the LAMP assay as compared to the turbidity measurement (Cao et al. 2015). However, it is important to note that some of the DNA intercalating dyes may inhibit amplification efficiency. For example, SYBR Green I, a very common standard DNA intercalating dye used in the PCR reactions, has a strong inhibition effect in LAMP reaction. Researchers have investigated several DNA dyes such as SYTO group, SYBR Green I, EveGeen, Miami group, etc., with different optical properties (Quyen et al. 2019). These investigations revealed the suitability and compatibility of DNA intercalating dyes for the LAMP reaction based on the amplification efficiency, inhibitory effects, and their quantum yield of fluorescence efficiency.

Unlike sequence-independent detection methods, fluorescence detection using sequence-specific probes have also been reported to detect specific amplified sequence of the targets (Becherer et al. 2020). Most currently reported strategies include self-dequenching loop primers (FLOS-LAMP) (Gadkar et al. 2018), HyBeacon probes (Howard et al. 2015), Guanin quenching (Takayama et al. 2019), universal quenching probe (QProbe) (Ayukawa et al. 2017), alternately binding quenching probe competitive LAMP (ABC-LAMP) (Tani et al. 2007), graphene oxide based FRET (Go-based FRET) (Waiwijit et al. 2015), multiple label techniques (detection of amplification by release of quenching (DARQ)) (Nanayakkara and White 2019), quenching of unincorporated amplification signal reporters (QUASR) (Ball et al. 2016), one-step strand displacement (LAMP-OSD) (Bhadra et al. 2015), molecular beacon-LAMP (Bakthavathsalam et al. 2018), light cycler-LAMP (Chou et al. 2011), mediator displacement LAMP (MD-LAMP) (Becherer et al. 2018), *Tth* endonuclease cleavage LAMP (TEC-LAMP) (Higgins et al. 2018), and multiple endonuclease restriction real-time LAMP (MERT-LAMP) (Wang et al. 2015).

4.3.2.5 Feasibility of LAMP for Multiplexed POC Diagnostics

Multiplexing has become a fundamental and most desired part of POC diagnostics and on-site testing. Multiplexed analysis based on nucleic acid amplification (DNA/RNA-based) is the most reliable technique in the analysis of food, environment, and clinical samples. The internal control assay is another test that is desired to be combined with the mainstream test reaction to validate the sampling and sample preparation processes. Several attempts have been made, in this direction, to enhance the capability of LAMP for multiplexed assay. TaqMan method provides multiple target gene detection in a single real-time PCR reaction. Similarly, fluorophore-quencher pair concept has been employed to monitor the LAMP reaction with a multiplex feature (Ball et al. 2016). Unlike TaqMan probe concept wherein both fluorophore and quencher are at the same oligonucleotide, the fluorophore and the quenchers in the LAMP reaction are located on different but complementary oligonucleotide strands. The 5′-end of the FIP primer is labeled with a fluorophore and a short oligonucleotide complementary to the 5′-end of the FIP was labeled with the quencher at its 3′-end. The vicinity of fluorophore and the quencher under hybridized FIP state results in the absence of any fluorescent signals in the beginning of the reaction or in negative reactions. In the presence of a target gene, strand displacement amplification mechanism removes the hybridized fluorophore-quencher pair either from the amplified product or from the intermediate loop region during the amplification. Further, in the newly synthesized oligonucleotide strand, the fold-back mechanism also releases the small probe that contains the quencher (Fig. 4.4).

Ideally, POC systems are desired to include all the steps necessary for sample preparation, amplification, and detection to make the test accessible and manageable by unskilled personnel. A centrifugal microfluidic device capable of detecting multiple foodborne pathogens (*Salmonella* Typhimurium, *Escherichia coli* O157, and *Vibrio parahaemolyticus*) was reported with LAMP and colorimetric detection strategy (Oh et al. 2016). The microfluidic chip consists of five identical structures and two kinds of patterned dispensing microchannels with zigzag shape to aliquot the LAMP components. The chip was capable of analyzing 25 samples simultaneously with a detection limit of 380 genomic copies of *Escherichia coli* O157:H7 within 60 min. Another centrifugal microfluidic platform integrated with sample preparation to detection was also reported for detecting *Salmonella*. There were 16 amplification chambers that could analyze four samples simultaneously (Ahmed et al. 2016). This device could detect DNA as low as 5×10^{-3} ng/μL. Multiplexed LAMP on a chip filled with dehydrated primers was demonstrated with 15 interconnected reaction wells (Tourlousse et al. 2012) and 4 arrays of 6×6 wells (Duarte et al. 2013). These microfluidic strategies could detect multiple foodborne pathogens that include *Salmonella*, *Campylobacter*, *Shigella*, and *Vibrio cholerae* in less than 20 min with a detection limit of 10–100 genome copies per reaction (Tourlousse et al. 2012) and *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella* spp. with a detection limit of 105 CFU/mL (Duarte et al. 2013). However, in both systems, sample preparation and nucleic acid purification

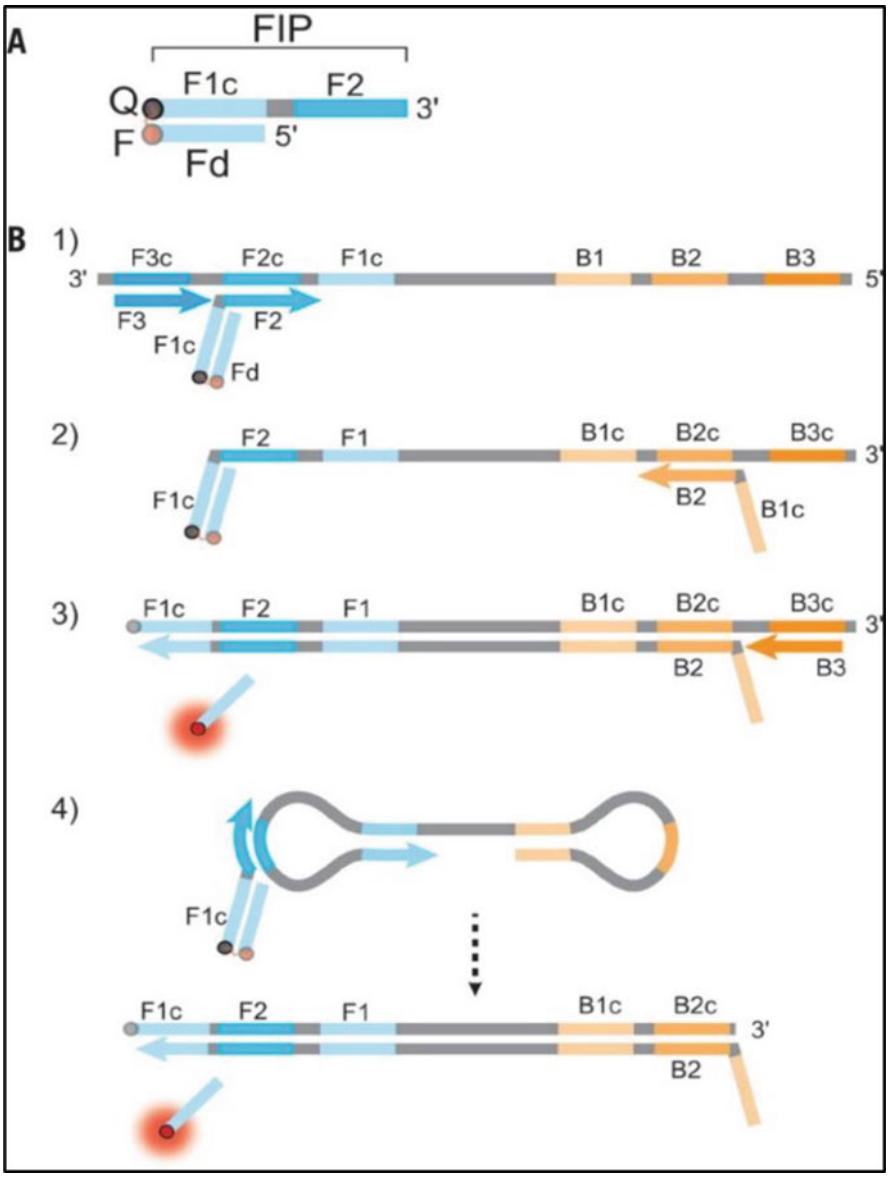

Fig. 4.4 Employing fluorescent and quencher molecules to perform multiplex and real-time LAMP assay (Tanner et al. 2012)

processes were performed off-chip prior to on-chip LAMP amplification. To date, several POC systems based on LAMP principle are commercially available (Table 4.2).

4.3.2.6 Merits and Limitations of LAMP in POC Diagnostics

LAMP exhibits several advantages that are ideal for its integration into the POC devices. At first, the amplification that occurs at a constant temperature between 60 and 65 °C eliminates the requirement of thermal cycling. Second, the dumbbell structure containing ssDNA at stem loops and self-priming at 3′-end provides the desired rapidity in the POC diagnostics. Third, LAMP reaction produces amplicons at an enormous amount that opens the way for adaptation of various detection strategies and facilitates simplified instrumentations. Fourth, the use of 4–6 primers that recognizes 6–8 distinct regions on the target DNA, makes the LAMP highly specific. Moreover, LAMP has been shown to be highly sensitive with a limit of detection (LOD) of a few copies per reaction (Zhang et al. 2014). Finally, the use of polymerase with high-strand displacement activity and the formation of stem-loops containing self-priming capability provides LAMP a higher tolerance to PCR inhibitors. LAMP is more resistant to inhibitors from most of the biological matrices such as food, feed, meat, feces, and body fluids such as saliva, sweat, serum, and whole blood. LAMP assay remained robust in the presence of 5% whole blood while normal *Taq* polymerase was totally inhibited at 5% whole blood (Francois et al. 2011). In the emergency conditions such as an outbreak or a pandemic LAMP can be a powerful alternative for PCR as the elimination of laborious and complex sample preparation steps elevate the testing capacity (Dao Thi et al. 2020). However, LAMP also suffers with high rates of false-positive results. The multiplexing capability of LAMP remains a matter of concern due to the requirement of multiple primers at considerably higher concentrations.

4.4 Challenges in the Development of POC Biosensors and Suggested Solutions

The development and implementation of POC biosensor has several challenges of which, sample preparation, enabling multiplexing capability, and compatibility of the developed assay with the mechanical and engineering section of the device are considered the most important. In most cases, POC biosensors have underperformed due to the lack of desired sensitivity and inhibition of the assays as a result of undesired matrix components. Magnetic bead-based pathogen concentration is an ideal approach for POC applications as the technique is robust, versatile, easily adaptable to other downstream protocols, and field applicable even at resource-poor laboratory settings. The most important advantage of the magnetic bead-based technique is its ability to handle larger sample volumes and removal of inhibitors, simultaneous with concentrating the pathogens. The combination of magnetic bead-based sample concentration with the biosensor can significantly elevate the assay performance and prepare the biosensor for POC applications with a sensitivity comparable to an advanced analytical/bio-analytical techniques.

Solid-phase amplifications (SP-PCR and SP-LAMP) are projected as new-generation molecular techniques ideal for their integration into POC biosensors. Unfortunately, the techniques also have some drawbacks. For example in SP-PCR, controlling the efficiency and undesired non-specific amplifications are the major challenges. Apart from these, SP-PCR is prone to three major factors such as masking effect, molecular crowding, and neighboring DNA interactions. In order to improve the efficiency of the SP-PCR, the length, density, and annealing position of the solid-phase primer should be carefully optimized. Density of the immobilized primer on the surface will have a significant effect on the amplification efficiency of the SP-PCR. In principle, solid-phase amplification relies on the primary amplified products of the liquid-phase amplification. These primary amplified products diffuse into the solid surface and interact as template with immobilized primers during solid-phase amplification. Therefore, under symmetric condition, liquid-phase primers dominate the amplification resulting in poor efficiency of the solid-phase amplification. In this case, the primers concentration and the density of reaction solution itself may become a limiting factor. One possible solution could be to reduce the concentration of the liquid-phase primer to create an asymmetric reaction environment. This approach may reduce the competition between the liquid-phase and the solid-phase primers during the annealing steps and enable better diffusion of the primary amplified products. Another possible approach could be to differ the annealing temperature between the liquid-phase and the solid-phase primers. Having the annealing temperature of the solid-phase primers few degree higher than that of the liquid-phase primers provides flexibility in controlling the kinetics of the liquid-phase amplification. These possible solutions may also eliminate the masking effect that usually arises due to the diffusion of the amplified products into the solid surface and could mask the adjacent solid-phase primers during annealing. Similarly, diffusion of the amplified products at higher concentration may also create molecular crowding effect resulting in the repulsion of each other during annealing to solid-phase primers. The repulsion is mainly due to the steric effect from neighboring solid-phase primers and the charge repulsion created by negatively charged DNA at higher densities. Under such circumstances, the free-floating amplified products that act as template in the solid-phase amplification tends to move away from the surface. High-density of the immobilized solid-phase primers may also create a neighboring DNA interactions effect wherein the DNA template may likely hybridize to an adjacent amplicon in preference to the primer resulting in preventing the primer extension. One way to overcome these effects is to increase the length of the solid-phase primer that may simultaneously reduce the masking effect and facilitate better hybridization of the primer to the target.

One of the major challenges in LAMP is the complexity in designing multiple primers. The outer primers (F3 and B3) are similar to regular PCR primers in length (18–20 bases) having a melting temperature near 60 °C. The other two inner primers (FIP and BIP) are longer (normally 40–45 bases) and each consist of two individually designed oligonucleotides (F1c and B1c) complementary to F1 and B1 locations on the target gene. LAMP requires at least these four primers. In addition, another pair of primer called “loop primers” (LF and LB) are generally used in order

to improve the assay sensitivity and to increase the speed of the amplification process. Therefore, a proper LAMP assay containing six primers will have a concentration of nucleotide around 5.2 μM that is much higher than that of the concentration of primers in regular PCR (0.8 μM). The challenge in designing such primers to be used at high concentration in a single reaction requires consideration of several factors that include the GC rate in the sequences of primers, the stability of 3′ or 5′ end, the length and melting temperature, as well as overcoming self-complementarity among primers and precise nucleotide length between F1 to F2 and B1 to B2 in inner primers (FIP and BIP). The later condition is considered as the most important factor for achieving self-priming and to generate a good "loop dumbbell structure" that dictates the efficiency and speed of a LAMP reaction. Although dedicated LAMP primer designing software such as PrimerExplorer (EikenChemical) facilitates primer designing, the challenge in overcoming false-positive reactions due to primer dimer is a bottleneck. Possible undesired interactions between these primers with no-specific targets in the real sample analysis and among primers of different targets might be an obstacle and a great challenge in the multiplexed LAMP analysis. This requires additional computational evaluation of designed primers as well as full proofed through practical experiments in a real-time assay. In addition, balancing between the sensitivity and reducing the reaction time to overcome the false-positive rate has been a major obstacle for LAMP in a way of becoming a popular molecular detection method.

LAMP produces amplicons with complicated structure that lack a definite molecular size, thus, it is difficult to identify the target-specific amplicons in a pool of products in the multiplexed assays. The best approach to address this problem is to integrate an array technology with LAMP-based POC biosensors. SAF micro-optic array discussed before in combination with SP-PCR is an example. Conventional multiplexed assays either use separated reaction chambers or multiple detector probes such as multiple fluorophores. Array format could eliminate the need for such multiple detector probes, thus, eliminating the necessity of having multiple optical filters and separated reaction chambers. Spatial separation of the reaction center can also overcome stearic hindrance between the reactants and improves the assay efficiency.

One of the pre-requisite for the design and development of a POC biosensor for pathogen detection is to integrate multidisciplinary competencies such as biomedical, bio-analytical, and engineering disciplines. There are several challenges in designing such biosensors to be potentially utilized at low infrastructure laboratories with limited resources and expecting the performance of a POC biosensor similar to an advanced detection technology. Therefore, it is very essential to align the biological assays with the device development because the complexities in the assay may have direct influence on the device engineering. Incorporation of a conventional PCR into POC biosensors poses engineering challenges. Requirement of the precise and repeated thermal cycles necessitates a complex, power-consuming, and bulky design, thus, making PCR an imperfect solution even at the micro-scale reaction level. LAMP is a better alternative to PCR with similar sensitivity and less complexities in its requirements. The interconnection and

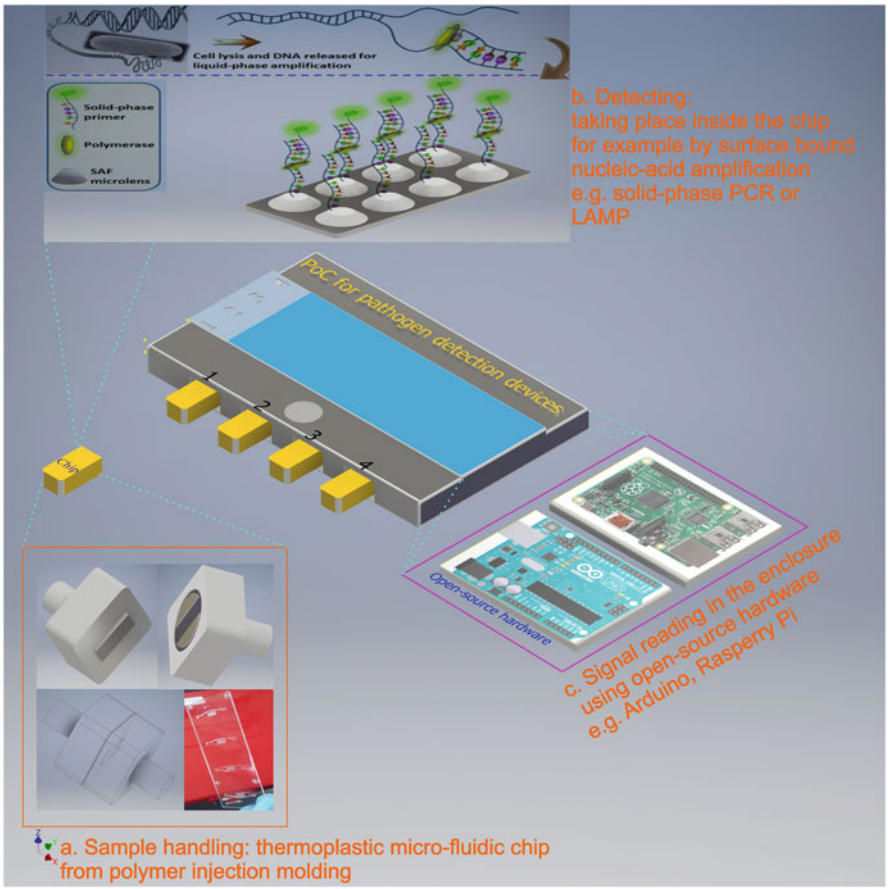

Fig. 4.5 The three essential components of POC devices for (multiplex) pathogen detections and the proposed solutions, include (1) the use of polymer disposal microfluidic chip, (2) surface-bound (or solid-phase) nucleic acid amplification, (3) using open source hard- (and soft-) wares for electrical readout and control. (Adapted with permission from Nguyen et al. 2020)

interdependency between the sample handling, detection, and signal reading were reviewed and discussed in detail by Nguyen et al. (2020). It was suggested that the major challenges in designing a POC biosensor can be solved with the following three components: (a) the use of disposal polymer microfluidic chips for sample handling, (b) applying surface-bound nucleic acid amplification for detecting targets, and (c) employing open-source hardware and software performing the test, e.g., signal reading, prompt the user, provide test results to the user, etc. (Fig. 4.5). Designing mechanics and electronics (the two major parts of a biosensor) is also difficult, challenging, and pose risk. Here, the advantage of using open-source solutions is that such designs can be adapted from an existing solution. Some of the obvious choices of open-source microcontroller boards are Arduino products,

Raspberry Pi, Teensy, Beaglebone, and Nucleo. These solutions generally run based on a Real-Time Operating System (RTOS, https://www.arduino.cc/en/main/software) or based on a General-Purpose Operating System (GPOS, https://www.raspberrypi.org/documentation/usage/python/) programs. Thus, during the initial stage of development of a lab prototype and proof-of-the concept units, open-source resources are of great help and are recommended for beginners.

4.5 Conclusion and Outlook

This book chapter provides basic guidelines for adapting new-generation molecular techniques in POC biosensors aiming at their implementation in different fields, such as multiplexed diagnostics, high-throughput screening, and food safety. POC biosensors have tremendous opportunity and are suggested to improve the healthcare facility at resource-poor and low infrastructure laboratories. For the successful development and implementation of a biosensor with an efficient sample concentration, next-generation molecular techniques that are simple, sensitive, and the advantage of open-source resources in the POC device development can be considered as primary steps. A focused research is paramount to integrate the engineering and biochemical technologies in a complex multidisciplinary area. Among the available advanced diagnostic strategies, LAMP is considered a highly promising technique to integrate into POC because of its higher sensitivity and compatibility in the device development strategy. LAMP and aptamer technology have initially shown compatibility and flexibility for the development of POC devices. It is also possible to adapt LAMP-based POC devices to microfluidic systems and connect to a smartphone-assisted remote communication technologies in the near future. In combination with an array technology such as SAF arrays, current POC devices can be enabled with robust multiplexing capability. It is worth mentioning that access to safe food, safe health, and quality of life are not far away even at a resource-poor section of the globe if efficient POC devices are properly implemented for infection surveillance and control. However, this also requires more efforts to transform the laboratory prototypes to an industrial mass production level. Thus, the next-generation POC biosensors could be simple, compact, and at the same time sensitive enough to that of the current advanced diagnostic tools.

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Chapter 5
Point-of-Care Biosensors for Glucose Sensing

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5.1 Introduction

The metabolic functions of cells provide the primary source of energy that supports all the needs for the sustenance of life. Cells utilize glucose as an essential source for producing energy apart from proteins, fats, vitamins, and other biomolecules obtained from the diet. The most prominent carbohydrate fuel is circulating glucose, emerging majorly from the diet in its complex polysaccharide form, which is then digested to monosaccharide glucose. It also serves as a building block for forming glycoproteins and glycolipids which are large structural molecules playing essential functions in the brain, muscles, and several other organs. Glucose in the blood at optimal levels goes unnoticed, whereas it shows devastating, permanent, and profound effects in sub-optimal or higher levels. Besides being the chief metabolic energy source, it is also an essential signaling entity to control neuronal and hormonal secretory activity. Glucose equilibrium or homeostasis is maintained by hormonal, nutrient-related, or neuronal cues (Steinbusch et al. 2015). The metabolic controls, when deregulated, cause several medical complications like diabetes mellitus, celiac disease, cystic fibrosis, nephropathy, renal failure, heart disease, or even cancer (Bruen et al. 2017). Preventive measures of such conditions are yet not known, thus tracking glucose level in blood as a disease marker is currently the most

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successful means to control and extend life expectancy by managing episodes of hyperglycemia and hypoglycemia (Steiner et al. 2013).

As per estimates, approx. 40% of blood testing involves the measurement of glucose (McMillin 1990). Several conventional tests are used to test glucose levels like the fasting plasma glucose test which can be done in the morning after overnight fasting that can help determine how much the body manages blood sugar levels in the absence of meals like when we go several hours without eating, the body will release glucose into the blood through liver, and indeed the body's insulin should assist to normalize blood sugar levels. Another glycated hemoglobin test also known as HbA1C provides us with an average blood glucose level over the past 2–3 months. Oral glucose tolerance test in which overnight fasting glucose level is measured and then glucose level is checked up after taking a sugary drink/diet in hours. All these tests are single-point measurements and do not give real-time data. A single test cannot confirm a diagnosis, it is of low value, therefore cannot be regarded as useful for monitoring chronic conditions. HbA1C tests also have limitations in patients with abnormal red cell turnover (Boland et al. 2001). In a healthy adult around 2×10^{13} red blood cells transfer O_2 and CO_2 between the lungs and tissues, of which approximately 1.7×10^{11} are regenerated every day, resulting from an average circulatory lifetime of approximately 120 days. Cellular longevity is the outcome of an evolutionary trade-off between the energy requirements of keeping cells healthy and functional vs. cell renewal whereas a shorter RBC life lifetime would result in lower HbA1c levels at a given average whole blood glucose concentration as compared to a normal patient (Manodori and Kuypers 2002).

These shortcomings of conventional techniques and the relentless need of industry to enhance stability, accuracy, simplicity, miniaturization, power control, intelligent system usability, and scalability of the diagnostic methods drive toward the invention of glucose biosensors. Glucose biosensors have experienced an exponential market of growth due to their ability to precisely measure and monitor blood glucose levels. As per market blogs, the biosensor market is valued USD 25.5 billion, which is estimated to reach USD 36.7 billion by 2026, growing at a CAGR (compound annual growth rate) of 7.5% from 2021 to 2026 (Wei et al. 2009). With a rapid increase in point-of-care (POC) diagnostic market, India advanced at a CAGR rate of 9.3% in 2013–2018 and estimated to raise up to 11.9% during 2018–2023 (Konwar and Borse 2020). To facilitate rapid treatment and decisions for hospitalized patients lower turnaround times are desirable. POC devices have become useful as they only require a small amount of sample volume, are user-friendly, have long-time stability, and can even be non-invasive. These POC devices are portable, convenient, and give quality assurance. The main focus of this chapter is to present fundamental science and technological advancements over decades in glucose biosensing, overcoming obstacles and opportunities to achieve its ultimate objective of a highly stable and efficient, minimally invasive, or non-invasive monitoring of glucose in real-time. Accurate POC glucose testing necessitates consideration of several parameters before, during, and after the tests which is further discussed in Sect. 5.4 and some examples of the POC devices are shown in Fig. 5.1.

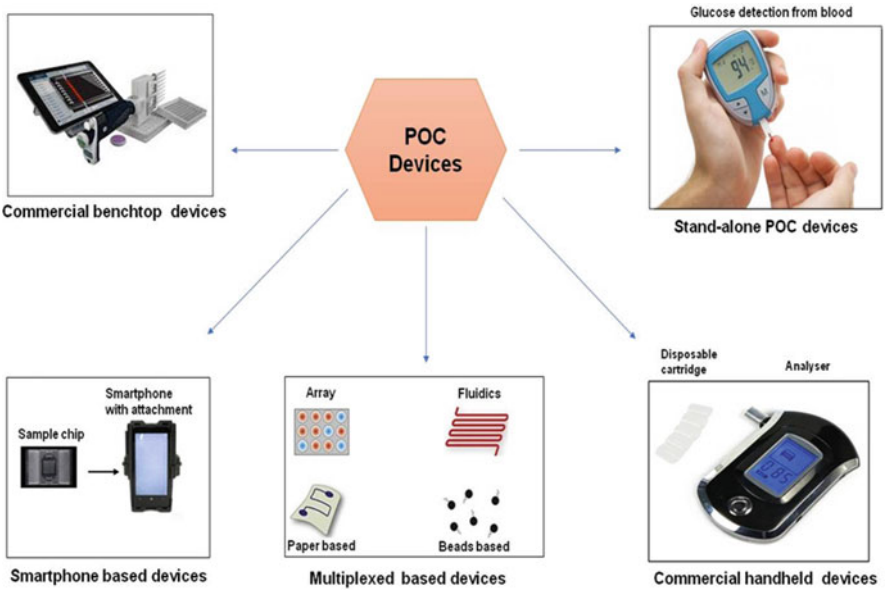

Fig. 5.1 Some examples of POC devices: Commercial bench top devices, standalone POC devices, smartphone-based devices attached with disposable sample chip, multiplexed like array, micro fluidics, paper, and beads-based devices for multianalyte detection, commercial handheld devices with disposable cartridge and analyzer

5.1.1 Importance of Glucose Levels in the Human Body

The maintenance of glucose levels at the optimum range is strictly regulated by our body playing a significant role in nurturing body functions effectively. Anomalously, its change in the level causes serious complications that can be life-threatening.

Human body is structured in such a manner that keeps glucose level constant in blood. Specific β cells of the pancreas regulate blood sugar constantly. Its level rises, whenever we uptake something resulting in secretion of insulin in bloodstream by beta cells. Insulin works as key to glucose which enters only when it unlocks cells of muscles, liver, etc., to generate energy and for storage, where excess residual energy accumulates as little bundles of glycogen (Dhara and Mahapatra 2017). People having diabetes either don't have sufficient insulin or their cells aren't capable of responding to it properly; causing a higher glucose level in their blood.

Blood glucose level drops significantly when we don't eat for hours, signaling the islets of Langerhans to slow down the insulin churning process. Other cells called α cells present glucagon which triggers the signal of breaking glycogen into glucose in the liver causing maintenance of glucose levels even during fasting. Physiological blood sugar level is around less than 100 mg/dL (milligrams per deciliter) between meals, known as fasting blood sugar level. Range of 90–130 mg/dL before eating

and less than 180 mg/dL after eating is considered a healthy range (Güemes et al. 2016).

In juvenile diabetes, the immune system targets pancreatic cells (responsible for producing insulin) and kills them, causing a lack of insulin in the body. But in adult-onset diabetes, the pancreas secrete insulin for glucose movement in the cells; however, the cells are unable to react with insulin adequately, which eventually results in the affected pancreas failing to produce insulin as per body's requirement (Ralston 2002). Approximately 200 mg/dL of glucose after 2 h of a meal or more than 125 mg/dL in the course of fasting is known as hyperglycemia. Low glucose level is known as hypoglycemia; its considerable range is under 70 mg/dL (Richter et al. 2018).

End products of carbohydrate digestion at the gastrointestinal tract are exclusively glucose, fructose, and galactose. Suitable enzymes present in liver cells facilitate inter-conversions between the monosaccharides. When liver produces monosaccharides, the resultant is always glucose due to the dynamics of the enzymes like glucose phosphatase which breakdowns glucose-6-phosphate into glucose and phosphate which transferred back to liver cell membrane via bloodstream. Glucose is a universal source of energy with chemical formulas $C_6H_{12}O_6$ (Fig. 5.2a), essential for both aerobic and anaerobic cellular respiration. In a sequence of biochemical reactions, glucose is broken down releasing ATP to power almost every energy-intensive process.

Most of the energy in eukaryotes comes from aerobic processes beginning with glucose molecules. In aerobic conditions, pyruvate can be used to produce energy-rich electron transmitters that help in producing ATP via electron transport chain (ETC) (Dunning 2016) (Fig. 5.2b). Glucose is first broken down in anaerobic phase of glycolysis, which produces ATP and pyruvate as a byproduct which turns into lactate (Bonora et al. 2012).

5.1.2 Fundamentals of Biochemical Analysis of Glucose

Biochemical measurements of glucose can be made in whole blood, serum, or plasma samples. Glucose concentration in whole blood is around 15% lower in comparison with plasma or serum under normal conditions, because of greater water content of cellular fraction (McMillin 1990). Reducing methods, condensation methods, and enzymatic methods are the three fundamental techniques for laboratory calculation of blood glucose concentration (Table 5.1) (Hall et al. 1983). The oldest methods use redox properties of glucose where glucose is oxidized to change the status of a metal ion. Reduction procedures are non-specific, and any strong reducing agent may interact to generate false high values by other strong reduction agents. While steps can be taken to remove most cross-reaction reduction agents, this procedure has largely been discontinued in the clinical laboratory. The aldehyde group of glucose can be converted to a colored substance by condensation with aromatic compounds. *O*-toluidine binds with glucose resulting in glucosamine,

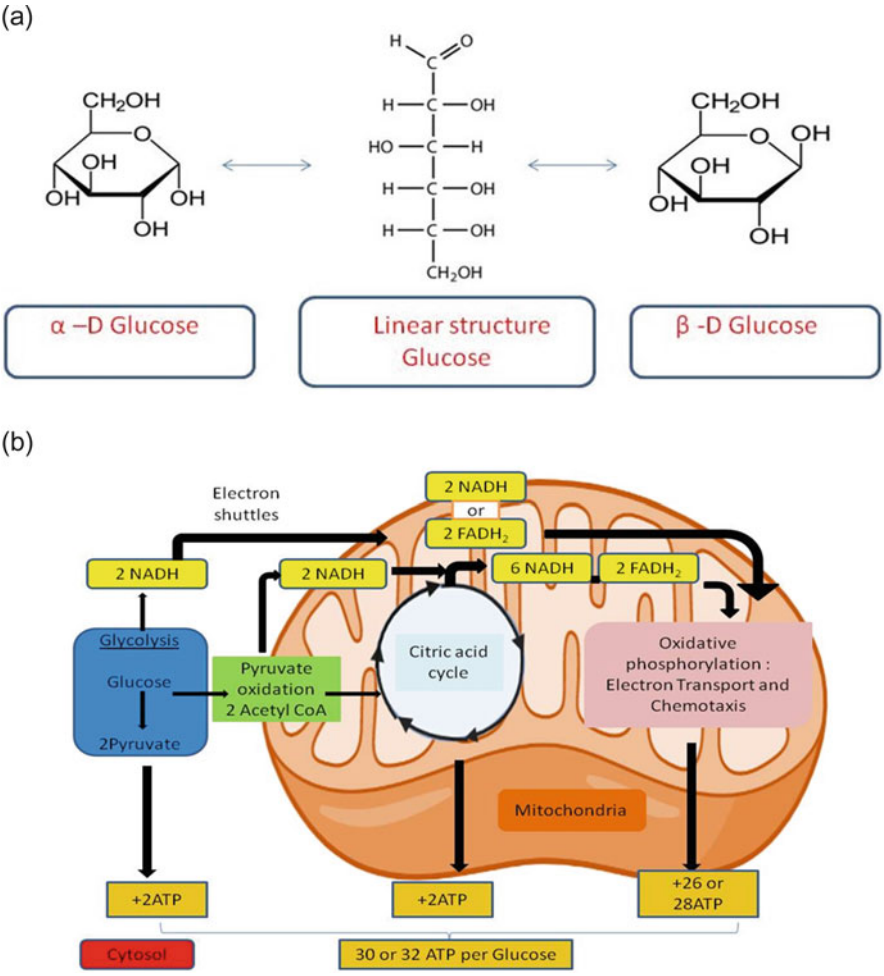

Fig. 5.2 (**a**) Structure of glucose Haworth and Fischer's projection of α and β-D glucose ($C_6H_{12}O_6$), from its linear structure, (**b**) physiology of glucose metabolism: Set of reactions involved in production of energy and transfer to cells from glycolysis to citric acid cycle and oxidative phosphorylation

turning the color of the condensation reaction into green. Afterward, the hue is analyzed for glucose levels by spectrophotometric analysis. The reaction is quick and highly sensitive to the bright color. Although other aldoses will also cross-react, only galactose and mannose generate colored substance (McMillin 1990).

Enzymatic assay approaches based on glucose oxidase and glucose dehydrogenase are nearly solely used in blood glucose monitors. Photometric or electrochemical detection of reaction products is used. In the presence of water and oxygen, glucose oxidase converts glucose to gluconic acid. The enzyme cofactor flavin adenine dinucleotide (FAD), reduced to FADH, serves as a first electron acceptor

Table 5.1 Fundamental biochemical techniques for detection of glucose concentration

S. No.	Method	Principle	Reaction	Color obtained	Absorption wavelength (in nm)
1.	Reduction	While glucose is being oxidized, the reducing properties of glucose are used to modify the state of a metal ion.	Glucose with 3,5 dinitrosalicylic acid producing gluconic acid and 3-amino-5 nitrosalicylic acid.	Brown Red	540
2.	Condensation	The aldehyde group of glucose can be converted to a colored product by condensation with aromatic compounds.	Primary aromatic amine (*o*-toluidine) reacts selectively with glucose in acetic acid solution.	Green	620–660
3.	Enzymatic	Glucose catalyzed by the presence of enzymes (e.g., GOx, GDH).	Enzyme GOx reacts with glucose, H_2O, and O_2 to form gluconic acid and H_2O_2. H_2O_2 further in presence of peroxidase reacts with 4-aminophenazone and phenol to form Quinoneimine.	Pink	540

(Trettnak and Wolfbeis 1989). The final electron acceptor, oxygen (O_2), then oxidizes FADH to form H_2O_2. Electrochemical approaches to calculating O_2 intake or H_2O_2 formation as per Eq. (5.1) and colorimetric method by GDH using NAD as cofactors may be used as per Eq. (5.2) (Wahl and Koschinsky 2018).

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{D-Gluconolactone} + H_2O_2 \quad (5.1)$$

$$\text{D-Glucose} + NAD^+ \xrightarrow{\text{Glucose dehydrogenase}} \text{D-Gluconolactone} + H^+ + NADP \quad (5.2)$$

These biochemical analysis methods unlike aren't single reading techniques, even do not provide real-time analysis and dynamic information about the higher and lower glucose levels. The reducing method is even non-specific; any strong reducing agent can cross-react to yield spuriously elevated values. Condensation method is rapid and gives intense color formation, but contains *o*-toluidine which is toxic and corrosive in nature, the enzymatic method although is specific and inexpensive but cannot be used for urine samples as uric acid and creatinine seems to have effects on results. Owing to these limitations, biosensors are introduced which provide more selectivity, sensitivity, and more efficient reproducibility.

5.2 Glucose Biosensors

A biosensor can be described as a compact analysis system or unit that integrates or is connected to a biologically derived recognition unit and a sensitive detection part (Yoo and Lee 2010). A typical biosensor is shown in Fig. 5.3. It consists of an **analyte** needed to detect a substance of interest like glucose. A **bioreceptor** can be an enzyme, cell, DNA, aptamers, and antibodies to directly identify the analyte and produce a response in the form of light, heat, pH, or charge. The **transducer** provides signals proportional to analyte-bio-receptor interactions that occur in an element that transforms energy from one kind to another. These signals are then displayed after amplification and conversion of the signal from analog to digital form by the **electronic** part. This **display** part is a combination of hardware and software parts having user interpretation systems like LCDs or direct printers where signals are displayed in a numeric, graphic, image, or tabular form as per requirements (Yoo and Lee 2010). The use of biosensor systems can play an essential role in disease monitoring, vaccine research and identifying pathogens, disease-causing microorganisms, and disease markers for body fluids. M. Cremer proved that acid concentration in a solution corresponds to the electric potential between portions of fluid on the opposite banks of a glass membrane in 1906 (Bhalla et al. 2016).

Each biosensor has specific static and dynamic attributes which define its effectiveness. The efficiency of the biosensor is reflected in the optimization of these properties, i.e., **selectivity, reproducibility, stability, sensitivity, and linearity**. Selectivity is the ability of a bioreceptor in a sample containing other admixtures and pollutants to detect a particular analyte accurately. The simplest example is the relationship between an antigen and an antibody. Reproducibility is the biosensor's capacity to provide the same results to several measurements. Precision characterizes

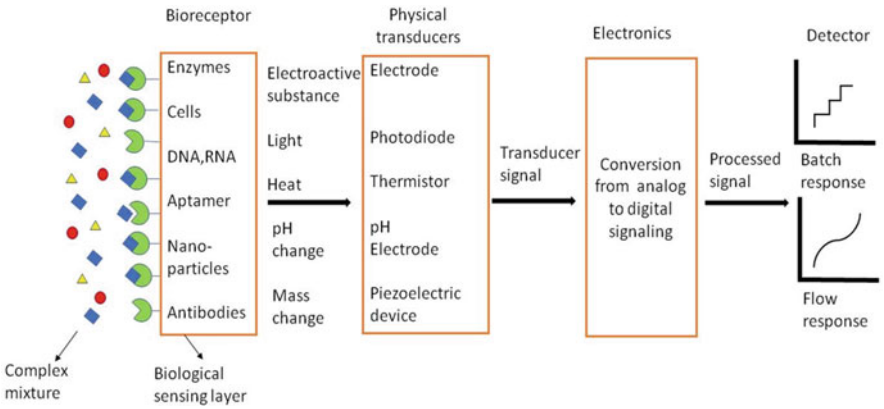

Fig. 5.3 Typical biosensor components and functionality: Biosensor consists of biological sensing layer of enzymes, cells, DNA, RNA, nanoparticles, aptamers, or antibodies which provide signal to transducers like photodiode, thermistors, pH electrode, or piezoelectric devices converting analog signals to digital and show batch/flow response on detectors

the reproducibility of transceivers and electronics in a biosensor for producing the lowest errors in measurements. Stability is the degree to which the biosensing mechanism is susceptible to environmental disruptions (Choudhary et al. 2019). These disturbances may trigger a drift in a biosensor's output signals. The minimum quantity that a biosensor can detect determines its detection limit. Linearity is a function which means proportionality of signal responses with changes in concentration which can be mathematically interpreted as a line equation of $y = m(x) + c$, where x is the research concentration, y is the signal of production, and m the biosensor sensitivity (Updike and Hicks 1967). In recent decades literature about biosensors shows that biosensors are not only desirable in academia but also in industry. Biosensor processing uses the specific characteristics of a biological identification case for a transducer. The interaction between the analyte and the bio-receiver would, in this case, becomes a user-friendly production (Bhalla et al. 2016).

5.2.1 *Types of Glucose Biosensors (Enzyme-Based, Colorimetric-Based)*

Clark and Lyons were the first to suggest the glucose biosensor principle in 1962. They invented the chrono-amperometric platinum electrode for glycemic control because they were involved in preparing artificial blood and wanted to track blood analytes to maintain the device in homeostasis (Clark and Lyons 1962). The idea behind the biosensor was simple: the electrode with immobilized glucose oxidase on its surface calculated oxygen consumption as a co-substrate (Martinkova and Pohanka 2015).

Glucose biosensor technology has dramatically advanced over the past 50 years, including point-of-care devices, continuous tracking system for glucose, and non-invasive monitoring systems. However, the accomplishment of precise, effective, and constant glucose testing continues to present many obstacles. Further technological advances are expected in glucose biosensors, standardization of analytical objectives, and the ongoing evaluation and formation of consumers (Yoo and Lee 2010). An increased number of platforms for glucose sensors have been developed for new products, glucose oxidase immobilization processes, and physical signal determination methods. Concerning new materials, organic salts, electrical connections, and nano-materials, enzymatic biosensors and non-enzymatic biosensors have been developed as described in Fig. 5.4 (Martinkova and Pohanka 2015).

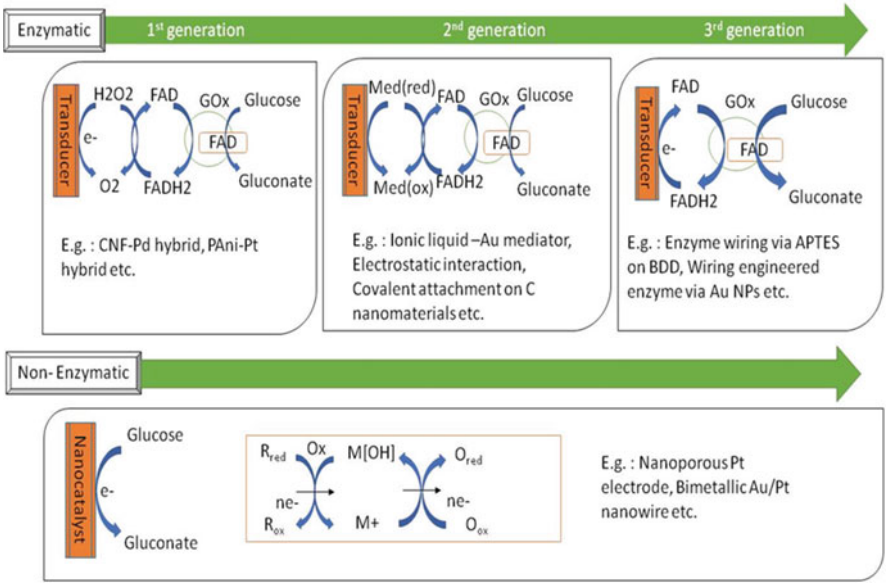

Fig. 5.4 Types of glucose biosensors and their mechanism: Evolution of different generations of electrochemical glucose sensors as enzymatic and non-enzymatic biosensors. These enzymes are classified based on their cofactors: FAD, PQQ, and NAD are tightly bound to the enzyme. Non-enzymatic sensing has widely been exploited by various nanostructures such as metals, metals oxides, and their hybrids with carbon nanomaterials

5.2.1.1 Enzyme-Based Glucose Biosensors

Amperometric biosensors are based on the redox reactions, or the oxidation/reduction of electroactive species formed in an enzyme reaction. Immobilization of glucose oxidase on an oxygen electrode produced the first amperometric biosensor. In a polyacrylamide gel, the enzyme was taken and then subsequently allowed to react to the electromagnetic oxygen. Glucose oxidase on the cellulose acetate membrane was immobilized by cross-linking with the bi-functional reagent glutaraldehyde, which was reliant on the H_2O_2 determination of a Platinum electrode. The main step in manufacturing a stable biosensor is to repair enzymes without losing stability or biological functions. One way is to use the leading composite polymer (CP), which is essential for designing electrochemical biosensors for the polymer substrate with a detection limit of 1.0–100 μM, sensitivity 1.3023 μA/mM, and R^2 is 0.99 (Lee et al. 2019).

For glucose amperometric biosensing, glucose oxidase has been put in a polymeric polyrrole or polyaniline film. Another way to test glucose using optical biosensors is to use screen-printed biosensors based on horseradish peroxidase (HRP) and glucose oxidase (GOx). Luminol chemiluminescence may be used in the glucose analysis in the presence of H_2O_2, generated in the glucose-oxidase catalyzed reaction (Pandey et al. 2019). Glucose dehydrogenases are another

important class of enzymes used in glucose analysis (GDHs). The classification is based on the cofactor of these enzymes. The flavin adenine dinucleotide (FAD) and pyrroloquinoline (PQQ-)-GdH enzymes are strongly linked to the FAD and PQQ cofactor, while the NAD cofactor in nicotinamide adenine dinucleotide (NAD)-GDH is not structurally related. Since GDHs are separate from O_2, FAD and PQQ-GDHs have lower actual glucose than GOx (Joshi et al. 2011).

Approaches of biosensors from generation to generation overcoming drawbacks considering economical and practical factors with new evolutions are discovered (Table 5.2). Extensive studies on different methods of immobilization and enhanced efficiency of electrode-enzyme electron transfer have resulted in the establishment of various sensory platforms that have continued to develop as state-art nanostructures and their nanocomposites are discovered. For example, gold nanoparticles, carbon nanotubes, carbon/graph quantum dots, and chitosan hydrogel composites are all used for their contribution to improving the immobility process and electrocatalytic against the glucose as a component in biosensors (Juska and Pemble 2020).

According to Scheller et al., imidazolium-based ionic liquid may be counter-anionized by Au nanoclusters that are redox active, and this liquid can be used to create an enzyme-amperometric glucose biosensor in the second generation. Such Au nanoclusters have recently been shown to be useful in the biocatalytic oxidation of glucose using GDH (Scheller et al. 1991). It is possible to detect salivary glucose by using nanostructured biosensors. The glucose content of each sample was validated using a conventional spectrophotometric methodology in second-generation biosensors. Their detection range is 0.5–20 mg/dL in buffer solution, and their reaction time is 30 s. SG levels between 1.1 and 10.1 mg/dL were effectively identified in ten healthy individuals (Du et al. 2016). An electron deposited gold nanoparticle-decorated glassy carbon electrode is used as a third-generation glucose biosensor based on cellular dehydrogenase. It was further enhanced using a self-assembled monolayer of 4-aminthiophene (4-APh) and 4-mercaptobenzoic acid (4-MBA), which was cross-linked with glutaraldehyde (GA). As a glucose biosensor, the improved electrode has a detection limit of 6.2 M, an expanded linear range from 0.02 to 30 mM, great stability, and high selectivity (Bollella et al. 2017).

5.2.1.2 Non-enzymatic (Calorimetric)-Based Glucose Biosensor

Though enzymatic sensors dominate the current glucose sensors market, various disadvantages mainly related to the internal characteristics of enzymes need to be addressed, particularly low reproductivity and long-term stability (Teymourian et al. 2020). The enzymatic sensors are generally very costly and unreliable and have various factors such as temperature, pH, and ion intensity that influence their catalytic activity. Therefore, because of its low cost, fast reaction, and exceptional sensitivity, this non-enzymatic process has attracted interest during the last few years (Lee et al. 2019). To prevent deficiencies caused by the existence of the enzymes, there is substantial effort to create non-enzymatic glucose sensor systems dependent

Table 5.2 Summary of types of biosensors, their evolution, and drawback during generations

S. No.	Types of biosensors	Evolution in biosensors	Drawbacks	Reference
1.	First generation	• Clark and Lyons initially proposed the concept in 1962. • They were composed of oxygen electrodes and inner oxygen semi-permeable membrane. • Thin layer of GOx and outer dialysis membrane. • Based on natural oxygen substrate. • Detection of produced H_2O_2.	• H_2O_2 measurement requires high selectivity. • Restricted solubility of oxygen in biological fluids. • Fluctuates oxygen tension, called 'oxygen deficit'.	Freeman et al. (2013)
2.	Second generation	• Oxygen was replaced with non-physiological electron acceptor/redox mediators. • Able to carry electrons from enzyme to surface of the working electrode. • Mediators as ferrocene, ferricyanide, quinines, TTF, TCNQ, methylene blue to improve sensors. • Introduction of commercial screen-printed strips for SMBG modified electrodes.	• Highly toxic mediators. • Did not lead to the rapid application of amperometry.	Hall et al. (1983)
3.	Third generation	• Is reagent less. • Based on direct transfer between enzyme and electrode without high toxic mediators. • Transfer using organic conducting materials relying on charge transfer complexes. • Implantable, needle-type devices for continuous in vivo monitoring. • Conducting salts like TTF-TCNQ mediates the electrochemistry of GDH-PQQ as well as GOx.	• Enzymes like peroxidase exhibit direct transfer at electrodes.	Jungheim and Koschinsky (2002)

Abbreviations: *H_2O_2* hydrogen peroxide, *TTF* tetrathialfulvalene, *TCNQ* tetracyanoquinodimethane, *TTF-TCNQ* tetrathiafulvalene-tetracyanoquinodimethane, *GDH-PQQ* glucose dehydrogenase pyrrole-quinolinequinone enzymes

upon direct electromagnetic glucose oxidation on the surface of the electrode (Tian et al. 2014). Nano-structured porous metal-based glucose sensors have a longer lifespan than enzyme sensors, since they do not include a biological component (Strakosas et al. 2019). As nanomaterials with enzyme-like activity are used as nanozymes, the popularity of sensor applications has increased dramatically. Catalytic nanoparticles are placed in the sense of artificial enzymes (Tian et al. 2014).

In general, two widely known hypotheses describe the electro-oxidation of glucose by metal oxide comprising electrocatalysts. The activated chemisorption model was proposed by Pletcher et al., which indicates that electrocatalytic glucose oxidation is carried out through a concentration step, i.e., glucose molecular adsorption on the surface of the electrocatalyst including metals and the hemiacetal hydrogen abstraction (Pletcher 1984). Adsorption potentially entails the establishment of appropriate connections to the adsorbate and the metal substrate with d-electrons and d-orbitals. Thus, each time the adjacent metal-based active center is absorbed, leading to a kinetic increase of glucose oxidation on the surface of electro-catalysts with appropriate geometry.

Another model is Burke's IHOAM model (incipient hydrous oxide atom mediator) (Burke 1994). According to this model, the formation of hydrous species on the electrode surface begins with electric glucose oxidation followed by chemisorption. The hydrous pre-monolayer will subsequently transmit to a significantly low potential the electro-mechanism of glucose consumed by the regeneration of the surface of the metal. The surface of the "functional" metal is re-oxidized by oxygen species with a certain potential during the repeated cycling process. A glucose oxidation reaction catalyzed by a variety of electric catalysts, including metals (Au, Pt, Pd, etc.), metal oxides (Co_3O_4, CuO, RuO_2, etc.), and alloys (PtPb, PtRu, etc.), (nanotubes of carbon, boron drugged diamond, etc.), is based on non-enzymatic glucose-biosensors (Si et al. 2013).

We discussed several enzymatic and non-enzymatic biosensors for the detection of glucose. Although enzymatic sensors are susceptible, selective than non-enzymatic one, and attain linear range from 1.0 μM to 30.0 mM with a detection limit of 0.29 μM, they still have high fabrication cost, difficulty in enzyme immobilization procedures and are poorly stable (Teymourian et al. 2020). In contrast, a non-enzymatic biosensor consists of high electrode cost and less chemical/thermal stability. Still, the ability to attain good reproducibility, low detection limit, prompt response, simple preparations, and low cost.

Some other types of glucose biosensors are also discovered like a strong covalent interaction between 1,2- or 1,3-diols and boronic acids has been demonstrated, allowing saccharides at mM or sub-mM levels to be bound, making boronic-acid-based saccharide sensing in biologically relevant scenarios possible. This work was supported by the National Science Foundation and the National Institutes of Health. As a result, boronic acids can distinguish structurally identical saccharides based on the orientation and relative location of hydroxyl groups. Monoboronic acids have been found to have a natural preference for fructose among monosaccharides (Li et al. 2012). Through the particular binding affinity to carbohydrates, lectin proteins have been widely utilized in the development of optical and electrochemical biosensors. Concanavalin A (Con A) is the most often utilized glucose- and mannose-selective lectin among lectin proteins. When immobilizing enzymes such as glucose oxidase (GOx) and horseradish peroxidase (HRP), such as glucose and hydrogen peroxide sensors, Con A is helpful since these enzymes are coated with inherent hydrocarbon chains (Wang and Anzai 2015). Metallic oxide and strontium

palladium perovskite sensors, which don't require costly enzymes in the system, also have better sensitivity.

Using fluorescent quantum dots (QDs) or metal nanoparticles (NPs) immobilized in phenylboronic acid (PBA)-functionalized microgels, Li et al. produced a variety of inorganic-polyester hybrid microgels for optical glucose detection (Li and Zhou 2013).

5.3 POC Devices for Glucose Biosensing

Treatment and individualized patient services have significantly helped with the use of point-of-care medical devices to monitor ECG, oxygen saturation, blood pressure, and temperature. Among the equipment used by POC applications are disposable box sets, such as quick assay dipsticks, handheld devices reusable for glucose tests, and multifunctional benchtop devices primarily found in laboratories (Gauglitz 2014). Colorimetry, potentiometry, fluorimetry, electrochemistry, microfluidics, and other concepts and mechanisms are used in these devices. In most POC diagnostic instruments, biosensors are the most essential devices. Nanomaterials such as gold and silver, quantum dots, magnet nanoparticles, carbon dots, and other nanomaterials in the development of biosensors have revolutionized in the nanotechnological field of POC tests (Konwar and Borse 2020).

POC monitoring instruments also improve diagnosis capability for several serious diseases and disorders. The mechanism of POC devices is shown in Fig. 5.5. Most POC systems, such as blood pressure, glucose, and oxygen screeners, use high-end digital channels to reduce the workload of health care professionals. The automatic multiplex analysis tools of the unit interpret the findings, so no technical specialist is necessary. Blood glucose detection and cardiological testing are the two key fields contributing to the significant POC diagnostic industry. Therefore, new diagnostic methods, like laboratory-based high-end applications, need to be updated to rapid, simple, and cost-efficient POC tests. One difficulty, which impaired the precise and reproducible nature of paper-based diagnostics, is overcoming significant variations in liquid evaporation and sample retention. Quantifiable data, particularly for multiplexed paper-based diagnostics of POCs, are often difficult to obtain (Wang et al. 2016).

There were some predefined sets of specification based on each device including factors like heat stability, portability, daily throughput, cost, power source, ease of use, quality control availability, and having access to maintenance and after services. The assessment of POC devices is based on these criteria like the limit to which a device can be utilized in settings with finite infrastructure are the POC features of equipment (i.e., portability, power source, and throughput). Another criterion is the degree up to which the product consumables were appropriate to use (i.e., shelf life of reagents, heat stability), its ease of use like how suitable the device for simplicity of testing procedure and minimal technical training to operators. It must have accessibility in providing quality control mechanism satisfactorily and compatibility

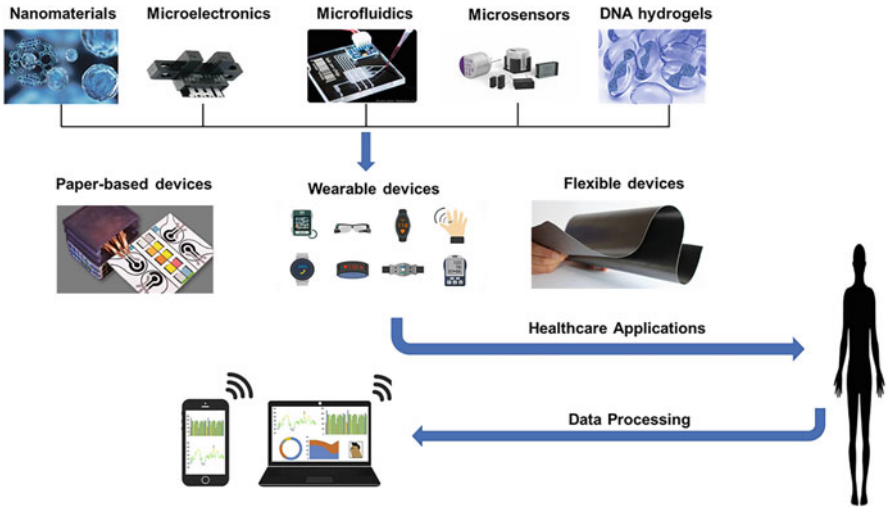

Fig. 5.5 Components and mechanisms of POC devices: Conductive polymers, nanomaterials, DNA hydrogels, microfluidics, micro-electronics, or microsensors are used in paper-based, flexible/wearable devices which pave the way for personalized therapies or healthcare applications after data processing with resultant sensing output

with external quality assurance schemes. The device must be cost efficient and have availability of after-sale technical services and local area distribution. Based on these specifications, scorecards are given to the devices showing their utility in low resources and primary care settings (Lehe et al. 2012).

In emerging and developed countries, rising healthcare costs and limited coverage are a cause of concern. Developing robust diagnostics that allow decentralized analysis (at home or the point of care) is essential for shifting the healthcare paradigm. Early and correct diagnosis of an illness is necessary for successful maintenance of health throughout the disease (Dincer et al. 2017). Diagnostics at the point of care (POC) are significant in healthcare delivery, particularly in the health context in developing countries. Because of their affordability, disability, and mass production capabilities, paper substrates are currently the most prominent (Wang et al. 2016). POC monitoring refers to collecting specific clinical results and criteria at the point of treatment where the patient is tested. POC has several meanings, but they all mean the same thing: fast analysis of test findings so that the patient can be handled appropriately as soon as possible (Konwar and Borse 2020).

Due to their low cost, quick, easy to use, and device-independent point-of-care diagnostics, they play an essential role in timely patient attention in primary care.

In non-laboratory settings where skilled technicians and specialized laboratory equipping have not been possible because of their benefits compared to

laboratory-based diagnoses, POC diagnostic is used for the diagnosis and prevention of infectious diseases as well as to track health conditions (Tian et al. 2016). End consumers can use paper-based POC diagnostics at home, such as glucose strips or pregnancy checks, without specialized instruction. There is still ongoing research in paper-based diagnostics to improve sensitivity, reduce batch-to-batch heterogeneity, and increase multiplexing capacity (Yamada et al. 2017).

Various instruments are used for the simultaneous identification of medicinal chemistry parameters such as blood gases, electrolytes, acute metabolites, and immunoassays. These systems, however, are either cumbersome or costly bench-top bioanalyzers, or they can only detect a small number or type of analytes. In contrast, POC devices significantly compete with them with qualities like low sample consumption (finger prick), and are accessible to non-invasive samples (saliva, urine, sweat). Wide area of information can be obtained from distinct specimens, automated system operation with minimal user intervention, rapid turn-over times (result in hours), prolonged reagent shelf lives, more accurate results then laboratories, portable, low cost, disposable strips or test cards. Nowadays cell phone-based or equipment-free systems is also preferable (Dincer et al. 2017). Table 5.3 signifies evolution in point-of-care devices and their drawbacks which are fulfilled in the next generation.

5.4 Commercially Available POC Devices for Glucose Biosensing

A plethora of POC devices have been evolved to generate distinctive statistics concerning the patient's medical condition. Numerous POC devices have been explored for years with varying detection techniques, principles, and detectors. These substantial endeavors throughout the earlier decades led to major systematic, factual, and hi-tech innovations which continued their progress approaching advanced continuous glucose observation platforms for monitoring of diabetes. Developing an inexpensive and straightforward point-of-care devices are becoming auspicious tools for evolving countries either in the provinces or for home use settings. There are several commercially available invasive blood glucose sensing POC devices manufactured. Some of them along with their detection range and response time are discussed in Fig. 5.6.

In particular, during specific everyday tasks, such as physical activity or hypoglycemia sleeping time, continuous glucose control is highly needed or when glucose levels are not being tracked by blood metering. Recently, the problems inspired the research of portable, non-invasive devices that constantly regulate glucose levels, such as sweat, urine, tears, and saliva, in outside body fluids, without disrupting the skin's integrity. There is a continuous development of POC devices for glucose sensing in different non-invasive fluids having the ability to achieve less

Table 5.3 Types of POC devices and their drawbacks following evolution

S. No.	Types of POC devices	Evolution in POC devices	Drawbacks	Reference
1.	Continuous glucose monitoring systems (CGMS)	• Real-time data of an internal insulin release system. • Surface contamination of electrodes by proteins and coagulation factors causes a risk of thromboembolism. • Subcutaneously implantable needle-type electrodes are developed.	• Are less accurate than traditional.	Cox (2009)
2.	Non-invasive glucose monitoring system	• Optical and transdermal approaches are most common. • Uses physical properties of light in the interstitial fluid of the eye. • The approach includes polarimetry, Raman spectroscopy, infrared absorption spectroscopy, optical coherence tomography.	• The reliable noninvasive method is still not available.	Tamada et al. (1999)
3.	Glucose biosensor for point-of-care testing (POCT)	• Rely on disposable, screen-printed enzyme electrode test strips. • Plastic or paper strips have electrochemical cells, contain GDH-PQQ, GDH-NAD, GDH-FAD, or GOx/redox mediator.	• The practical and economic factors.	Cartier et al. (1998)

Abbreviations: *GDH-PQQ* glucose dehydrogenase pyrrole-quinolinequinone enzymes, *NAD* nicotinamide adenine dinucleotide, *FAD* flavin adenine dinucleotide

detection limit and quick response time. Some developed non-invasive devices are summarized in Table 5.4.

Although these sensors with non-invasive diagnostic fluids have relatively easy sampling, less complex procedures, fewer detection limits, compliance for frequent clinical monitoring, even doesn't require any specialized instrument for sample collection but it needs large sample volumes, pH variations occur and have less glucose concentration than blood. Multiple steps of sample processing of saliva need to be done, is a very tedious and lengthy process. Sweat analysis also requires individual calibration for reliable results; composition also varies in tear samples that are why results aren't stable for a long time. Every device has their own advantage and disadvantages, some generalized implications, advantages, and disadvantages of POC devices are shown in Fig. 5.7.

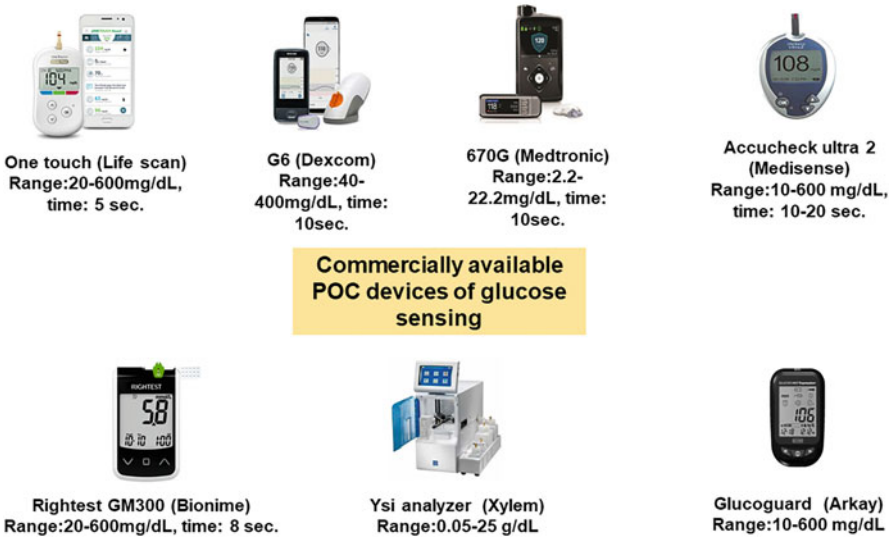

Fig. 5.6 Some commercially available POC devices for invasive glucose sensing: YSI analyzer a trademark of Xylem Inc., its proprietary immobilized enzyme electrodes allowing rapid, accurate measurement in a min., Accu check ultra 2 finger pricker, small and slender device with alternative site testing spacer, Medtronic 670g system enables the personalization of glucose with adjustable target setting as low as 2.2 mmol/L, Dexcom G6 system have slim sensor continuously measures glucose level beneath the skin and sends data wirelessly on a display device. One Touch life scan color sure instantly shows when sugar numbers are in or out of range and syncs data seamlessly with oneTouch reveal mobile app. Glucoguard Arkay has safe eject button and auto coding function for before and after meal results, Rightest GM300 by bionime is safe, convenient with wide LCD screen, and smart accuracy testing within 8 s

5.5 Sources of Errors in Glucose Monitoring

Glucose monitoring is an essential part of diabetes treatment and monitoring; however, certain limitations of detection methods lead to errors in measurement. Precision can be limited due to variations in the manufacture of strips, handling, and aging. They can also be affected by weather conditions such as temperature or altitude and patient factors such as repetitive coding, insufficient washing of hands, and altered hematocrits. The precision of a blood glucose meter is determined by the degree to which the average values correspond to the reference value. Accuracy refers to the repeatability of a set of numbers, regardless of their similarity to the reference (Freckmann et al. 2015). Some sources of errors have been shown in Fig. 5.8 (Jungheim and Koschinsky 2002).

Table 5.4 Some POC devices for non-invasive glucose sensing and their performance

S. No.	POC device	Substrate	Technique used	Detection limit	Response time	Reference
1.	Cavitas sensor	Glucose in saliva	Enzymatic method	5–1000 µmol/L	1.0 s	Arakawa et al. (2016)
2.	Mouth guard sensor	Glucose in saliva	Enzymatic method	0.1–1 mM	60 s	Kim et al. (2014)
3.	On chip nano-biosensor	Glucose in saliva	Enzymatic	0.1–20 mg/dL	30 s	Zhang et al. (2015)
4.	Pacifier sensor for newborn	Glucose in saliva	Electrochemical method	0–250 mg/dL	200 s	García-Carmona et al. (2019)
5.	Nafion composite film and NPs	Glucose in serum	Electrochemical method	0.1–2.2 mM	1.0 s	Yang et al. (2009)
6.	Bi-enzyme glucose biosensor with magnetic nano-composite	Glucose in serum	Enzymatic method	0.05–8.0 mM	5 s	Chen et al. (2011)
7.	Plasma polymerized film	Glucose in plasma	Enzymatic electrochemical method	0–60 mM	8–10 s	Muguruma et al. (2000)
8.	Thermo-responsive needles	Glucose in sweat	Electrochemical method	0.1–0.6 mM	0–10 s	Lee et al. (2016)
9.	Micro-fluidic device	Glucose in sweat	Colorimetric	1.5–100 mM	<1 min	Koh et al. (2016)
10.	Tear glucose analyzer	Glucose in tear	Quantitative chromatography	0–20 mmol/L	5–10 min	Lane et al. (2006)
11.	Nano-particles embedded contact lens	Glucose in tear	Spectroscopy	0.0–2.4 mM	5 min	Kim et al. (2020)
12.	Contact lens with embedded biosensor	Glucose in tear	Enzymatic Amperometric method	0.1–0.6 mM	20 s	Yao et al. (2011)
13.	Photelometer	Glucose in urine	Photoelectric method	0–200 mg/100 cc	7 min	Hoffman (1937)
14.	Biochemical sensor	Glucose in urine	Electrochemical method	0–200 mg/dL	0–2 s	Park et al. (2005)
15.	Micro-fluidic paper based	Glucose in urine	Electrochemical method	0–20 mM	5 s	Zhao et al. (2013)

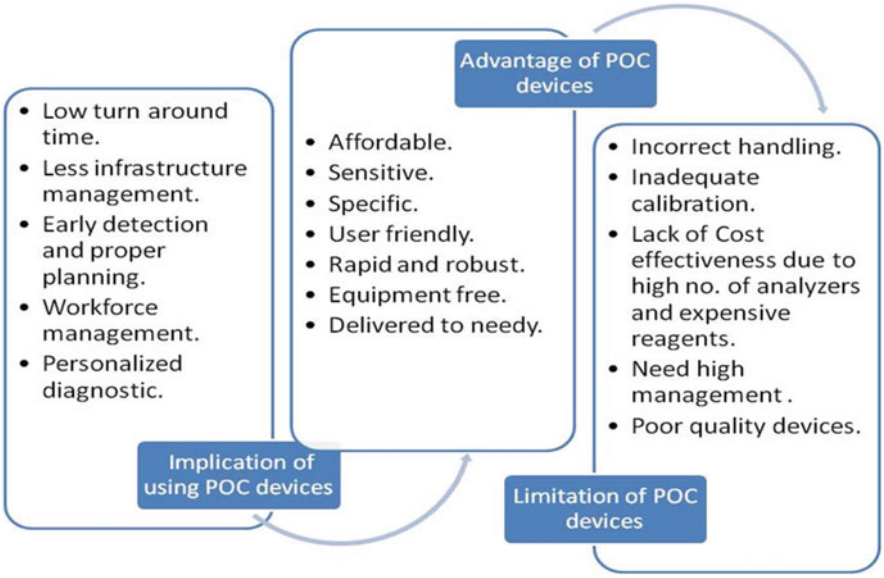

Fig. 5.7 Few implications which are needed in healthcare industries like less instrumentation, low turnaround times, proper planning, and personalized diagnostic are done in POC devices making them affordable, sensitive, user-friendly, and rapid. But these POC devices have some drawbacks like it needs high management, lack of cost-effectiveness, needs inadequate calibrations, etc.

1. **Strip factors**: There is always a strip-to-strip variation in manufactured products, resulting in inaccurate readings. In some strips, the sample chambers or wells are small (2–3 mm) with a size variation of 50 μm, leading to a 3–4% error. Enzyme coverage also influences accuracy. An excess enzyme is present in the strip; that's why alteration at a large proportion correlates with the reading. A slight decrease in the amount of enzyme doesn't alter glucose values.
2. **Physical factors**: Various physical variables affect the precision of bands such as altitude, which differ in temperature with monitoring systems accordingly. Glucose oxidase biosensor strips typically are oxygen sensitive. The value of the active mediator would be below expected, as the electrode detected the mediator only if the oxygen content of the sample is high. Even the meter may provide an inaccurate measurement when the oxygen level is low. The effect of temperature also can lead to instability, and can cause variations to an extent of 5–7% (Haupt et al. 2005).
3. **Patient's factors**: The willingness of the patients to correctly use their blood glucose meter may have a significant impact on its performance. While most glucose meters of the blood require coding, some of the newer versions do not. Coding determines the connection between the electric signal from the strip and the blood glucose measured. The precision of blood glucose control systems depends on the patient's measurement technique, but modern measurement facilitates the accuracy of patient data. Any naturally occurring substances in

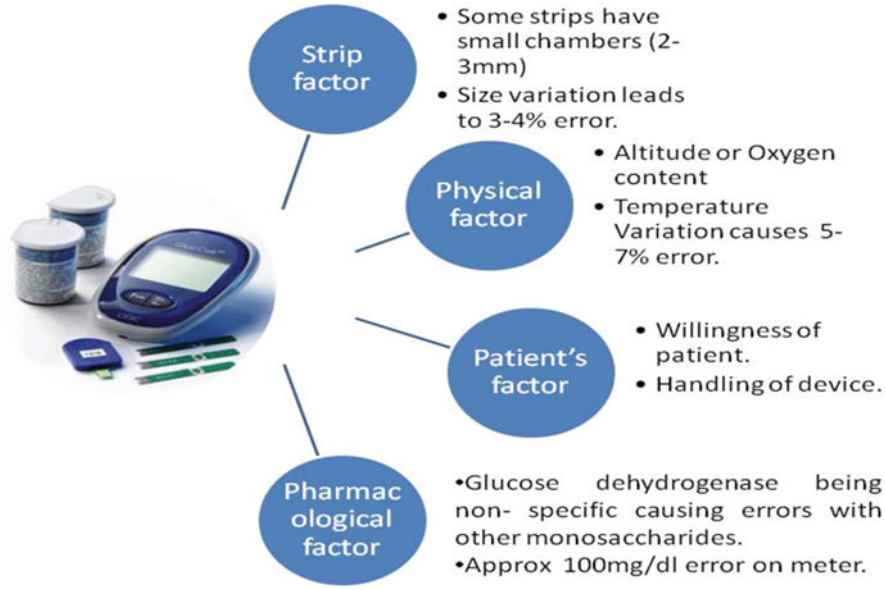

Fig. 5.8 Source of errors in point-of-care glucose monitoring: Various factors for sources of errors either it be physical due to temperature/altitude, or due to patient's handling technique, or error due to strips or pharmacological factors like enzyme being nonspecific for the process, etc.

the body can also skew blood glucose monitoring results (Somogyi 1928). Three natural compounds, which interact with electrochemical oxidase-dependent bands of glucose, include triglycerides, oxygen, and uric acid. When triglycerides space up and decrease glucose levels in the capillary volume, meters can be highly inaccurate (Ervin and Kiser 1999). Oxygen is competing with an enzyme electron mediator. As strips are normally calibrated for concentrations of capillary oxygen, the higher levels of oxygen found in arterial samples are less optimistic. The electrode oxidizes uric acid but only at very high concentrations (Ginsberg 2009).

4. **Pharmacological factors**: Glucose dehydrogenase, a less specialized enzyme, can compete with some naturally occurring sugars. On glucose dehydrogenase, galactose, xylose, and maltose compete with glucose and can give false values. On the other hand, glucose dehydrogenase sensors are less likely to shift the concentration of oxygen (Kost et al. 1998). The effect of maltose and xylose is minor, but icodextrin is the most dangerous medicine, which is used by some peritoneal dialysis fluids that can increase the glucose level by over 100 mg/dL per meter (Tang et al. 2001).

5.6 Safety and Handling of POC Devices

The capacity of managing working load and timelines depends on its staff and processes to be efficient, effective, and reliable in critical care units. Errors can lead to preventable impairment, including inadequate care in critical patients. Precise and timely diagnosis can lead to the replacement of more invasive instruments with less invasive instruments, thereby reducing the probability of complications, including infection. Many clinical hospitals from around the world have long advocated this to improve patient safety and quality results.

POC diagnostics can decrease workload by shifting tests from a centralized laboratory to the bedside, decreasing the time of diagnosis and decreasing preliminary test errors. Their use can lead to positive effects including reducing blood drawn from patients, reducing the blood consumption required for testing, and quicker diagnostic results to ensure precise treatment protocols. Patient detection errors are reduced when the procedure is done at the bedside (Plebani 2010).

5.7 Robustness and Ease of Use

It has become more challenging than ever for healthcare providers to respond to changes in care delivery. These are the problems that POC devices overcome, e.g., they lower the expense of treatment and help lower the total costs of providing care by the direct integration of diagnostic tests into the pathway of patient care. It improves access to treatment, and new models emerge that enables healthcare to be accessed anywhere, anytime. Innovative diagnostic solutions transform care delivery through quick, reliable observations and the better experience of patients along with improving care quality (Titler 2008).

The POC tests provide fast results, enabling adequate management to be initiated promptly by reducing the turnaround time of the results by reducing delays and errors in transport and processing. A more comfortable and less stressful test with minute blood or non-invasive fluids drawn results in potential patient satisfaction. Some POC analyzer features that help in ease of use and minimizing error risk: long-life electrodes or disposable sensor packs without repair, user interface touch screens, Including user and patient recognition software, built-in scanners for bar code, clot identification in the analytical chamber, instead of gas bottles they consist of liquid calibration systems, automated measurements, automated sampling of quality control, comprehensive QC programs like data analysis, remote monitoring and controlled connectivity of information systems integrated instructional recordings.

Possess the ability to actively handle particular circumstances and to conduct frequent tests, using less medically intrusive POCT procedures as well as smaller samples and reagent quantities. POCT can deliver a simpler covered entity for example to the elderly in remote places, where accessibility to a lab is constrained,

economic—while POCT is normally quite costly than lab tests, with a lower array of medical visits, decreased hospitalization, and lower hospital admissions, it can deliver larger financial benefits, greater patient participation in your treatment, and patient's experience improved (Gomez 2013).

5.8 Safety and SOP

In one health facility, POC check-ups led to a 44% decrease in residence time, reducing the risk of side effects such as healthcare-associated surveillance and other extending stay complications. Financial health facilities can also be improved as POC testing in one hospital showed a 47% reduction in inpatient care costs (Nichols 2003). To improve quality and safety, monitor costs, and enhance transparency, further programs are being implemented by federal and state governments, as well as accreditation and quality organizations, to focus on patient outcomes, care delivery, and staffing. These policies affect not only reimbursement and tax health but also the ability to advertise itself to its communities as a viable and secure treatment center (Burgener 2017).

All POC operations should indeed be conducted so that the safety and well-being of the patient or operator are not jeopardized: The gadgets should then be run by the guidance of the producer. The gadget should be disinfected at the specified time or promptly after any over-contamination of blood or body fluid, as directed by the manufacturers or local legislation.

POC users and team leaders should always remember the significance of conventional infection control practices, preventive measures of work exposure to bloodborne viruses, wearing of gloves or other body armor and avoidance of sharp injury, prevention of trans infection with viruses transmitted to blood, along with the choice of proper launching instruments, safe practices, and disposal management (Belkin 1988).

Standard Operating Procedures (SOP) All POC tests engaged at work should be subject to SOPs. An SOP aims to perform the processes adequately and consistently. The line staffs need to ensure that personnel using POC instruments are compliant with the relevant SOP that is prepared for each POC device and process, in compliance with clinical pathology accreditation (UK) standard. SOPs to every equipment will prove essential: medical background, analyzing approach, safety standards which include: COSHH (control of substances hazardous to health regulations), appropriate waste management, disease prevention; incident notification, pre-analytical reporting, chemicals, benchmarks, quality control and regulates, testing procedures, samples, analysis, results in calculations, predictive validity, and record keeping.

All new devices shall be purchased following organizational norms and subject to discussion with the medical devices group. POC units utilize software applications and stakeholders must make it accessible with other administrative software, such as

the laboratory information management system or hospital information technology, with which interaction may be required. The consequences of all the relevant technical details shall be considered by stakeholders making POC procurement choices (Kasparick et al. 2016). For the intended function, all POC devices must be suitable, they must meet criteria of efficiency and security, be supported appropriately, produce outcomes that can be documented relatively safely, compliant to standards and data transmission norms; only employees certified for their use shall operate (Choudhary et al. 2019).

5.9 Waste and Disposal

All those working in POC testing are responsible for ensuring that all the trash created during their job is safe and correctly disposed of. Inappropriate and irresponsible garbage dumping into the drains, collection of rubbish by the local authority or in the environment is prohibited under law. In compliance with the NHMRC rules for managing waste in the healthcare sector, handling and disposal of harmful products should be carried out. Sample collection lancets and reagents (cuvettes/bands) designated dangerous "sharps" and disposal in an authorized sharps container deemed dangerous. Additional pollutants such as blood or body fluid, contaminated tissues, or swabs shall be discarded and burnt in a (yellow) trash bag (Ali et al. 2017). The recent arrival of biodegradables has presented enormous possibilities to alter medical technology by allowing sensors that gradually decay upon usage.

Degradable elements can also help environmentally friendly sensor arrays minimize certain prominent nature concerns by minimizing the amount of electrical or clinical wastage and carbon footprints. The rates of material degradation and biocompatible device dissolving behavior might vary dramatically in distinct biological processes. For practical implementation, customized transitory materials are essential for monitoring deterioration at pre-set rates. In addition, to build a naturally derived platform, the development of elevated and miniatured transmission lines, circuits, and chips is necessary on biodegradable surfaces and cordless transmission. The energy consumption of future embedded sensors must be minimized and devices can take the energy required from bodily motion or heat from other in situ power sources like the biofluid (Kim et al. 2019).

5.10 Challenges and Troubleshooting for POC Devices

Approaching patient diagnostics provides healthcare equipment producers and Pharmaceutical Corporation's enormous business potential. It also offers a variety of issues that can jeopardize the commercial viability of the POC device unless it's been addressed early in the development process.

- **Cost**—There is indeed multiple hidden point-of-care testing costs, which are typically not taken into account in clinical sectors, including validation instruments reagents, product quality materials, and cost of competence tests. The link among POC system software or gadgets is also connected with a considerable expense for the laboratory information management system and/or the electronic health records (EHRs) (Nosanchuk and Keefner 1995).
- **Quality assurance**—Quality problems with POC are also evident. The procedures used are often less comprehensive than those at the central labs and far more vulnerable to interference than conventional laboratory procedures. POC glucose techniques, for instance, frequently use glucose measuring enzymes that were not strictly glucose-specific and can also be impaired by certain sugars like maltose or galactose (Nichols 2003).
- **Accuracy**—The reliability of POC assessments is also based on the fact that POC is often carried out by hurried medical team personnel and not by qualified laboratory experts. Non-laboratory trainees are generally unaware of the relevance of quality assurance and controlled maintenance. Moreover, these professionals focus on the care of the individual and sometimes bring additional strain from an already demanding job in quality treatments related to POC testing. The POC satellite laboratory paradigm of the medical laboratory technologists is an alternate option to POC tests conducted by clinical personnel (Shaw 2016).
- **Errors in measurement**—Any error may occur at any moment during the test cycle, like in any laboratory experiment. Recent research has evaluated error margins among screening POC and clinical laboratory for pre-operational, analytical, and post-diagnostic components. In comparison to central laboratory analysis, a greater proportion of pre-analytical mistakes were reported with POC tests. Especially about positive patient identification, pre-analytical inaccuracy is very often detected. Patient medication identifiers for 45% of POC tests performed could not be confirmed by operators (Cantero et al. 2015).
- **Longevity of product**—The shelf life of POC devices is expected to be limited, contrary to laboratory-based equipment. The device needs to attract the user and keep up with rising trends and mode does not endure forever. Developers need devices to be developed so that updated models become a viable business approach so they can meet the needs of gadgets that survive and thrive in a "consumer" driven commercialization (Asghar et al. 2016).
- **Product portfolio**—POC tools have to meet the patient demands and patients are consumers, unlike lab-based diagnostics devices, which mainly have to meet patient expectations, purchasing needs, and requirements of domain's expert users. It is quite improbable that a single device will serve the needs of all desired users; therefore, it is necessary to examine a portfolio of devices.
- **Training of stakeholders**—In the recruitment and utilization of a POC device, the number of other people involved is far more than those participating in a standard laboratory-based diagnostic tool. In decision-making, patients will be crucial, and caregivers, nurses, and even doctors are likely to be major players that will impact the performance of the commercial equipment (Menon et al. 2020).

- **Need to disrupt**—The need to disrupt POC tests implies developing a device that disrupts, not necessarily a technological disruption that is designed to replace existing procedures with a more convenient method. It is not easy to create disruptive devices. For example, to achieve comfort together with trust for quality and consistency of findings. This is extremely difficult if a well test/working flow is to be replaced by the new device (Anwar et al. 2015).
- **User needs**—The nature of POC testing implies that the operating environment and user types of the device are significantly diversified. Process optimization, elimination of the requirement for qualified technical staff who work in regulated procedures, adds added value by effective POC devices. To achieve so, the POC gadget must handle non-specialist personnel in all stages of sample collection, processing, and presentation. These factors may cause the target user and process stages to be misdefined, a problem sometimes combined with the early phase of solving the key technology's technical issues (Yamada et al. 2017).

5.11 Conclusion

The blood glucose range became one of the world's most confronting issues during the past few decades. Consequently, blood sugar monitoring is an ongoing tool that helps the doctor interpret results, comprehending blood glucose monitoring techniques. Because of this rapidly rising requirement, numerous glucose sensing and monitoring technologies are being conducted to solve these problems. To summarize, three basic methodologies, i.e., reduction, condensation, and enzyme methods are used for laboratory calculation of blood glucose concentration. Measurement of blood glucose inaccuracy, its causes, mechanism, possible strategies to increase patient reliability, and the advancement of electrochemical glucose surveillance over the last decade which has taken great steps are marked.

As stated, biomedical diagnostics are now everywhere, which provide energy to monitor therapy and progression of illness in a point of care. Its connection with high-affinity biomolecules permits a variety of analytes to be sensitive and specific. Biosensor components, efficiency characteristics, and biosensing methods have been discussed in general. As the prevalence of diabetes has increased, new technologies have been created throughout for the glucose biosensor of POC devices, CGMS, invasive and non-invasive glucose surveillance. In novel materials, organic salts were produced; metal electrode technological connections and nanomaterials are established. After an orientation to the relevance of (continuing) glucose sensing, and a short glance back, enzymatic and non-enzymatic biosensors were made accessible.

Over 40 years after its wide-ranging debut, the volume of POC tests has gradually expanded. This growth will probably continue, spurred by innovations in healthcare that seek to provide cheaper healthcare near to the home of the patient. For this reason, recent progress has been made toward non-invasive and continuous glandular monitoring devices, with a special emphasis on glucose level monitoring in

substitute physiologic interstitial fluids in the blood such as saliva, serum, plasma, sweat, tears, and urine.

Last but not least, we streamlined SOP processes, followed by simple safety measures and sturdy POC equipment, and addressed waste and disposable features, which are obstacles to the commercial viability of POC equipment throughout the development phase. In subsequent efforts, glucose sensors with developing materials and equipment should then be progressively integrated to enhance multi-parameter assessment and increase the breadth of the body surveillance system. At around the same time, the integration of machine teaching with multiplexed systems can improve the effective management of diabetes considerably and certain incremental changes with current technology are likely to persist, as a result of continued miniaturization of electronic devices and elevated computer power on other markets, such as consumer items.

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Chapter 6
Overview of Affordable Upfront Point-of-Care Testing for Cancer Detection

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6.1 Introduction

Point-of-care (POC) diagnostic biosensor is a clinical analysis tool and it is used to get diagnosis results at the site of patient care (Omidfar et al. 2020; Slovis et al. 2020). In 1550 BCE, the first POC test was described in the scientific literature to measure glycosuria in diabetes mellitus suspected patients, using urine samples (Yang et al. 2019). In the current century, the main aim of POC biosensor is to offer fast, suitable, user-friendly and easy-to-perform analytic test that reduced the therapeutic turnaround time once associated with a routine diagnostic test (Fig. 6.1) (Sachdeva et al. 2020; Chakravarthy et al. 2021). POC biosensors can make available quick diagnosis in a clinic, remote areas and in resource-limited nations that do not have basic pathology facilities. Consequently, it offers immediate clinical

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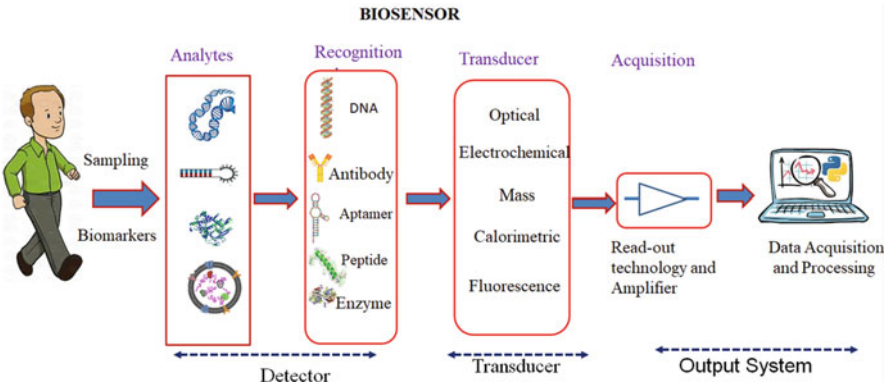

Fig. 6.1 Schematic illustration of biosensor comprising its components detector, transducer and output system

management decisions to improve the avocation of drug therapy along with direct changes in therapeutic practice. Practice of these POC devices to monitor and diagnose critical diseases like cancer is a major challenge in the medical setup. This chapter highlights the significance and importance of cancer and cancer biomarker(s) identification in the biological fluid. In this chapter, we tried to intricate the overview as well as the benefits and limitations of the bio-analytical methods involved in the diagnosis of cancer. The prospects of future clinical research and improvement in the field of medical biosensors for cancer detection are deliberated.

6.2 Cancer and Their Biomarkers

Cancer is a set of diseases causing uncontrolled growth and spread into surrounding cells, and consequences in death if its spread is not controlled (Hayes et al. 2018). Impairment to a gene or/and specific molecule could interrupt communication between cells, eventually leading to the termination of all vital functions that must be needed to sustain a living organism (Min 2005; Tao et al. 2012; Yamada et al. 2019). Environmental factors such as tobacco, infectious organisms and an unhealthy diet, and internal factors, such as inherited genetic mutations, hormones and immune conditions are evoked and responsible for cancer diseases. Factors may work together or in order to cause cancer, and manifestation of the disease may occur at variable periods between the exposure to external carcinogenic factors and detection of cancer markers (Tao et al. 2012) or after changes in the genetic code and the formation of cancer cells.

Biomarkers of cancer/cancer disorders are biomolecules present in the body fluid or tissue that are indicative of normal or abnormal physiological processes, or of a disease or disorder (Song et al. 2014). Cancer biomarkers are molecules secreted by cancerous/tumour cells or in other words a specific response of the body in the

Table 6.1 Specific biomarkers used to diagnose the different cancer types

Type of cancer	Biomarkers	Bioassay-identification
Breast cancer	HER2-oncogene	FISH, IHC
Colorectal cancer	Mutations in the K-RAS-gene stimulate RAS-RAF-MEK signal pathway	PCR
Esophago-gastric adenocarcinoma	HER-2 oncogene	FISH, IHC
Chronic myeloid leukaemia	BCR-ABL oncogenestimulates the tyrosine kinase activity	Cytogenetics, FISH, RT-PCR
Lung adenocarcinoma	BRAF and EGFR (HER1) stimulate the mutations in tyrosine kinase domain of HER2-oncogene Over-expression of	Sequencing
NSCLC	EGFR and HER1 stimulate mutations in tyrosine kinase domain	Sequencing, FISH
Skin cancer-melanoma	Mutation in BRAF oncogene, stimulate the downstream mediator of RAS, it leads to downstream activation of MEK and ERK	Sequencing

FISH fluorescence in situ hybridization, *IHC* immunohistochemical staining, *PCR* polymerase chain reaction, *NSCLC* non-small cell lung cancer

presence of cancer. Various cancer biomarkers are reported in the scientific domain such as proteins, enzymes, nucleic acids (microRNA or non-coding ribonucleic acid (RNA)), peptides and antibodies (Gubala et al. 2012). Cancer biomarkers can also be a pool of alterations at a genetic, proteomic and metabolomic level. On the other hand, alteration in gene level could be hereditary and identified as sequence alteration in the nucleic acid of germline, which is isolated from biological fluids such as blood, buccal cells sputum. Moreover, genetic variation may be somatic as well as it could be recognized as mutations in genetic material originated from cancer tissue (Simon 2010; Gubala et al. 2012; Lommen et al. 2020). Some cancer biomarkers are allied with the skin and smell of breath in addition being found in biological fluids and cancer tissues. The correlation has been shown in scientific literature, connecting breath smell and disease (Silveira-Moriyama et al. 2008; Horvath et al. 2009; Matsumura et al. 2012). These cancer-specific biomolecules are very impulsive organic compounds and volatile in nature, ranging in ppb concentrations. Metabolic products generated by these biomarkers are used as a 'signature array' of the diseased state in cancer biology (Simon 2010). Detection of the exact candidate as a biomarker will be useful to diagnose the cancer. These candidate biomarkers will associate with the pathological condition of the biotic system, which aids better disease treatment outcomes. Moreover, it can point out the disease progression of the patient and provide the best suitable medical opinion (Chen et al. 2015; Syedmoradi et al. 2019). Different stages of the related disease or different diseases can be monitored/diagnosed by a unique biomarker. In this chapter, some of the significant biomarkers are mentioned in Table 6.1, which can be utilized to distinguish various cancer types. These exceptional biomarkers can be used for various biomedical

applications such as screening, diagnosis, prognosis, monitoring, and choice of treatment (Ludwig and Weinstein 2005; Lin et al. 2013).

6.3 Biosensors for Detection of Cancer

In this chapter, the overview of the type of point-of-care test in cancer and highlighted most common types of cancer according to the National Cancer Institute and American Cancer Society are listed (National Cancer Institute n.d.-a, n.d.-b). In this chapter, mention types of cancers are chosen as of their high incidence and mortality, and potential application of POC biosensor to monitor these types of cancers.

6.3.1 Breast Cancer Biosensors

Worldwide, breast cancer is the most commonly diagnosed cancer among females and it has been observed that 1 in 28 Indian women is prone to develop breast cancer during her lifespan. However, it is more for urban women than the rural group (Cytecare n.d.). It has been reported that more than 30,000 new cases projected in 2020 in the United States alone (NIH n.d.). The curable probability of breast cancer is found to be more than 80% when compared to lung cancer in which approximately 30% of the cases have been cured (American Cancer Society 2016; Matzke and Watson 2020). In the last few years, it has been reported that the incidence of breast cancer cases has reduced. Breast cancer has been reported as the second major cancer disorder among women with a very high mortality rate. Among all cancers, lung cancer is the leader with the highest mortality rate.

In breast carcinoma, ductal carcinoma is the most common type which originates in the lining of the thin tubes, also known as milk ducts which transport milk from the lobule to the nipple. Other common types of breast cancers are lobular carcinoma and invasive breast cancer.

In brief, the nucleic acid integrated biosensors utilize the interface between the target to be analysed and the complementary nucleic acid, which is immobilized on the transducer surface; it acts as a biorecognition layer. The sensor is very specific since there is a definite sequence for the nucleic acid (complementary), thus making the recognition highly specific. Therefore, after the biorecognition element recognizes the specific sequence, signal transduction can be done using various mechanical (Quartz Crystal Microbalance and acoustic sensors), optical as well as electrochemical techniques (Soper et al. 2006; Tothill 2009). There is an increase in the signal if the molecules of guanine increase in number. Thus, these sensors can be useful in the cancer rapid screening in case these recognized nucleic acids are expressed in this disease.

Since breast cancer is a very dangerous disease and requires early detection in a fast and simple manner for better prognosis, the use of nucleic acid sensors-based

diagnostic approach signifies huge potential. Using a combined optical and electrochemical technique, an ultrasensitive method was created to detect the mRNA for the proto-oncogene c-Myc in the breast cancer cell line, which is commonly known as Michigan Cancer Foundation-7 (MCF-7) cells. In these sensors, in the recognition element, the anodic pole was modified with the antisense DNA which detected the tumor's cell nucleic acid on a bipolar electrode made of indium tin oxide in a poly (dimethylsiloxane) microchannel (Wu et al. 2012). The electro-chemiluminescence biosensors were capable of detecting 2203 nucleic acid copies in the MCF-7 cell lineage while the immortal hepatic cell line control could only detect 13 copies. This indicates that these miniaturized devices are a good for breast cancer detection. BRCA1 and BRCA2 genes are used to detect breast cancer disorder and these genes lead to the production of tumour suppressor proteins which are responsible for repairing the damaged DNA and thus these genes play a key role in stabilizing the genetic material of the cell. Scientists have found a strong association of breast cancer syndrome with BRCA1 and BRCA2 mutations. Also, the mutations in the BRCA1 are linked to the increased risk of cancer. Thus, the BRCA gene mutation detection can be a worthy approach for the identification of breast cancer. BRCA1 gene mutation was detected by nucleic acid coupled electrochemical biosensor where zinc oxide nanowires have been used for the immobilization of bio-recognition element/probe (Mansor et al. 2014). This technique exploited the differential pulse voltammetry procedure to detect the targeted molecule. Some other techniques use similar nucleic acid sensors for the detection of BRCA1 (Li et al. 2012; Rasheed and Sandhyarani 2014). Not only is this technique useful in cancer diagnosis but it is also important in monitoring cancer development.

One of the most important biomarkers for aggressive breast cancer is HER2, i.e. human epidermal growth factor receptor 2. This is an important marker for choosing the treatment options for breast cancer. This protein marker belongs to the class of tyrosine kinase and is responsible for controlling the growth factors as well as hormones. Since any HER2 alterations are related to the abnormalities in the cells, these can be used as biomarkers for different types of cancer such as abnormal processes in the cell, indicating their use as biomarkers for various cancer types, such as ovarian cancer, breast cancer, etc. (Zwick et al. 2001; Gohring et al. 2010) proposed a biosensor for the detection of HER2 in the serum of humans in combination to the optical ring resonator architecture with microfluidics. This biosensor was made by attaching the antibodies to the surface of the sensor. Since this sensor was able of measuring HER2 concentrations which ranged from 13 to 100 ng/mL, this device was considered as medically appropriate for the real cases.

Jokerst et al. in 2009 developed an optical sensor for the simultaneous quantification of three cancer markers that were carcino-embryonic antigen (CEA), cancer antigen 125 (CA125) and HER2 (Jokerst et al. 2009). This sensor allowed the quantification of the markers in both saliva as well as serum. In this device, semiconductor nanoparticle quantum dots (QDs) were integrated into a microfluidic biosensor. As these QDs have a narrow range of emission peaks, analysis of multiplexed is possible along with a significant increase in the signal-to-noise ratio indicating their advantage over the organic fluorophores in POC devices (Soper et al.

2006). However, these QD conjugates face challenges in terms of stability and aggregation in biological systems as demonstrated by Soper et al. (2006). In this regard, QD bioconjugates capping and optimization of the conditions have proven to be successful in cancer diagnostics (Soper et al. 2006). The detection of different biomarkers simultaneously is considered best by some researchers for the diagnosis as well as treatment of patients (Harris et al. 2007). The levels of CEA and CA125 are also elevated in tumours. However, these are nonspecific antigens for different cancer types.

6.3.2 Biosensors for Colon and Rectal Cancer Diagnostics

Cancers of colon and the rectum are known as colorectal cancer and which is the most fatal cancer in the world and especially in developed nations like the United States after lung cancer. In this direction, point-of-care screening tests can be a useful tool to fight and avoid colorectal cancer incidence. Most colorectal cancer has been detected or identified as a result of irregular cell growths or cell mass in the colon or rectum region of the body. Such abnormal masses can be apart before turning cancerous or at early stages, curing the cancer in most of the case.

Due to late detection of colorectal cancer among the cancer cases, consequently with highest morbidity and mortality rates reported in colorectal cancer (Bray et al. 2018; Nikolaou et al. 2018). According to the reports of 2018, 1.8 million new colorectal cancer cases have been reported globally whose incidence rate is the third highest in the human population (Bray et al. 2018; Rawla et al. 2019). Present routine diagnostics are not user-friendly, normally invasive and time-consuming with variable sensitivity and specificity. In the pathological detection of colorectal cancer, carcinoembryonic antigen (CEA) is the only certified blood biomarker used for prognostic screening of cancers of colon and the rectum. So, development of biosensor is needed to overcome the cancer disease/disorder, with a high sensitive, specific and rapid diagnosis device. Currently developed biosensors are mostly antibody-based, which restricts their commercialization and utility in the medical field due to stability, cost and variability batch-to-batch (Tie et al. 2015, 2016).

Advanced diagnostic tools such as biosensors-based analytical tools can target cancer biomarkers even in small amounts of samples/cells and make them very important in the clinical diagnostic setup. These analytical tools are useful in biomedical applications, exclusively to detect and examine the hidden unusual and rare cells in a massive heterogeneous cell population (Zhang et al. 2014a). These POC bioanalytical tools are very fascinating due to their applications of rapid, fast, user-friendly, and portable. Moreover, medical professionals and patients can know and can apply the results of the measurements obtained from these biosensor devices and which are helpful in treatments. Electrochemical-based biosensing approaches have been reported as useful diagnostic tools as POC biosensors for animal testing. This biosensor device is capable of differentiating between cancerous and normal tissues (Vernick et al. 2011) and was established for the identification and

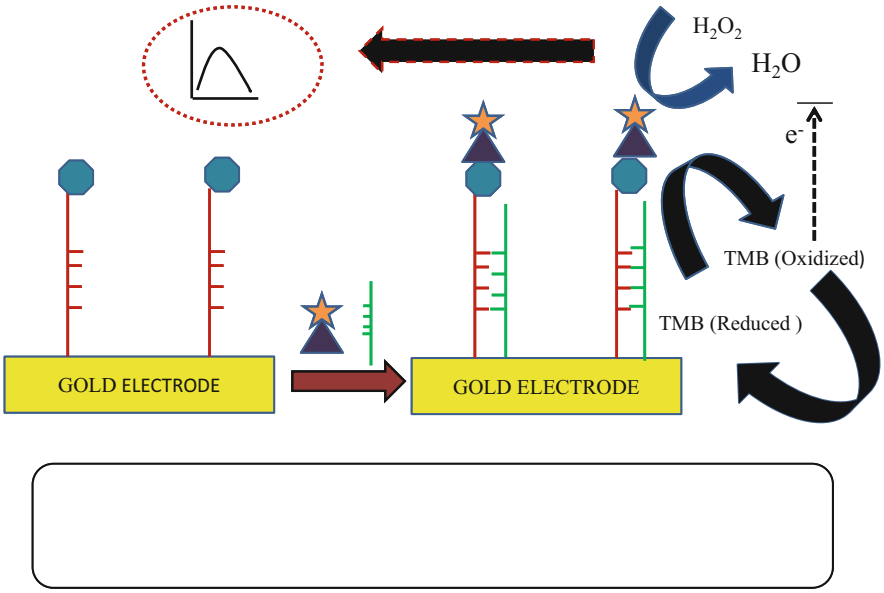

Fig. 6.2 Schematic presentation of enzyme-based nucleic acid biosensor. Target nucleic acid binding and the formation of the duplex make the avidin label HRP binding, which catalyses the electrochemical reaction with the reduction of hydrogen peroxide

quantification of alkaline phosphatase (ALP) enzyme activity. It has been reported that ALP is recognized to be down-regulated in cancer cells and in colorectal cancer cells, enzymatic activity of ALP was measured and it was found to be 0.7 unit/mg protein (Gum et al. 1987). In cancer patient's sample ALP activity was measured on a screen-printed carbon electrode, within a few minutes with the help of a handheld cheap portable device. In this electrochemical assay, gold was used as a working electrode and Ag/AgCl was used as a reference electrode. Recently, a common electrochemical type of DNA-based biosensor has been reported in the scientific literature. Wang et al. (2008) reported that the K-ras gene found to be highly associated with colorectal cancer. This cancer was diagnosed through an electrochemical sensor based on the addition of a sulfhydryl group (–SH) group as modification in horseradish peroxidase (HRP)-labelled probe and chemically adsorbed on the gold electrode through self-assembly. The detection took place through the hybridization of HRP-labelled oligonucleotide complementary to other target DNA parts. Further, the hydroquinone presence as a mediator was used to amperometrically measure the electroreduction current for H_2O_2 which was catalysed by HRP (Fig. 6.2). Feng et al. in 2006 used a glassy carbon electrode which was modified and contained a nanoporous CeO_2/Chitosan composite film (Feng et al. 2006). This film was used for the immobilization and hybridization of the DNA probe. Further, signals were recorded using the indicator as methylene blue and also by the technique of differential pulse voltammetry. This method has various advantages such as low detection limit, good reproducibility, and higher sensitivity

for the target gene sequence detection. Various other optical sensors for cancer detection include microwave, magnetic, and fluorescence-based assays (Tao et al. 2012). The analysis of the electromagnetic properties for the target constitutes the main principle of the sensors. It has been reported that benign and malignant tumour cells were detected with help of electromagnetic signatures, which is exclusively present in the cancer cells or tissues. (Zhang et al. 2014a). Also, he showed the estimation of the different phases of the tumour as the cancer diagnosis depends on the tumour stages. With the increase in the cancer stage, i.e. from 0 to 4, there is an increase in tumour aggression and more chances of proliferation and metastasis into the remote tissues. The data from the five different colon cancer cell lineages which were in their different stages were taken with the help of 15 passive biosensors as well as 4 adjustable sensors. The above-mentioned study has illustrated that analysis of the intracellular activity in terms of dielectric permittivity by the use of microwave frequencies, which is used to evaluate the degree of aggressiveness of malignant cells. This POC biosensor is indeed a promising tool for detecting the abnormal or sporadic cells and which is offering an early, rapid diagnosis with the aim to measure and avoid cancer spread, consequently stop the metastasis development.

6.3.3 POC Biosensors for Bladder Cancer

Due to the chronic condition of inflammation and irritation, there are chances of development of adenocarcinomas and squamous cell carcinomas in the inner lining of the bladder (National Cancer Institute n.d.-a, n.d.-b). As per the data by the National Health Services in the United Kingdom, the elder population is affected to a larger extend with the bladder cancer. Although this cancer is not always preventable, few of the lifestyle changes example changing the dietary habits and smoking cessation may help reduce the risks. The early cancer diagnosis can be helpful in minimizing the patient sufferings. There are several literature reports that focus on this purpose. A method for detecting the specific bladder cancer DNA sequence was demonstrated by researchers from China (Zhang et al. 2014b) where by modifying the surface of GC nucleotides with CdTe QDs-semiconductors, they developed an electrochemical biosensor. Few factors that help ease the sensor operation are good sensitivity as well as selectivity, low cost, rapid response and help in the achievement of good results.

6.3.4 POC Biosensors for Kidney Cancer

One of the most common kidney cancer types is renal cell carcinoma. It is estimated that nine out of ten cancers of kidney belong to renal cell carcinomas (American Cancer Society n.d.). A cancer developed in the small tube lining in the kidney is known as renal cell carcinoma, and small tubes lining in the kidney are responsible

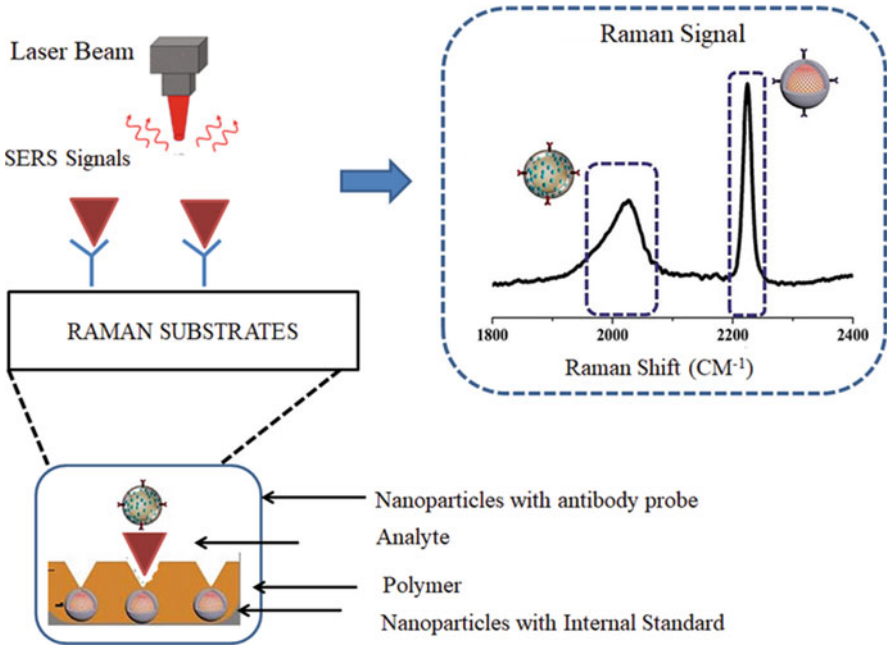

Fig. 6.3 Schematic illustration of POC device integrated with SERS technology for quantitative detection of targeted cancer biomarkers

for the filtration of the blood and removal of waste products. A particular type of cancer that is formed in the kidney centre where there is collection of the urine is called the transitional cell cancer. Another type of cancer developed in the children's kidney of age less than 5 years is called the Wilms' (based on information of the National Cancer Institute). As per the 2015 data, there is an estimation of about 61,560 renal kidney cancer cases in the USA. This analysis mainly includes the renal cell carcinomas along with up to 5% renal pelvis behaving more like the bladder cancer as well as 1% cases of Wilms' tumour (1%) (American Cancer Society 2015). The major limitation is the absence of any urine/blood tests that could detect the kidney tumour presence. For the complete evaluation of the suspected tumour, a combination of the ultrasound and CT scan is required (Urology Care Foundation n.d.).

Although there are only limited studies on the biosensors for the kidney cancers, a patent from America offered a label free technique for the renal cancer diagnosis at an early stage. This approach was based on the surface-enhanced Raman spectroscopy (SERS) as well as the localized surface plasmon resonance (LSPR) as transducer platforms. These techniques are based on reading the protein fingerprints which further allows the exact measurement of protein in the urine. Rapid screening of kidney cancer was assessed with SERS and LSPR-based biosensor using metal nanostructure, and which used adipophilin (ADFP) and aquaporin proteins that are considered as non-invasive biomarkers for kidney cancer detection (Fig. 6.3)

(Singamaneni 2012). Another POC-based paper strip integrated plasmonic technology was adopted in which kidney cancer-specific proteins were targeted and found to be very sensitive for kidney cancer screening (Tian et al. 2012). Therefore, the creation of the biochip provides various benefits including the easy storage, lower cost as well as diagnosis of POC. A major hurdle in the development of POC devices for cancer diagnosis is the transformation of paper-based LSPR substrates to printable microfluidic devices, which is offered to detect multiple analytes in complex biological fluids.

6.3.5 POC Biosensors for Leukaemia

On the basis of the type of cell and growth rate, leukaemia can be further categorized into four groups: (1) acute lymphocytic leukaemia (ALL), (2) chronic lymphocytic leukaemia (CLL), (3) acute myeloid leukaemia (AML) and (4) chronic myeloid leukaemia (CML). About 91% of the leukaemia patients mainly include the age group of 20 years and above (American Cancer Society n.d.; Abdulhalim et al. 2008). Thus, an efficient treatment of this disease, the diagnosis is required to be very accurate and sensitive. The current detection methods for this cancer involve the use of PCR, flow cytometry as well as the measurement of the fluorescence (Shan et al. 2014). In view of that, there is a new proposal of a sensitive and quantitative biosensor for the diagnosis as well as treatment with POC properties (Liu et al. 2009) based on the combination of the optical properties of gold nanoparticles. Single-stranded oligonucleotides are known as aptamers, which bind to a certain target molecule, having the advantage over the conventional molecular probes such as the ease of production, stability as well as high specificity as compared to others (Jayasena 1999). The interaction of lymphoma cells (Ramos cells) with gold nanoparticles conjugated-aptamers letting the rapid cancer cell detection in the bloodstream at a lower price. Also, there is a possibility of using different aptamers for detecting several cancer cell types in the near future. The optical transducers are commonly used in the research for cancer biosensors. Field-effect transistors-based biosensor is also reported for cancer detection, using the fluorescence and nanoparticles (Soper et al. 2006). Surface plasmon resonance (SPR) is an optical technique and offers the detection of target molecules with help of change in the refractive index of biomaterials or nanoparticles (Abdulhalim et al. 2008). This biosensor coupled with cited spectral imaging technique is useful for the examination of the type of leukaemia with the help of specific myeloid antigen and CD33 (Fang et al. 2011). This antigen is useful in the diagnosis as well as determination of myeloid leukaemia treatment as it is a cell surface marker. The results obtained are further compared to the standard flow cytometry test and are corroborated statistically. Also, this technique has high reproducibility, takes less time, enables its direct analysis and is also cheap as compared to the conventional methods.

6.3.6 Melanoma Biosensors

As per the National Cancer Institute (NCI) and American Cancer Society (National Cancer Institute n.d.-a, n.d.-b), melanoma is the malignancy of the cells producing the skin pigmentation called the melanocytes. This is one of the most dangerous forms of skin cancer. It is more common in the fair skin people; however, it may occur in all types of skin colours. The melanomas depict as a dark spot like a mole, only it has an irregular border. This disease may be hereditary and the overexposure to the sun increases the risk of this disease. The diagnosis of this cancer type involves tyrosinase monitoring which is a cytoplasmic melanocyte differentiation protein. This protein is a major enzyme in the synthesis of melanin and is listed as an important melanoma biomarker. Mossberg et al. (2014) developed an electrochemical biosensor platform with an amperometric detection mode for the detection of the trosinase enzyme activity in fresh biopsy samples without pre-treatment of the samples. The combination of this method with modern portable devices can provide interesting POC sensors in the future. D'Amico et al. in 2008 developed a technique which used an electronic nose sensor with a good sensitivity towards volatile organic compounds emitted by skin lesions of melanoma patients (D'Amico et al. 2008). The method was effective in the identification of malignant lesions. The gas sensor used gas chromatography mass spectrometry detection and showed satisfactory accuracy. This approach combined chemometric tools with a sensor that can be a favourable approach to detect a pattern in POC biomedical devices.

6.3.7 Biosensors for Lymphoma Cancer

The lymphoma is a type of cancer that begins in cells of the immune system. It is categorized into two basic categories: Hodgkin's lymphoma, depicted by the presence of a Reed-Sternberg cell and in non-Hodgkin's lymphoma which consists of a large varying group of cancers of immune cells. The non-Hodgkin's lymphomas are divided into two wide cancer groups: first having an indolent (slow-growing) course and second that have an aggressive (fast-growing) course. Mansur et al. in 2014 have revealed that the imminent applications for in-vitro identification of non-Hodgkin's lymphoma (NHL) tumours using novel multifunctional immunoconjugates composed of QDs as the fluorescent inorganic core and antibody-modified polysaccharide as the organic shell (Mansur et al. 2014). The QDs/immunoconjugates have shown binding affinity to antigen CD20 (aCD20) expressed by malignant B-lymphocytes.

6.3.8 POC Biosensors for Lung Cancer

Lung cancers form the lining in the cells of the air passages of the lungs. The two main categories are small-cell lung cancer and non-small-cell lung cancer. This categorization is based on the appearance of the cells under a microscope (NIH n. d.). thymidine kinase 1 (TK1) and CEA are the major biomarkers of this cancer type. CEA is an acidic glycoprotein and found to be elevated in ovarian, breast, liver and lung cancers. It is useful for monitoring the reoccurrence of cancer after surgery as well as for follow-up of patients during therapy. In healthy individuals, serum CEA levels are lower than 5 ng/mL (Ronkainen and Okon 2014). The studies on the levels of CEA have illustrated a strong link of elevated marker levels with metastases and poor prognosis (Ronkainen and Okon 2014). Wang Li et al. in 2010 developed an important biomarker for the detection of nitrated ceruloplasmin in lung cancer, cardiovascular diseases and stress response to smoking (Li et al. 2010). The scientists developed a portable fluorescence biosensor based on QDs and a lateral-flow test strip. The results for this hold good for POC and in-field analysis of protein biomarkers. With the help of the same biomarker, Gao et al. (2011) constructed an amperometric immunosensor for detecting the CEA, utilizing uniform carbon nanotubes (CNTs)-based film with gold nanoclusters and anti-CEA immobilized antibodies having a detection limit of 0.06 ng/mL and good stability. On the contrary, Alegre et al. in 2014 developed a traditional enzyme-linked immunosorbent assay (ELISA) for detecting TK1 in serum (Alegre et al. 2014).

6.3.9 POC Device for Pancreatic Cancer

The formation of tumours in the endocrine cells and exocrine cells of the pancreas are different. Also, these cells produce different symptoms and have varied risk factors, thus it is very important to distinguish between exocrine and endocrine cancers of the pancreas (Kim et al. 2004).

The measurement of serum carbohydrate antigen (CHA) 19-9 has shown satisfactory sensitivity and predictive potential in pancreatic cancer patients (Kim et al. 2004). Zhang et al. (2014c) demonstrated a sandwich-type electrochemical immunosensor for the detection of CHA 19-9 antigen based on the immobilization of primary antibody (Ab1) on a three-dimensional ordered macroporous magnetic (3DOMM) electrode (Zhang et al. 2014c). The 3DOMM electrode was fabricated by the introduction of core–shell Au–SiO_2@Fe_3O_4 nanospheres onto the surface of a three-dimensional ordered macroporous Au electrode. Chang et al. (2013) developed a high-throughput biosensor based on metal-enhanced fluorescence technique for detection of a pancreatic cancer marker, UL16-binding protein 2 (ULBP2), in diluted human serum. The authors have described this biosensor as a cost-effective high-throughput sandwich immunoassay; compared with the limit of detection (LOD) of

the conventional ELISA method, the LOD of the proposed biosensor for ULBP2 is significantly improved 100-fold under the same conditions.

6.3.10 POC Biosensors for Prostate Cancer

For prostate cancer identification, prostate-specific antigen (PSA) is used as the main biomarker; it is a serine protease which is synthesized in the epithelial cells prostate gland and its expression is regulated by an androgen receptor. In the human body standard range of PSA is found to be 0–4 ng/mL and it is elevated in the case of prostate cancer or disorder (Jolly et al. 2015). Its tissue specificity along with cancer sensitivity makes PSA the most valuable accessible tumour biomarker for rapid screening as well as managing prostate cancer (Ronkainen and Okon 2014). SPR integrated biosensor has been reported and used for measuring real-time quantitative monitoring of PSA. In this immunoassay, interactions of specific antibodies with different epitopes of free and complex PSA in the test samples were assessed using microfluidic immunoassay devices (Damborský et al. 2015). It was also described that SRP-based biosensor is a selective, sensitive and reliable POC device for prostate cancer diagnosis. In another study, a CNT-based electrochemical immunoassay was reported for PSA detection. Sandwich-based immunoassay was performed on a carbon electrode for simultaneous and sensitive evaluation of PSA and interleukin 8 (IL-8), which is another cancer biomarker (Wan et al. 2011). Mohd Azmi et al. (2014) developed a handheld, POC device, in which a silicon nanowire immune-sensors chip is wire-bonded to a bio-smartcard as well as consequently inserted into an electronic readout system. These immune-sensors were used to detect 8-hydroxydeoxyguanosine (8-OHdG), an oxidative stress biomarker, which has been associated with prostate cancer risk.

6.3.11 POC Biosensors for Thyroid Cancer

The thyroid gland is an organ that produces hormones that maintain the physiology of biological systems such as control blood pressure, heart rate and body temperature. Thyroid cancer is broadly divided into four main groups, follicular, papillary, medullary and anaplastic thyroid cancers, based on the appearance of cells by using microscopic study. Thyroglobulin (Tg) is a protein biomarker, which is used as tumour biomarker for thyroid cancer/disorder detection (Burne et al. 2005; Krahn and Dembinski 2009). Moreover, it is also used as a post-surgical biomarker to observe disease relapse and it describes POC assays for autoantibodies to thyroglobulin and to thyroid peroxidase (TPO). Both immunoassays are inhibition-based assays, in which binding of monoclonal antibodies to TPO or to Tg proteins is inhibited due to the presence of auto-antibodies in test samples. These assays don't require any specific equipment and results are observed in 10 min.

Microfluidic technique-based biosensing platform for the detection of Tg using competitive protein adsorption has been reported by Choi and Chae (2009). In this assay researchers designed two surfaces which are covered with two known protein fibrinogen and immunoglobulin G (IgG), having different affinities (Choi and Chae 2009). Immunoassay-based microfluidic techniques are fluorescent labelling-based technologies which provide selective protein detection by a target Tg protein and these proteins are used to assess exchange and adsorption of auto-antibodies in the samples. Therefore, POC immune-sensors can be beneficial for the onsite diagnosis and used to detect cancer with the aid of different cancer-specific biomarkers. Additionally, research in POC biosensors is necessary for the development of rapid diagnosis technique to be suitable and available to the public. Furthermore, the field of clinical research in the area of diagnosis also needs to work on these modern approaches to develop feasible POC for public health monitoring use in the remote area (Nayak et al. 2017).

6.4 Future of POC-Based Biosensors

The POC-based biosensor has truly transformed the current diagnostics approach in the field of clinical research. Keeping the importance of POC-based biomedical diagnosis research and its future in perspective, prepare a list with essential key points that the scientific community should be deliberated for the coming development of POC devices.

Following points are necessary for future development of POC biosensor for biomedical application: (1) a molecular signature or a panel of signatures may be used for the development of POC biosensors that need early rapid detection of cancer. In this context, it is critical to include the compulsion of (2) integration of POC biosensors with physio-chemical approaches along with pattern recognition tools to assess this complex molecular signature such as gene, protein, receptors, etc. These mentioned methods can consist of Artificial Intelligence (AI), Artificial Neural Networks (ANN), Principal Components Analysis (PCA), and Soft Independent Modelling of Class Analogy (SIMCA), (3) enhance the specific selectivity of POC device owing to the complexity of sample media, (4) developing POC biosensor work for tissue samples analysis, (5) small amount of sample required for the analysis, (6) increase sensitivity as well as less expenses. In addition, develop biosensors incorporate POC with easy processing along with sample handling to improve companion devices for biomedical application and research. The combination of multivariable calibration is vital to make devices for rapid and simultaneous diagnosis of different types of cancers.

6.5 Conclusion

Biomarkers of cancer/cancer disorders are biomolecules present in the body fluid or tissue that are indicative of normal or abnormal physiological processes. Cancer biomarkers can also be a pool of alterations at a genetic, proteomic and metabolomic level. On the other hand, alteration in gene level could be hereditary and identified as sequence alteration in nucleic acid of germline, which is isolated from biological fluids such as blood, buccal cells sputum. These cancer-specific biomolecules are volatile and impulsive organic compounds, which are found at ppb range concentrations. Detection of the exact candidate as a biomarker will be useful to diagnose the cancer. In this chapter, we highlighted the potential applications of POC biosensors for the diagnosis of the most common types of cancer according to the National Cancer Institute and American Cancer Society.

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Chapter 7
Point-of-Care Diagnostic Testing in Urgent Cardiac Care

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7.1 Introduction

Cardiovascular diseases (CVDs) are a category that groups together a variety of heart and blood circulation distresses including coronary heart disease, rheumatic heart disease, peripheral arterial disease, deep vein thrombosis, etc. As per World Health Organization, these disorders together are the leading causes of deaths globally, amounting to almost 32% of the total deaths worldwide (17.9 million deaths in a year 2019). These fatalities not only affect the developed countries and low-income group countries in human life loss but also lead to economic losses. In last few decades, the mortality rate has reportedly increased in developing countries and the condition gets much worse in developing and underdeveloped countries, which contribute to greater than three-fourths of total CVD fatalities worldwide (WHO CVD factsheet 2021). Out of these, cerebrovascular diseases (e.g., Stroke) and coronary heart diseases (e.g., Heart attack) amount to 85% of CVD deaths. This difference in mortality rate is the result of a wide gap in the availability of advanced health-care facilities in middle- and low-income nations environmental and socioeconomic status, etc. (Leigh et al. 2016). In order to improve these statistics and upgrade cardiovascular health in people, there is an immediate requirement for technologically advanced diagnostic and treatment systems that are easily accessible, cost-efficient, and portable to point-of-care (PoC) centers. Analysis and estimation of

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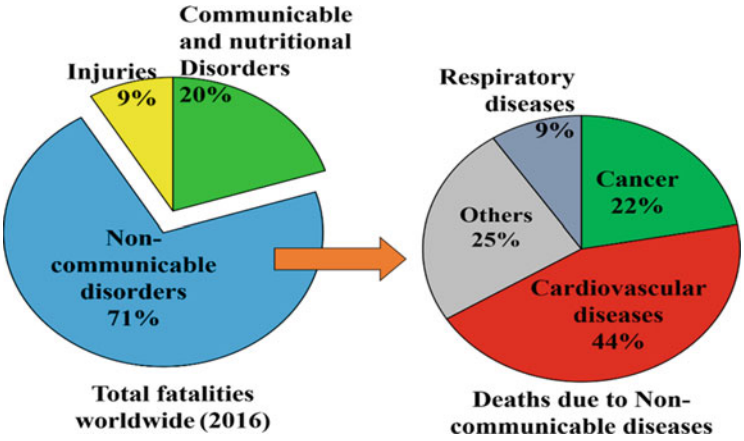

Fig. 7.1 Distribution of worldwide fatalities (year 2016) by WHO

cardiovascular biomarkers is critical in PoC for better prognosis, monitoring, and management in patients (Fig. 7.1).

7.2 Point-of-Care CVD Testing

Traditional diagnosis methods for CVDs performed in laboratories are strenuous, time-consuming, and need highly skilled technicians and sophisticated instruments (Baryeh et al. 2017). To overcome these hurdles, point-of-care testing (PoCT) systems are developed, which are simple and easy to perform. During the development of efficient biosensors, it is highly desirable that they are usable in PoCT. PoCT refers to the practice of diagnosing and monitoring any disorder in patients for fast and high accuracy results. These tests also need to be low-cost, simple, and should not require high-skilled technicians and/or sophisticated laboratory instruments. The PoC tests need to present on the spot analysis and are less sensitive to environmental changes. This kind of PoCT plays a very crucial role in developing or undeveloped nations where lack of financial capabilities, limited infrastructure, unskilled technicians, etc. can cause an increment in causalities.

7.3 Biomarkers in Cardiovascular Diseases

A biomarker is described as a "measurable and quantifiable biological parameter" for example, an enzyme, any protein fragment, or specific hormone concentration present in a clinical sample (e.g., blood, tissue, urine etc.), which presents itself for physiological and health examinations like disease prognosis, environmental

distress, neurological disorders, etc. The primary function of a cardiovascular biomarker is to enable the health-care personnel in optimal management of patient. For example, a biomarker (Dobutamine stress echocardiogram) may be expected to enable the detection of patients with severe chest pain from an ischemic etiology or angina in a person with chronic or atypical chest pain. A successful identification of biomarkers could aid in distinguishing cases with acute myocardial infarction (AMI) from those with acute pulmonary embolism, unstable angina, or aortic dissection (e.g., trans-esophageal echocardiogram) in a timely manner to encourage targeted management in an emergency department admission presented with acute severe pain in chest (suspected acute coronary syndrome).

7.3.1 Stand Alone and Unique Biomarkers

N-terminal propeptide B-type natriuretic peptide (NT-proBNP), Cardiac troponin (I (cTnI), T (cTnT)), B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (hs-CRP), and mid-regional pro-atrial natriuretic peptide fragment (MR-proANP) are six well-established, stand alone, and unique biomarkers preferred for the detection of myocardial damages and cases of heart failure.

7.3.1.1 Cardiac Troponin

Cardiac troponin is the most favored detection marker in detecting damaged myocardial tissues and diagnosing acute coronary syndrome/myocardial infarction. It is a group of proteins (troponin I, T and C) present in heart myocardial tissues, which regulates muscle contraction. When the cardiomyocytes are damaged, cardiac troponin is a marker released into the bloodstream, and both cTnT and cTnI can be linked to recognize damage to cardiac myocytes that may be caused by heart failure (HF) or acute coronary syndrome/myocardial infarction (ACS/MI). For ST-segment elevation myocardial infarction (STEMI) (O'Gara et al. 2013) and Non ST-elevation acute coronary syndrome (NSTE-ACS) (Amsterdam et al. 2014), cTn is the main, most sensitive, and precise biomarker. The level of troponin starts to increase as soon as the symptoms start to appear and remain elevated up to many weeks. Therefore, it is highly targeted in all the patients displaying the signs similar to ACS when brought to emergency, to at least a few hours after symptoms emerged. Myocardial damage can be identified as the rise in cardiac troponin level above the accepted clinical limit of the 99th percentile, and this value is assay dependent. In addition, regular monitoring of troponin I levels after the patient has been admitted and up to 4–6 h after admission of all patients presented with ACS like symptoms for early risk stratification in ACS in order to detect an increasing and/or dropping trend of values (Amsterdam et al. 2014).

7.3.1.2 Brain Natriuretic Peptide (BNP) and N-Terminal proBNP (NT-pro-BNP)

NT-pro-BNP and BNP are highly targeted diagnostic markers for early heart damage stratification, where risk assessment in cases suspecting acute coronary syndrome can be contemplated. European Society of Cardiology (ESC) guidelines (Roffi et al. 2016) reported that Natriuretic Peptides (NPs) deliver additional cardiac troponin diagnostic data for acute coronary syndrome (Thygesen et al. 2012). When the myocytes stretch and get damaged, BNP is secreted and therefore more applicable to heart failure biomarker. BNP is prepared in the form of a prohormone called pro-BNP, which further cleaves in an active C-terminal (BNP) and an inactive N-terminal fragment (i.e., NT-proBNP) with a higher half-life than former (Weber and Hamm 2006).

7.3.1.3 C-Reactive Protein (CRP)

One of the most researched and analyzed biomarker in case of CVDs is the high-sensitivity C-reactive protein (hs-CRP) and it is useful in risk stratification in CVDs. Hs-CRP is a critical protein released in case of primary as well as secondary CVDs (Adukauskienė et al. 2016). In case of cardiovascular disorders, several inflammatory markers like CRP are linked with future risk calculation in visibly healthy population, as evident through more than 50 studies (Ridker 2014).

7.3.1.4 Mid-Regional Pro-Atrial Natriuretic Peptide (MR-proANP)

As a diagnostic marker for acute heart failure in the emergency department, MR-proANP is prescribed as an alternate diagnosis to offer efficient identification of AMI from other noncardiological sources of acute dyspnea with a typical higher concentration of 0.12 ng/mL (Ponikowski et al. 2016). MR-proANP levels can vary with factors like sex, age, body mass index race, or conditions like atrial fibrillation, like NT-proBNP and BNP biomarkers (Maisel et al. 2010).

7.3.1.5 Copeptin

When paired with cTn, Copeptin has proved to be clinically significant in diagnosing NSTE-ACS and MI. Copeptin is stoichiometrically cosecreted with vasopressin, but because of a higher blood half-life value, it is greatly favored over vasopressin and easier in measurement (Morgenthaler et al. 2008). Copeptin also provides better analytical data helpful in ruling out myocardial infarction if hs-cTn values are available. However, it is still important to thoroughly elucidate the incremental value of copeptin over hs-cTn assays.

7.3.1.6 Galectin-3

Fibrosis and inflammation are associated with Gal-3 such that they can be associated with development, initial risk analysis, treatment, and advancement of heart failure. The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) recommends Gal-3 in outpatient and acute settings as an evolving marker of myocardial fibrosis for HF having advantages like advanced risk assessment, since it can assess the requirement of hospitalization in extreme cases (Yancy et al. 2017).

7.3.2 Biomarkers that Provide Additive Information in Cardiovascular Patients

Usually, in the guidelines, these proteins cannot provide decisive data for predicting cardiac ailments due to their nonspecificities and low sensitivities toward CVDs and therefore not regarded as standalone cardiac biomarkers or because of restricted reproducibility as evident in patient trials. However, their usefulness in multibiomarker approaches is highly observed in real patient cases and can offer excellent sensitivity and precision in combination.

7.3.2.1 Myoglobin

Myoglobin (MB) is a muscle oxygen storage protein, which is mostly secreted in muscle and cardiac tissues. During muscle injury, myoglobin is released into the blood stream from damaged muscle. Therefore, an increase in myoglobin levels in blood stream is an indicator of muscle damage. In the pathogenesis of the phenomenon of being released into the blood, myoglobin can be used to fulfill the detection requisites of acute ischemic myocardial injury disease in patients, as early as possible using rapid diagnosis criteria.

7.3.2.2 Creatine Kinase-Muscle Brain (CK-MB)

Creatine kinase (CK), previously called as Creatine phosphokinase, is an intracellular enzyme found in the skeletal muscle, myocardium, and brain. Before cardiac troponin, CK-MB was major biomarker used for detection of CVDs. CK is a dimeric molecule consisting of the two subunits M and B assigned. Combinations of these subunits form the CK-MM, CK-MB, and CK-B isoenzymes. The primary locus of CK-MB is the myocardium. A substantial CK-MB isoenzyme concentration is located almost exclusively in the myocardium, and the presence of elevated serum CK-MB levels is highly specific and susceptible to damage to the myocardial cell

wall. Standard reference values fall in the range 3–5% (percentage of total CK) or 5–25 IU/L for serum CK-MB.

7.3.2.3 Myeloperoxidase

Myeloperoxidase (MPO) is a commonly used inflammatory biomarker for predicting adverse cardiac outcomes. After the AMI occurs, for first 24 h, MPO levels are observed to be elevated in the serum. The rupture of an atherosclerotic plaque results in several ischemic cases, rendering MPO an important biomarker for diagnosing fatal heart conditions such as AMI, ACS, etc. This biomarker can be used without need of any other diagnostic biomarkers like cTn or CK-MB (Nicholls and Hazen 2005).

7.3.2.4 Low-Density and High-Density Lipoprotein

Individual levels of HDL and LDL, as well as the LDL to HDL ratio, offer vital role in prediction of coronary heart disease and their risk stratification for differentiating different kinds of CVDs (Honikel et al. 2018).

7.3.2.5 Interleukin-6 (IL-6)

Elevated levels of IL-6 has been associated with congestive heart failure (CHF), since inflammation is one of the major occurrences during heart tissue damage. In chronic CHF patients, IL-6 could rise due to several reasons like heart tissue rupture and increase in blood IL-6 could be a direct indicator of risk associated with the damage in tissues.

The overall biomarkers responsible for cardiovascular diseases are depicted in Fig. 7.2.

7.4 Biosensors for Cardiovascular Diseases

Sensors are fabricated for variety of applications including PoCT, industrial, laboratory bench-top setting, or home-testing. The type of application targeted dictates the detection method, size, and sample to be analyzed. In PoC settings, biosensors for CVD biomarkers hold several setbacks in terms of sensitivity, performance, or technical viability of detection methods.

Usually, biosensors are an assembly of separate components put together into an integrated system. The function of overall biosensor setup is to convert a biosignal into a clinical data, which can be employed for diagnosis and monitoring the

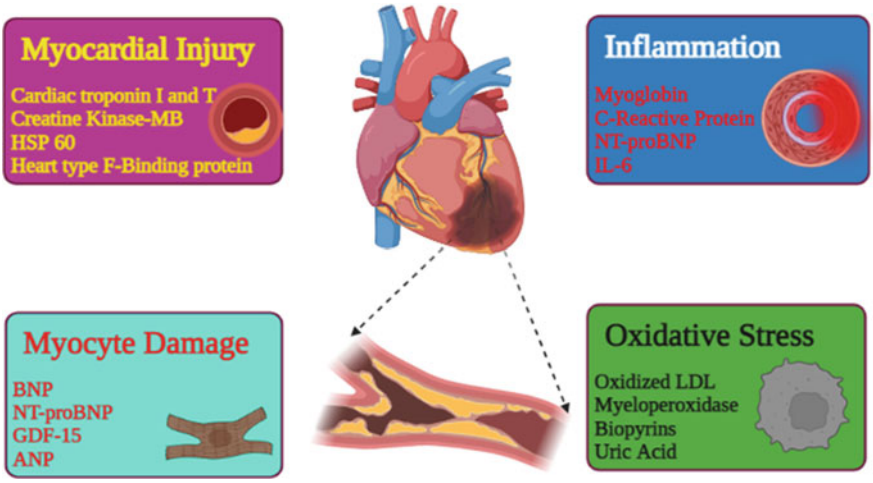

Fig. 7.2 Cardiovascular disease biomarkers. (Created with BioRender.com)

disorder. Biosensors have three predominant components, namely biorecognition probe, signal transducer, and signal-processing unit.

The biorecognition agent in a biosensor function as an identifying receptor surface, which identifies the analyte in biosample. The biorecognition surface is functionalized using a highly specific recognition agent like antibodies, enzymes, DNA/aptamers, cellular structures, microstructured materials like carbon nanotubes, etc.

The biological reaction is transformed by the signal transduction agent into a readable or measurable signal. Signal transduction techniques could vary between them. Techniques, varying from the levels of current produced by the system to deflection of laser beam, could be used in biosensors. The processor amplifies the signal received by the transduction unit and the final analysis, which is corresponding to the biomarker-in-interest, is displayed using an interface.

Biosensors are generally categorized according to their use of a label, in addition to transduction techniques. Labeled techniques use a secondary or tertiary physiochemical reactions for predicting various concentrations of biomarkers indirectly. The most common examples of this are use of enzymes like horseradish peroxidase (HRP) and glucose oxidase (GOx). Methods of label detection have many advantages, such as amplification of responses and improved sensitivity and specificity. Label-free methods of detection do not require preparation of samples or analytes with detection species such as fluorescent, enzymatic, or nanostructured labels, making it much user-friendly than other methods.

7.4.1 Clinical Biosensors for CVD Detection

Many standalone biomarkers have been already used in commercially available cardiac distress sensing with approval from Food and Drug Association (FDA) and other national and international agencies. These systems may often calculate single biomarkers, although many platforms use multiple markers in a single device for specific diagnosis, such as MI.

7.4.1.1 Available Point-of-Care Devices for CVD Biomarkers

Point-of-care testing can be portable hand-held or small bench-top systems, using simpler biosample collection and processing and is able to generate fast and sensitive results, which equip clinicians for better patient diagnosis and monitoring. POC testing has resulted in reducing total time required from 110 min (centralized laboratory tests) to 17 min for common cardiac biomarker measurements (Gaze 2016). Patient trials have shown that the cases, which received early CVD diagnosis using PoCT, have usually shorter hospitalization duration and faster recovery (Goodacre et al. 2011). POC testing may be utilized to broaden the quantitative estimation of data for wide range of citizens, specially low- and middle-income groups, beyond those who have easy access to cardiovascular clinical care, and thereby increase the efficacy of PoC studies (King et al. 2016). With several benefits, there are still few setbacks, which prevent adaptations of PoCT, for example, robustness, sensitivity, installation at clinical centers and their use in real time, analysis of results by clinicians in contrast to laboratory reports, etc.

Commercial PoCT for CVDs can be categorized into following four categories (Ouyang et al. 2020):

1. Hand-held device
2. Large clinical laboratory setups
3. Bench-top device
4. Qualitative tests readable by eye

Primary CVD testing using laboratory approach takes patient's venous blood testing them using large laboratory setups and provides advanced analytical outcomes, a bench-top system on the other hand generally uses shorter analysis time and is not as costly. Still bench-top needs semiskilled technicians to operate and analyze the instruments, thus limiting their usage in all PoC like ambulances or rural locations. A hand-held PoC on the other hand is fast with smaller effort in sample processing and analysis with usability outside hospitals or laboratories.

Devices like Cobas h232 (Roche), i-STAT (Abbott), and Minicure I-20 (Philips) are examples of handheld devices, which are used for detection of several biomarkers like cTnT, BNP, NT-proBNP, etc. In bench-top devices, PATHFAST (Mitsubishi Chemical), Mini VIDAS (BioMerieux), RAMP (Response Medical), and Fluoro-Checker TRF (Nano-Ditech) are examples of single biomarker analysis

systems available commercially. Laboratory systems like ARCHITECT by Abbott, BRAHMS by Thermo Scientific and rapid testing kits like Roche Cardiac by Roche, Nano-check by Nano-Ditech, etc. are available in current markets for PoCT purposes. These devices assess not only acute myocardial infarction or heart failure but also cardiac ischemia, etc.

All devices achieve a fair 15–20 min time response and utilize analytes in quantities ranging from 50 to 1000 μL. The quantitative system measurable concentration ranges diagnose the cut-off values according to the prescribed clinical ranges of Myoglobin, CK-MB, Brain natriuretic peptide, NT-proBNP, and h-FABP.

7.4.1.2 Multiplexed Biosensor for Cardiovascular Diseases

Multimarker approach takes into account more than one cardiovascular biomarker for the purpose of improving sensor performance. With the help of combined data from multiple biomarkers, sensors can be made more robust, sensitive, specific, accurate, and more definitive for the prognosis to be made. However, larger sample volume, increased material requirement, higher testing time, and increase diagnosis cost can be few expected drawbacks for this method.

Few examples of multimarker biosensors available commercially are as follows (Ouyang et al. 2020):

1. CardioDetect by Renesa for detection of cTnI and h-FABP
2. RapiCard InstaTest by Cortez Diagnosis for cTnI, CK-MB, and Myo
3. LifeSign 3-in-1 by Princeton Biomedicals for cTnI, CK-MB, and Myo
4. Randox Biochip Cardiac Array by Randox for cTnI, CK-MB, Myo, and h-FABP

These multimarker tests still lack the panels recommended for clinical samples and none of the current available devices address other significant biomarkers such as copeptin, hs-CRP, MPO, etc. In order to measure low cTn levels for heart failure determination, and very low concentrations for early MI detection, more sensitive cTn assays are required. It is important to create a multimarker, cost-efficient, portable ACS/MI sensor, usable even in an ambulance, or at the POC. In addition, there is a great need and shortage of inexpensive, handheld, multimarker IVD tracking items for POC devices.

7.4.2 Research Platforms for CVD Biomarkers

Although there are many biosensors available worldwide, the need for lower detection limit and high specificity with cost efficiency is still challenging. The research community has been highly invested in investigating cardiac biomarkers and publishing potential sensors with PoC applications. In past 10 years, majority of research focusing on fabrication of PoC sensors for cardiovascular disorders have used popular markers like cardiac troponin (Ceylan et al. 2018; Zhang et al. 2018;

Sinha et al. 2019), BNP (Ceylan et al. 2018; Li et al. 2019), CRP (Boonyasit et al. 2019; Ballard et al. 2020), and add-on biomarkers like myoglobin and Creatine kinase-MB (Zhang et al. 2018; Xu et al. 2020). On the other hand, other transpiring markers have been less lucrative to researchers. Some sensing platforms have been developed for biomarkers like copeptin (Li et al. 2020), Galactin-3 (Primo et al. 2018), and Myeloperoxidase (Wen et al. 2018), although MR-proADM and MR-proANP had not been explored much by researchers. In contrast to sensing devices, which are available commercially, where transducing element is primarily optical (fluorescent based or colorimetric), researchers prefer a wide variety of platforms like magnetic, electrochemical (capacitive, Electrochemical impedance spectroscopy), surface-enhanced Raman spectroscopy (SERS), etc. to improve sensor performance.

Another way to boost the signal beyond the use of a more responsive transduction feature is to use sophisticated computational analytical methods for multiplex optical sensing systems (Ballard et al. 2020). Few literature studies have also expanded their research by preferring other biorecognition materials like aptamers (Sinha et al. 2019) and chemical-interaction mechanisms like calcium-dependent binding affinity of CRP for phosphocholine (Boonyasit et al. 2019), in addition to trying to improve sensing capability. Instead of commonly used antibodies, aptamers decrease reagent costs, prepare stable platforms and higher shelf-life, particularly under ambient conditions (i.e. stable at room temperature). Although the efficacy of aptamers in a multispot platform has been demonstrated, their cross-reactivity still poses higher concerns.

7.4.3 Recent Research Advancements for Multiplexed Biosensors

Majority of multiplexing strategies employ spatial-based approach, which is using different lines or spots for separate analytes. These spots, functionalized using specific transducing agent, are used for fast and easy-to-read signal display. Other complex methods for multiplex biosensing platforms use multiple biorecognition agents in close proximities, therefore reducing footprint of the device (For example, characteristic Raman peak analysis using SERS for studying size and shape). These kinds of studies promise potential for better sensing but ultimately need bench-top reading systems for analyte detection.

7.5 Detection Platforms for CVD in Research

As mentioned earlier, there are several kinds of platforms, which can be used for development of a CVD biosensor including optical, electrochemical, magnetic, piezoelectric, etc.

7.5.1 Optical Biosensors

Optical sensors for detection of CVD bioanalytes are one of highest sensitive and widely used techniques. These sensors are dependent on alterations of color, amplitude, or phase. Optical biosensors can be classified into fluorescence based, colorimetric, SPR based, etc. In fluorescence- and colorimetric-based platforms, either target or transducer element can be labeled with color/fluorescence-sensitive dyes. The analyte reacts with these platform to give a visible or fluorescent signal, which is directly proportional to the concentration of target analyte and can hold the lowest detection sensitivity, sensitive to even change in single molecule (Fan et al. 2008).

The target molecules are not classified in label-free detection methods, and are observed in their native forms. These kinds of platforms are simpler and low cost and enable kinetic/quantitative analysis using techniques like SPR (Surface Plasmon Resonance) or optical fibers. Even though optical sensing platforms have high sensitivity and cover a wide variety of biomarkers, they are usually bulkier in size and require costlier setups and skilled manpower.

A wide array of optical detection methods has been used by researchers for detection of cardiovascular biomarkers. These days, the focus of researchers is shifting more toward microfluidic and multianalyte immune-sensing platforms with an intent to increase sensitivity and accuracy. A novel immune-sandwich assay for IL-6 functionalized with Rubpy-encapsulated fluorescent core–shell silica nanoparticles fluorescence was fabricated by Hun et al., where IL-6 was estimated using interaction between IL-6 antigens and anti-IL-6 antibody functionalized with fluorescent nanostructures (Hun and Zhang 2007). In another method, by using photonic crystals coupled with colloidal quantum dot emitters, a 20-fold increase in emitted light by fluorescent molecules was achieved, resulting in a high signal-to-noise ratio for cardiac marker (TNF) detection (Ganesh et al. 2008). Pultar et al. (2009) used C-reactive protein for detection of CVDs using on-chip sandwich immunoassay in microarrays tagged with fluorescent aptamers. In a recent study, Silica-Encapsulated CdSe/ZnS Quantum Dots were used for detection of cardiac troponin I. This system offered very high precision and low limit of detection in a fluorescence lateral flow immunoassay (Wu et al. 2020).

Surface Plasmon spectroscopy is a highly sophisticated method for detection of analytes in biosensing platforms. SPR uses the principle that when a laser beam passes through a prism, placed together with analyte, the beam reflection angle (resonance angle) changes according to changed refractive index of the

prism-sensor substrate system. When a target binds to the sensor biorecognition element surface, the change in refractive index is recorded. Biosensors based on SPR have been studied extensively for cardiovascular disease biomarkers. Pawula et al. prepared a biosensing platform based on SPR technology using cTnT antibody-conjugated with Gold nanoparticles and reported a low detection limit using direct and sandwich assays in buffers (Pawula et al. 2016). Another study by Lee et al. using multiple biomarker approach using Myo, cTnI, BNP, and CK-MB for high sensitivity cardiac disorders testing used label-free PANI-nanowire-based SPR detection system (Lee et al. 2012). In a more recent study, Galectin-3 was used for detection using a 4-bilayer of poly(diallyldimethylammonium chloride) and graphene oxide (GO) on a gold-thiolated surface, functionalized with anti-Gal-3 antibodies using SPR. The study focused on less studied biosensors and achieved higher level of sensitivity in biosensing system (Primo et al. 2018).

7.5.2 Electrochemical Based Biosensors

Electrochemical biosensors make use of electrochemical changes occurring on the sensing surface when the biorecognition element binds with the analyte. Several sensing approaches have been extensively studied in electrochemical biosensors including amperometric, potentiometric, impedimetric, etc. Several studies have been researched for several cardiac marker diagnostics like Troponin (Jo et al. 2015; Tuteja et al. 2016; Akter et al. 2017; Bhatnagar et al. 2017; Dempsey and Rathod 2018; Rezaei et al. 2018), C-reactive Proteins (Buch and Rishpon 2008; Ibupoto et al. 2012; Bing and Wang 2017), Myoglobin (Lee et al. 2015; Puri et al. 2015; Singh et al. 2016; Zhang et al. 2016), etc. Several kinds of transducers have been adopted for electrochemical sensing like nanoparticles (Gold, silver, carbon based), nanostructures like Nickel-copper, platinum, carbon, silica nanorods, etc., layered materials like graphene, etc. due to their conducting nature and high stability on electrode surfaces.

Shin et al. used electrochemical impedance spectroscopy (EIS) method for detection of trace amounts of Creatine Kinase-MB aptamers in cells and achieved ultralow limit of detection (Shin et al. 2016). In 2019, Sun et al. used Fe_3O_4@UiO-66/Cu@gold nanoparticles to detect Cardiac Troponin I with differential pulse voltammetry and achieved LoD as low as 16 pg/mL (Sun et al. 2019). In another study, Gold nano-dumbbell structures were integrated on electrode surface as transducers to detect cardiac troponin aptamers using DPV and recoded a detection limit as low as 8 pg/mL (Negahdary et al. 2017). In a more recent study by Sharma et al., cardiac myoglobin aptamers were detected using polyethyleneimine (PEI)-functionalized reduced graphene oxide (PEI–rGO) thin films and reported limit of detection as low as 0.97 pg/mL in buffers and 2.1 pg/mL in human serum (Sharma et al. 2020).

Electrochemical techniques with various conducting and semiconducting materials, like AuNP, AgNP, graphene and their derivatives etc., have been researched a

lot in past years for fabrication of biosensing platforms for cardiac biomarkers. However, there is still a need for these platforms to reach point-of-care platforms.

7.5.3 Magnetic Biosensors

Magnetic beads/particles have been used in biological assays for years now. Superparamagnetic beads functionalized with biomolecules like whole cells, DNA, antibodies, enzymes etc., have been used as labels for several detection platforms so far. These particles can be of varying sizes depending on the kind of technique being used and with the popularity of nanostructures; several magnetic nanoparticles have also joined this category. A rapid immuo-sensing platform was built by Luxton et al. (2004) for detection of CRP and CKMB, which removed the sample washing step using magnetic structure in order to provide results within 3 min. Magnetic beads have also been used by Miao et al. who used bound and unbound magnetic bead aggregation for detection of CRP (Miao and Bard 2004).

In more recent studies, researchers have focused on integrating magnetic separation with other techniques like electrochemistry. Djebbi et al. used nanoscale zero-valent iron core (NZVI©Au) magnetic nanocomposite-based electrochemical magneto-immunosensing for ultrasensitive detection of troponin-T cardiac biomarker (Djebbi et al. 2020) achieving a detection limit of 0.354 fg/mL. BNP-32 aptamer was taken up by Bruno et al. using aptamer-magnetic bead combination with buffer and human serum studies for development of electrochemiluminescence sandwich assay (Preliminary development of a DNA aptamer-magnetic bead capture electrochemiluminescence sandwich assay for brain natriuretic peptide).

Even though magnetic sensors use a labeled approach for biomarker detection, they enable real-time monitoring in biosensors and simultaneous detection of cardiac biomarkers. Also, magnetic probes functionalizing biorecognition agents can prove to be highly advantageous for multiplexed research platforms providing easy separation of biomarkers and enhanced sensitivity.

7.6 Challenges for Development of Biosensing Platforms in CVD Detection

As discussed through the chapter, even though there is extensive research being conducted through various sensing platforms for several biomarkers and multiplex sensing systems, there is still a huge gap between researched platforms to come to commercially available point-of-care devices. There are several PoC systems which are available for several cardiac disorders, still there is a need for improving issues of accessibility to middle- and low-income general population, remote areas, and varying skilled clinicians. Also, in the PoC systems, which are available, the issues

of high cost, sample processing time, probabilities of false negatives, and sensitivity variations need attention in order to address the cardiac prognosis and diagnostics demands worldwide. A lot of devices are becoming wireless, integrating techniques like Bluetooth so that they can be accessed using smartphones, enabling automation and control of the devices. There are several studies, which have integrated machine learning and artificial intelligence for enhancing the viability of test results achieved by detection platforms. Such devices if made low cost can be used by general population with much ease and prove to be life-saving and life-elongating in terms of cardiovascular risk assessment and treatment.

Multiplexing assay also provides a push toward higher sensitivity as we have already discussed. These sensors combine data from two or more biomarkers sensed on a single platform and together form a higher accuracy outcome for the assay using similar time than single biomarker, hence proving to be highly desirable for PoC platforms.

7.7 Conclusion

Analyses of biomarkers like Troponin, BNP, and CRP are preferred for decisive diagnosis of ACS/MI and heart failure. Especially cTnI and cTnT are targeted while detecting acute coronary syndrome or any other myocardial injury and BNP and pro-BNP are preferred for risk stratification when primary suspect is HF. In case of acute heart failure, MR-proANP can also act as a preferred diagnostic biomarker. Well-established biomarkers like CK-MB, Myo, and inflammatory biomarkers like IL-6 and CRP provide additional information to determine the diagnosis results and also new biomarkers like GAL-3, copeptin, etc. could provide viable data for assessment of CVD condition in patient.

Other than these, biomarkers like microRNAs (miR-1, miR499 etc.), fatty acids like Heart-Type Fatty Acid-Binding Protein (hFABP) are also reported as potential targets for myocardial infarction and heart failure (Wang et al. 2020).

There are several factors, which contribute to successful laboratory diagnosis of a cardiac biomarker and hence sensitivity, precision, and specificity of the estimation technique. Therefore, majority of the commercially available methods prefer to use established biomarkers like Troponin, BNP, and additional biomarkers like CK-MB and myoglobin for diagnosis purposes. Additionally, it can be stated that using multimarker approach rather than single biomarker can provide better diagnosing outcomes and therefore enhancing the efficiency of clinical decision making and risk stratification. Though there are commercially available panels focusing on multiple biomarker theme, majority of the manufacturers still target bench-top, single biomarker diagnosis platforms with costly accessories able to measure up to nanogram per milliliter concentrations of troponin, which is still not sufficient to exclude myocardial infarction.

Several biosensing platforms are currently being researched, which have reported extremely low detection limit using electrochemical, optical methods and integrating

nanostructures and highly sensitive biorecognition elements like aptamers in an attempt to increase the sensitivity of the diagnostic platform. Still, there is a huge gap in development and availability of such platforms especially for point-of-care applications in urgent cardiac care.

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Chapter 8
Point-of-Care Nanobiosensors for Determining Vitamin Deficiency

Hrishikesh Kalita, Mahima Kumari, Mayank Bhushan, Debananda Mohapatra, and Laishram Robindro Singh

8.1 Introduction

Vitamins can be classified into two groups depending on their solubility, as water-soluble (vitamin B, C) and fat-soluble (vitamin A, D, E, K) group of compounds (Huang et al. 2021). Both fat-soluble and water-soluble vitamins are necessary component of our daily intake of food in order to maintain the optimal health, because they play very crucial role in vital functions of our body like growth and metabolism (Kim 2011). Their role in restraining many deficiencies related diseases is very much established. For example, folic acid (vitamin B_9) and vitamin B_{12} are essential vitamins for neural development, hematopoiesis, and many other vital body processes, and their deficiencies can induce anemia (Zhao et al. 2006), cardiac malfunctions, and congenital defects in fetus such as hydrocephalus, cleft palate, encephalocele, and spina bifida (Crane et al. 1995; Santos and Pereira 2007; Berry et al. 2010). The consequences of lack of vitamin B_{12} and folic acid on embryological development and mother's health are of severe nature and therefore require nutritional supplements with prenatal diagnosis for genetic and neural tube disorders (Pacheco et al. 2009). Both these vitamins help in maintaining blood homocysteine level, therefore lower the risk of cardiovascular, cancer, and other age-induced chronic disease like dementia (Groff et al. 1995; Iyer and Tomar 2009; Russell 2012). Vitamin B_1 mainly acts as coenzyme for metabolic reactions, which involve

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transfer of activated aldehyde groups, and decarboxylation of α-keto acids. It exists in different forms namely thiamine, thiamine monophosphate, thiamine triphosphate, and pyrophosphate (Russell 2012). A lack of vitamin B_1 in body leads to diseases like beri-beri and amnesia, which may cause death of the patient due to brain damage in severe cases (Harper 2006). Vitamin B_2 exists in the forms of riboflavin, FMN (flavine mononucleotide), FAD (flavine adenine dinucleotide), and lumiflavin. It takes part in various metabolic reactions and pathways including reduction of oxygen to hydrogen peroxide, hydroxylation, and decarboxylation. Thus, it is an integral part of Krebs cycle, electron transport chain, and many other metabolic reactions (Russell 2012). The riboflavin and thiamine are important water-soluble vitamins very commonly used for fortifying food products (Boyaci et al. 2012).

There are three main forms (vitamers) of vitamin B_6, namely, pyridoxine, pyridoxal, and pyridoxamine, along with some other forms (Mihhalevski et al. 2013). The phosphorylation of pyridoxine (vitamin B_6) results into pyridoxal phosphate, which acts as prosthetic group for enzymes like synthases, transferases, dehydrases, racemases, and amino acid decarboxylases (Zand et al. 2012). The vitamers of this vitamin play an important role in the synthesis of hemoglobin, homocysteine, and their deficiency leads to protein-metabolism-related disorders (Rivlin 2007). All the cobalamins, which exhibit biochemical functions in the human body, are known by the generic term vitamin B_{12} (Eitenmiller and Landen 2008). The cobalamin exists in the form of octahedral cobalt complex, to which ligands are attached at equatorial chiral, lower axial, and upper axial positions (Randaccio et al. 2006). The most stable form of cobalamin is cyanocobalamin; thus, it is used in food fortification and dietary supplements (Kim 2011). There are many different vitamin-B_{12}-dependent enzymes (Eitenmiller and Landen 2008). Vitamin B_{12} is closely related with folates in their deficiency symptoms. Similar to folates, the deficiency of vitamin B_{12} may induce chromosomal aberrations by interfering with normal DNA synthesis and associated processes (Santos and Pereira 2007). Vitamin B_{12} is essential for the synthesis of neurotransmitters, creatine, phospholipids, proteins, and DNA. The vitamin B_{12} deficiency induces pernicious anemia and neurological disorders like seizure attack in epileptics and cerebellar ataxia (Ball 2006; Russell 2012; Thiel and Fowkes 2004; Morita et al. 2003). The structures of vitamin B_1, B_2, B_6, and B_{12} molecules are shown in Fig. 8.1.

Vitamin C is water soluble and exists in two forms, namely, L-ascorbic acid and dehydro-L-ascorbic acid (Eitenmiller and Landen 2008). For humans, the daily requirement of vitamin C must meet from food and dietary supplements. It has excellent antioxidant property and helps body in neutralizing all kinds of infections and toxicity. Vitamin C is essential in the body for the processes like synthesis of collagen, regeneration of other antioxidants, iron absorption, and wound healing (Brody 1994). The deficiency of this vitamin leads to scurvy (fatal skin disease), and damage of biological macromolecules by free radicals and reactive species (Olson 1999; Bhagavan 2001). Vitamin D consists a couple of fat-soluble secosteroids that facilitate the absorption of zinc, phosphate, and calcium in the body; thus, it is responsible for maintaining proper growth and functioning of bone and muscles

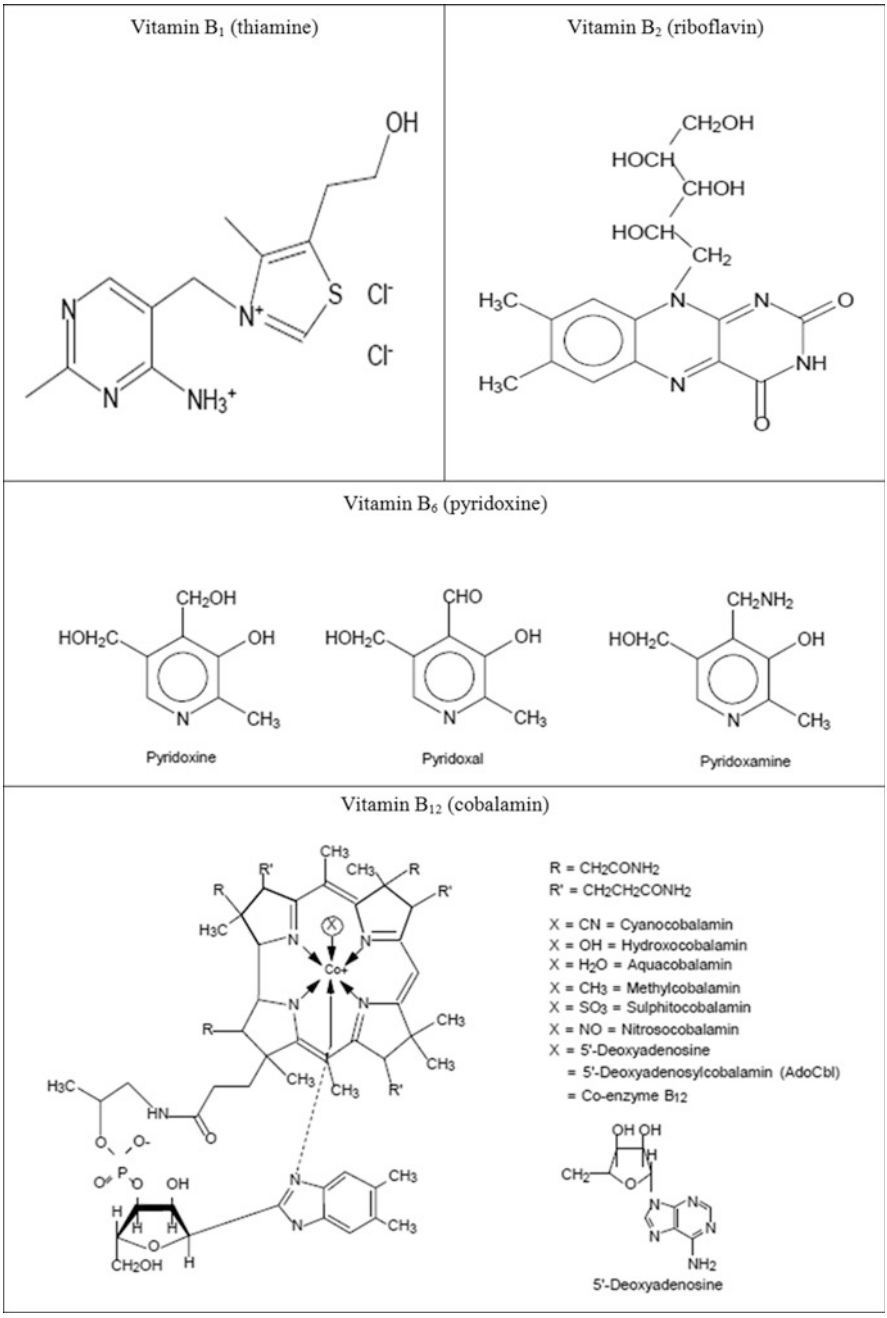

Fig. 8.1 Structure of vitamin B_1, B_2, B_6, and B_{12} molecules

(Norman 2008; Calvo et al. 2005). Its deficiency causes rickets in children, and osteoporosis, cancer, diabetes, and heart related issues in adults (Wagner and Greer 2008; Holick 2004). The biologically inert form "cholecalciferol" of this vitamin is synthesized in the skin, and then converted into 25-hydroxyvitamin D by undergoing hydroxylation in the liver, which gets circulated in the body. The supplements of vitamin D are available in the forms of ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) (Tripkovic et al. 2012).

Therefore, it is required to determine the quantity of vitamins present in food items and supplements (fortified foods and vitamin capsules) in order to ensure the proper supply of these essential nutrients to the body (Zhao et al. 2006). High-performance liquid chromatography (HPLC) is generally used for the qualitative and quantitative analysis of different vitamins in pharmaceutical formulations. Despite having advantageous features like high sensitivity and specificity, its high operating cost and hazardous environmental impact of organic solvents make it essential to look for alternative eco-friendly technology. The convergence of nanotechnology with biotechnology and information technology has opened new possibilities in electrochemical nanobiosensors. The amalgamation of graphene, gold, and magnetic nanoparticles within biosensing devices has provided brilliant analytical performances in the detection of vitamins.

Nanobiosensors can be categorized into three types based on the mechanism by which they sense analytes as potentiometric, amperometric, and cantilever type. Potentiometric sensors work on the principle of measurement of potential change due to the charge of the analyte molecule being sensed. Hence, potentiometric sensors are suitable for sensing analytes that carry a significant amount of charge. Amperometric sensors are used for analytes, which are charge-less and carry very small mass, whereas analytes carrying a considerable amount of mass are sensed by cantilever type of sensors. These three kinds of sensors have been designed to identify analyte molecules based on their intrinsic properties of mass, charge, molecular configuration, etc.

Different types of sensors have been developed by conjugating protein and DNA molecules on the surfaces of nanoparticles. Use of graphene-based sensors has shown promising results in detecting trace concentrations of vitamins B_{12} in the order of ng/mL in human blood samples (Lorsch and Szostak 1994). The aptamers required for conjugation with the nanoparticles are synthesized by a process known as SELEX, which is systematic evolution of ligands by exponential enrichment. The method works by exposing randomly synthesized strands of oligonucleotides to the target ligand molecule. The ligands strands, which bind with the target ligand molecule, are then amplified using other processes such as PCR and the ones that show no binding are removed. Vitamin B_{12}-binding RNA aptamers were isolated using enzyme amplification processes (Lorsch and Szostak 1994). Carbon paste electrodes modified with gold nanoparticles have been used to sense folic acid in human serum sample (Arvand and Dehsaraei 2013).

8.2 Nanobiosensors for Determining Vitamin Deficiencies

8.2.1 Nanobiosensors for the B-Complex Group of Vitamins

Deficiency of vitamin B_{12} has been associated with a number of cardiovascular as well as hematological disorders, and hence, it is essential to determine the level of this vitamin in blood as well as other food commodities for its regulation (Oh and Brown 2003). Determination of vitamin B_{12} has been carried out using a number of immunoassay techniques, spectrometry, electrochemical analysis, high-performance liquid chromatography, etc. (Moazeni et al. 2018). However, modern biosensors using nanomaterials have made it possible to detect trace concentrations of analyte molecules, thereby improving the detection limits of the nanosensors drastically when compared to costly conventional techniques. D-Phenylalanine nanotubes were integrated on the surface of gold electrode using liquid phase synthesis, and the resulting modified electrode was used for the detection of vitamin B_{12} (Moazeni et al. 2018). Vitamin B_{12} cyclic voltammetry measurements of the unmodified gold electrodes, as well as the layered D-phenylalanine gold electrodes exhibited reduction of peak current in the modified D-phenylalanine gold electrodes as shown in Fig. 8.2, which is attributed to restriction in interfacial charge transfer in the electrodes due to vitamin B_{12} binding on the surface of the modified electrodes (Moazeni et al. 2018).

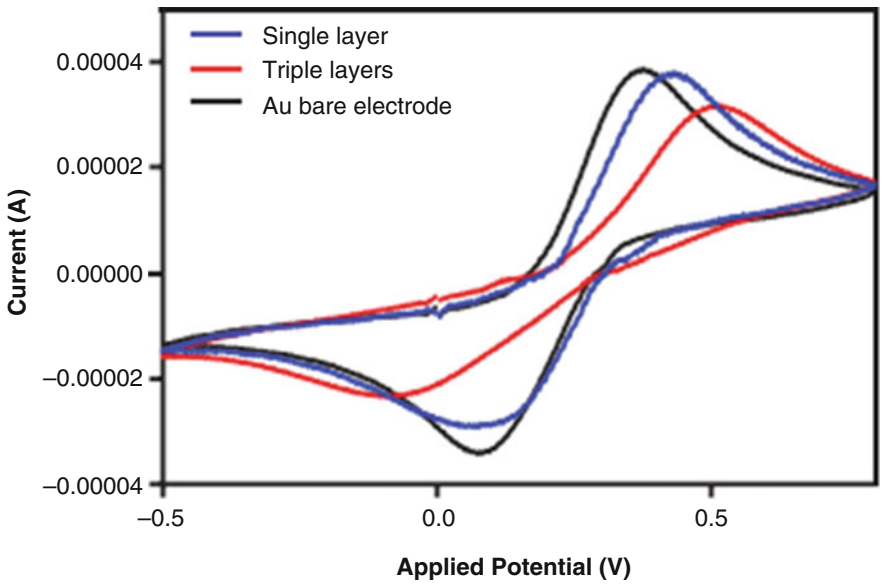

Fig. 8.2 Cyclic voltammograms (CV) of bare Au and phenylalanine nanotube (single layer and triple layer) modified electrodes (Moazeni et al. 2018)

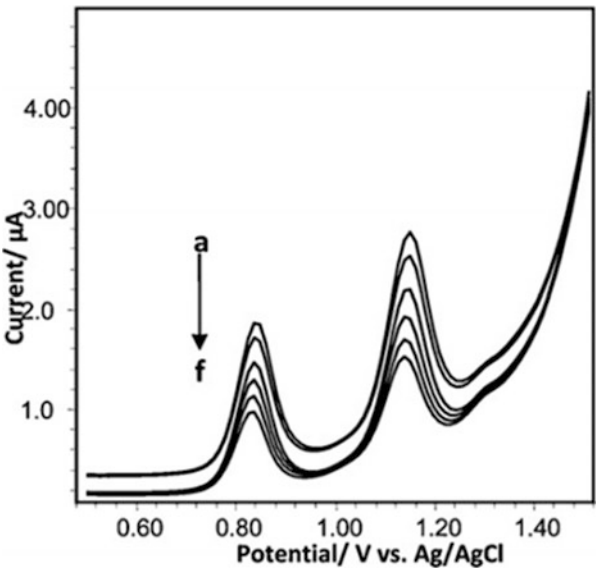

Fig. 8.3 Differential pulse voltammograms for the interaction of vitamin B_1 with ds-DNA-modified MWCNTPE; oxidation signal of guanine and adenine after interaction with (from up to down) 0.0, 1.0, 20.0, 45.0, 65.0, and 80.0 μg/mL vitamin B_1 at surface of ds-DNA-modified MWCNTPE (Brahman et al. 2013)

Vitamin B_1 or thiamine is another important member of the vitamin B complex group, which is essential for metabolic functions of the human body (Akyilmaz et al. 2006). Cost-effective and highly sensitive electrochemical sensing techniques using nanobiosensors have allowed for the study of biomolecular interaction and drug identification. Nucleic acids provide many opportunities for surface functionalization of electrodes as they show intercalation with a large number of drugs and their interactions have been widely studied (Gua and Hasebe 2012; Liping et al. 2006). Thiamine (vitamin B_1) nanobiosensors were made using multiwalled carbon nanotube paste electrodes (MWCNTPE), surface functionalized with nucleic acids such as double-stranded DNA molecules (Brahman et al. 2013). The concentration of thiamine was found to be proportional to the difference in oxidation signals of adenine and guanine of the nucleic acid of the sensor electrode. The oxidation current was determined by performing a positive differential pulse potential scan on the sample also known as adsorptive transfer stripping voltammetry, which has higher current sensitivity than cyclic voltammetry (Brahman et al. 2013). The nanobiosensor with vitamin B_1 intercalated on it shows decrease in current with increase in the concentration of vitamin B_1, which is evident from the DPV studies, shown in Fig. 8.3 (Brahman et al. 2013).

The peaks in the DPV curve denote the oxidation of potentials of guanine and adenine from left to right, respectively. The downward shift in the current curve shows that there is a decrease in oxidizing current due to reduction in the number of oxidizable groups of guanine and adenine due to their intercalation with the vitamin B_1 biomolecules (Ensafi et al. 2012). Therefore, the detection of vitamin B_1 is possible by using nucleic acid functionalized MWCNT paste electrode nanosensors (Ensafi et al. 2012).

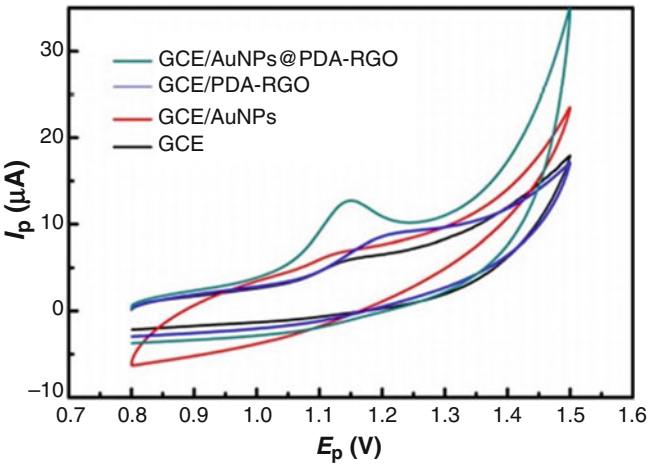

Fig. 8.4 CV of GCE based nanocomposite electrodes at scan rate of 0.05 V/s (Li et al. 2019)

Vitamin B_6 is another important vitamin of the B complex group of vitamins, which is utilized by the human body in enzymatic reactions for protein metabolism, and also for the production of hemoglobin (Annaraj and Neelakantan 2015). Numerous support materials for glassy carbon electrodes (GCEs) have been studied, out of which polydopamine (PDA) loaded reduced graphene oxide (RGO) is among one of the stable support materials for GCEs with high catalytic activity due to presence of positive charge density of functional groups present in PDA (Huang et al. 2014; Liu et al. 2014). Also, gold nanoparticles (AuNPs) are among the most widely used nanoparticles for electrode medication due to their impressive properties such as high surface area and excellent electrical conductivity (Oishi et al. 2008). Cyclic voltammetry study on GCEs modified with PDA-RGO support material and further functionalized with AuNPs were done to measure the electrochemical response of the nanocomposite electrode in presence of 50 μM vitamin B_6 in 0.1 M PBS solution at pH = 3.0 (Li et al. 2019). Comparison of voltammograms of the AuNPs @ PDA-RGO modified GCE, PDA-RGO-based GCE, AuNP modified GCE with unmodified GCE (Fig. 8.4) shows that the current response is highest in case of the AuNPs @ PDA-RGO modified GCE, indicating enhanced electron transfer due to vitamin B_6 at the nanoelectrode surface (Li et al. 2019).

Riboflavin or vitamin B_2, which is widely available in dairy and vegetable products, is essential for the growth of body tissues and for the immune system development, and the most common symptom of vitamin B_2 deficiency is anemia (Lane and Alfrey 1965). Although riboflavin has been detected by different techniques such as electro-chemi-luminescence (López-de-Alba et al. 2006), spectrophotometry (Perez-Ruiz et al. 1994), fluorescence (Shumyantseva et al. 2004), and electroanalytical determination using novel electrode materials provide cost-effective alternatives for riboflavin detection. A bismuth film-coated copper-based electrode (BiFE) sensor was used for cyclic voltammetric measurement of redox

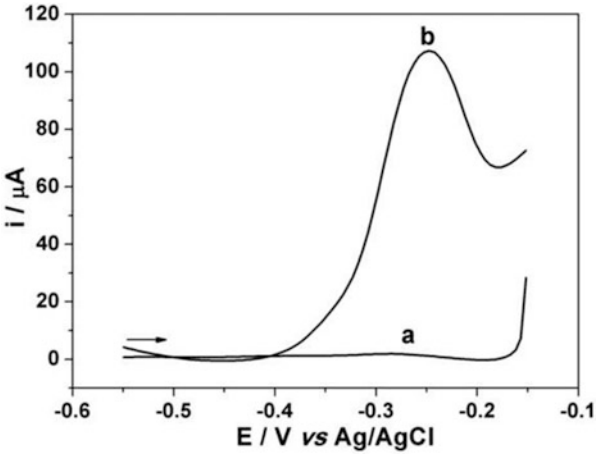

Fig. 8.5 Cyclic voltammetric curves with potential ramp from −0.1 to −0.6 for BiFE in the absence (a) and presence (b) of vitamin B_2 (Sá et al. 2015)

potential of vitamin B_2 using an electrochemical cell containing acetate buffer and a 100 μmol/L vitamin B_2 solution (Sá et al. 2015). The cyclic voltammograms exhibit well-defined peaks in the presence of 100 μmol/L vitamin B_2 solution as shown in Fig. 8.5 (curve 'b') and no oxidation peaks are observed in the absence of vitamin B_2 (Fig. 8.5 curve 'a') (Sá et al. 2015).

Vitamin B_9 or folic acid is a cofactor essential for the processes involving DNA and protein synthesis and its deficiency has been associated with neural tube disorders in newborns (McMahon et al. 2013). Therefore, proper quantitative assessment of the vitamin has to be made possible with considerable accuracy. Compared to conventional analytical determination techniques, which generally involve a number of time-consuming steps such as assays, electrochemical-sensing-based techniques provides easy and faster solution for folate detection with reasonably good accuracy (Jamali et al. 2014). Nanobiosensor for the detection of vitamin B_9 using metal oxide nanoparticle modified electrodes has shown promising results in numerous CV, DPV studies (Akbar et al. 2016). Glassy carbon electrodes, CPE, powdered graphite electrodes have been used for electrochemical studies (Akbar et al. 2016). Enhanced voltammetric response has been reported from CV studies on glassy carbon electrodes modified with α-Fe_2O_3 nanofibers used for folate detection when compared to its bare electrode counterparts (Maiyalagan et al. 2013). Similar results have been observed in the case of DNA modified graphite powder electrodes (Mirmoghtadaie et al. 2013). DNA molecules have the capacity to bind many smaller molecules due to their phosphate backbone, which is negatively charged, and double helix structure using noncovalent interactions (Mirmoghtadaie et al. 2013). The analyte interaction can be observed from the anodic peaks obtained from the DPV with a scan rate of 10 mV^{-1}, shown in Fig. 8.6, which exhibits decrease in the anodic peak current with increase in folic acid concentration (Mirmoghtadaie et al. 2013). The folic acid interaction with the ds-DNA structure causes the helix structure to destabilize and oxidation of the adenine and guanine bases can be done on a graphite-based electrode for quantification of the DNA

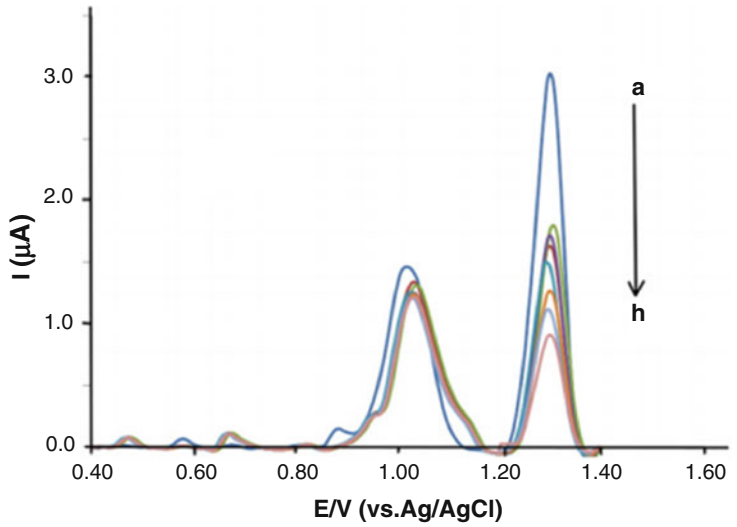

Fig. 8.6 DPV of adenine/guanine peak current on ds-DNA modified pencil graphite electrode for decreasing order of folic acid concentrations (from a to h given as 0.10, 0.50, 1.0, 2.0, 5.0, 7.0 μmol/L) in 4.8 pH acetate buffer solution (Mirmoghtadaie et al. 2013)

damage from exposure to folic acid (Ensafi et al. 2014). The increase in concentration of folic acid from 0.10 to 7.0 μmol/L leads to reduction in peak (Fig. 8.6) due to the loss of adenine/guanine signal from damage due to folic acid interaction (Mirmoghtadaie et al. 2013). Moreover, studies carried out with buffer solution of different values of pH showed that the interaction of folic acid with the ds-DNA helix is maximum for a pH of 8.5 (Mirmoghtadaie et al. 2013).

Carbon paste electrodes modified with Pt:Co nanoalloy in an IL liquid (*n*-hexyl-3-methylimidazolium hexafluoro phosphate) buffer solution (pH 9.0) recorded higher oxidation peak signals and reduction in oxidation potential for vitamin B_9 concentration of 300 μmol/L in CV studies at a scan rate of 100 mV/s compared to bare CPE without Pt:Co nanoalloy modification (shown in Fig. 8.7), which is attributed to enhanced catalytic oxidation from the nanoalloy, and also because of the faster electron mobility due to ILP buffer solution (Jamali et al. 2014).

Vitamin B_3 or niacin is utilized by cellular processes for the breakdown of sugars into energy and its balanced intake is essential for maintaining healthy digestive system, eyesight, and nervous system (Farmanzadeh and Ghazanfary 2013). Determination of niacin has been carried out in food items using HPLC, capillary electrophoresis, and liquid chromatographic techniques (Windahl et al. 1999). Nanotubes of boron nitride (BNNT) have found wide utility in the area of biosensing due to their excellent electrical properties owing to their high electron delocalization and high surface to volume ratios (Moscatello et al. 2008). BNNTs are found to be biocompatible with a host of biomolecules including amino acids, and their functionalization with organic molecules has been widely reported for sensing applications from numerous studies (Farmanzadeh and Ghazanfary 2013).

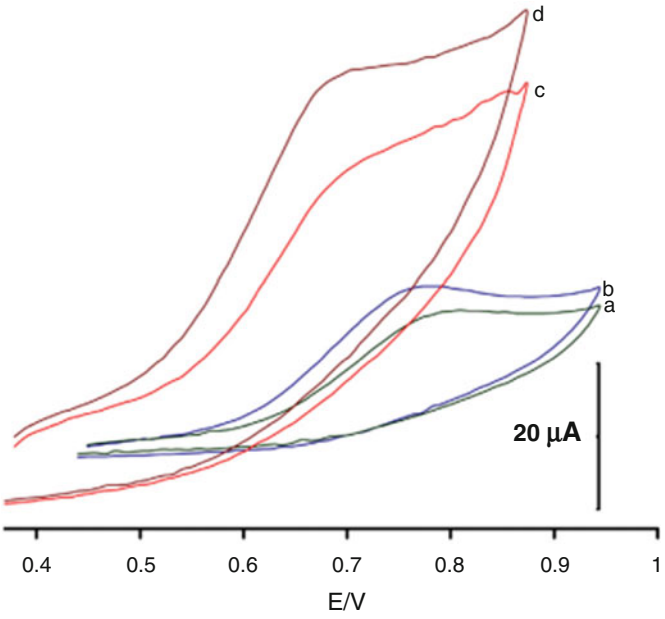

Fig. 8.7 CV of (a) bare CPE (b) Pt:Co modified CPE (c) bare CPE in IL (d) Pt:Co modified CPE in IL (Jamali et al. 2014)

Optimized single walled BNNTs were studied for their adsorption capabilities of vitamin B_3, which happened due to the noncovalent interaction of the N-atoms of BNNT with H-atoms of the hydroxyl group of vitamin B_3 (Farmanzadeh and Ghazanfary 2013). The adsorption sensing mechanism of the analytes on the surface of BNNTs makes them excellent materials for fabricating reusable catalytic biosensors.

8.2.2 Nanobiosensors for Vitamin C Detection

Vitamin C is one of the most essential vitamins for human metabolism and immunological functions, deficiency of which is related with ailments weakness, joint pain, and tiredness (Jiang and Du 2014). Some of the major methods used for determination of vitamin C are spectroscopy (Fong et al. 2016), amperometry (Su et al. 2017), and voltammetry. Carbon paste electrodes (CPEs) are among the widely used electrodes used for voltammetry-based biosensing due to their excellent conductivity and modification abilities (Beitollahi et al. 2015). CPE electrodes were functionalized with a layer of hydroxyapatite (HAP), and further coated with metal oxide (zinc oxide and palladium oxide) nanoparticles and the resulting modified electrode was used for the determination of vitamin C (Shahamirifard and Ghaedi

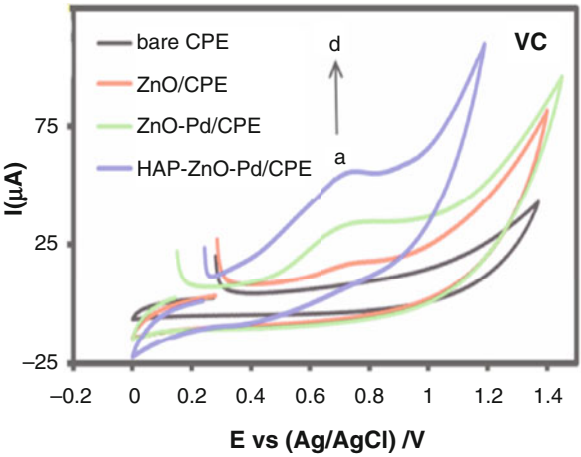

Fig. 8.8 Voltammograms of (a) HAP-ZnO-Pd functionalized CPE (b) Zno-Pd/CPE (c) ZnO/CPE (d) bare CPE at scan rate of 0.1 V/s (Shahamirifard and Ghaedi 2019)

2019). The palladium oxide complex nanostructures supported on HAP over CPE electrodes have shown enhancement in the catalytic efficiency for oxidation reactions with alcohol (Mori et al. 2002), due to the excellent biocompatibility, mechanical stability, slow biodegradation of HAP (Yang and Zhang 2011), and high surface to volume ratio of the metal oxide nanoparticles (Liu et al. 2017). The ZnO functionalized electrodes exhibited an increased oxidation potential for vitamin C detection due to vitamin C/ZnO interaction facilitated by the carboxylic or hydroxyl groups present in vitamin C biomolecule (Rochford et al. 2007). Voltammograms obtained by cyclic voltammetry studies in the potential range of −0.1 to 1.5 V carried out on the HAP-ZnO-Pd/CPE electrodes along with other bare electrodes (unmodified CPE and HAP/CPE) in the presence of vitamin C exhibit oxidation peaks for the functionalized electrodes as shown in Fig. 8.8. The higher oxidation of vitamin C at HAP CPE surface is aided by their increased adsorption due to the presence of ZnO NPs in the CPE (Shahamirifard and Ghaedi 2019).

Conduction polymer materials have gained considerable attention for their utility in biosensors due to their stability and nontoxicity (Holbrey et al. 2002). An electrochemical nanobiosensor was synthesized using poly(3,4-ethylenedioxythiophene)-ethyl sulfate or PEDOT-$EtSO_4$ modified GCE and exhibited very good biocatalytic property for the oxidation of vitamin C (Wen et al. 2012). The biosensor showed response to applied potential of 0.2 V over a wide range of vitamin C concentrations (Li et al. 2010). The inherently conducting polymer materials (ICPs) for electrode conjugation prepared in nontoxic ionic liquid solution exhibited increase in biosensor sensitivity to vitamin C determination when further modified with nafion layer (Wen et al. 2012). Metal oxide nanosheets such as ZnO/CuO modified with carbon paste electrodes have received significant attention due to their high performance and catalytic activity, which make them suitable for biosensor applications (Beitollahi et al. 2016). An electrochemical sensor based on carbon paste electrode modified with 2-(ferrocenylethynyl)fluoren-9-one and ZnO/CuO

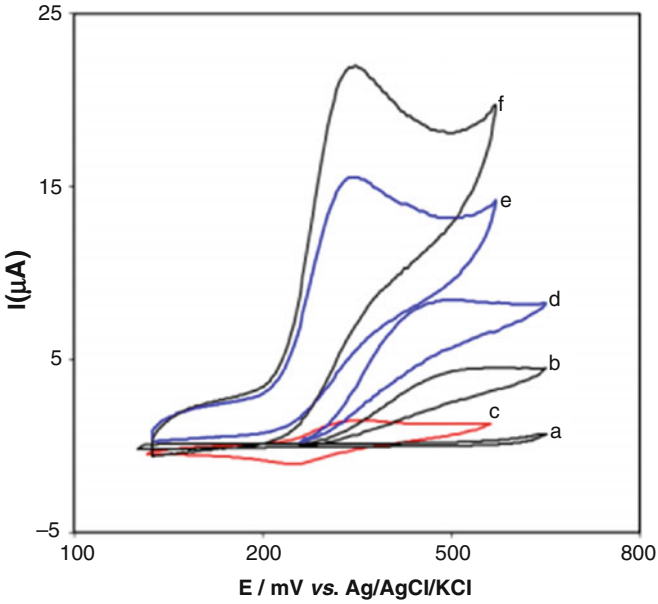

Fig. 8.9 Cyclic voltammograms of (a) unmodified CPE, (b) unmodified CPE in presence of vitamin C, (c) 2FE/ZC/IL/CPE, (d) ZCPE in PBS in vitamin C, (e) 2FE/IL/CPE in vitamin C and (f) 2FE/ZC/IL/CPE in vitamin C (Mehdi Motaghi 2016)

nanosheets in ionic solvent, (2FE/ZC/IL/CPE) was prepared and its electrochemical behavior was studied by cyclic voltammetry measurements at a scan rate of 10 mV/s (Mehdi Motaghi 2016). A greater amount of increase in the peak anode current for the modified 2FE/ZC/IL/CPE was registered when compared to the unmodified electrodes variants used for CV measurements in PBS solution in the presence of 450.0 μM of vitamin C as shown in the Fig. 8.9.

The increase in peak current in modified electrodes is caused by the enhancement in oxidation at the surface and is responsible for the increased sensitivity of detection of vitamin C of the 2FE/ZC/IL/CPE nanosensor (Mehdi Motaghi 2016).

8.2.3 Nanobiosensors for Vitamin D Detection

The deficiency of vitamin D has been found to be associated with multiple diseases in children as well in adults such as cardiovascular disorders (Mozos and Marginean 2015). Vitamin D is essential for the metabolism of calcium in the human body. Vitamin D concentrations have been analyzed using techniques like ELISA (enzyme linked immune sorbent assay), radioimmunoassay, etc. (Jafri et al. 2011). It has been found that a concentration of vitamin D below 30 ng/mL in blood plasma is associated with disorders related to the deficiency of vitamin D (St-Arnaud et al.

1997). Metal oxide nanoparticles have also been used widely for the development of biosensors for vitamin detection. Surface functionalized metal oxide nanoparticles of rare earth metals have been found to be especially suitable for electrochemical sensor applications due to their improved dispersivity and biocompatibility with other biomolecules (Shim et al. 2002). Surface functionalizations have been carried out using different chemical species such as biopolymers, proteins, and amino acids, among which amino acids have been found to be very effective due to presence of functional groups in them that aid in the binding of biomolecules. A vitamin D sensor was built by surface coating ITO electrode material with gadolinium oxide nanorods and then further surface modification with aspartic acid (Chauhan et al. 2019). The resulting nanocomposite electrode was used for the detection of vitamin D_3 (Chauhan et al. 2019). Gd_2O_3 nanoparticles were synthesized by the coprecipitation technique, using $Gd(NO_3)_3$ and NaOH as precursor materials (Chauhan et al. 2019). After carrying out sonication process of the Gd_2O_3 nanorods dispersed in aqueous solution of aspartic acid (Asp), aspartic acid gets conjugated onto the surface of the Gd_2O_3 nanorods due to electrostatic interaction between the positive charged Gd_2O_3 nanoparticles and the COO^- groups present in aspartic acid (Chauhan et al. 2019). The Asp functionalized Gd_2O_3 nanorods were electrodeposited onto the surface of the ITO electrodes to obtain the desired Asp-Gd_2O_3NRs/ITO electrodes, which were then used for the purpose of immobilizing vitamin D_3 (Chauhan et al. 2019). The capturing of Vit-D_3 on the Asp-Gd_2O_3NRs/ITO electrodes is possible due to covalent bond formation between the activated COO^- group of Vit-D_3 molecule and positive NH_3 groups present on the surface functionalized electrodes (Schwaminger et al. 2015). It is evident from differential pulse voltammetry (DPV) measurements of the electrodes that the electrodes with Vit-D_3 captured on their surfaces show higher current measurement, when compared to the nonconjugated, and plain unfunctionalized ITO electrodes (Chauhan et al. 2019). Figure 8.10 shows the comparison between different electrodes. The possible explanation for the observed higher current in DPV measurements for modified ITO electrodes can be attributed to the increased electrostatic interaction between the free NH_3 groups present in the conjugated electrodes and redox carriers present in the electrolyte solution (Ali et al. 2014).

8.2.4 Nanosensors for Vitamin E Detection

Vitamin E are a group of eight fat-soluble compounds, which serve important functions in the human body like oxidation of free radicals, regulation of nervous and immune systems. Common symptoms for deficiency of vitamin E include weakness and anemia. Vitamin E is widely available from foods such as green vegetables, dairy, and poultry products. Vitamin E is conventionally determined using HPLC, chromatography techniques; however, the development of conductive polymer-based nanocomposite materials for biosensing applications might as well provide a better and easily reproducible alternative for vitamin E detection (Lin et al.

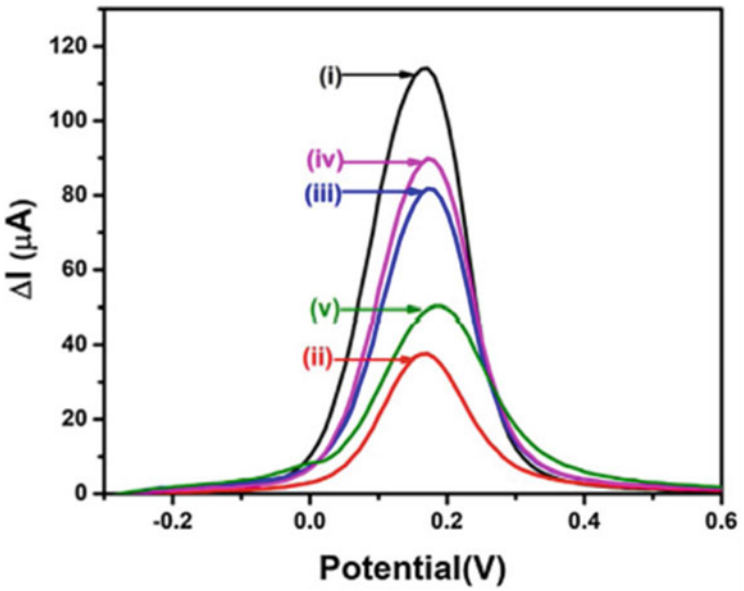

Fig. 8.10 DPV curve of (i) ITO (ii) Gd_2O_3NRs/ITO (iii) Asp-Gd_2O_3NRs/ITO (iv) Ab-VD/Asp-Gd_2O_3NRs/ITO and (v) BSA/Ab-VD/Asp-Gd_2O_3NRs/ITO immunoelectrode (Chauhan et al. 2019)

2015). Conductive polymers (CPs) such as polyaniline (PAn), which are obtained by the electro-polymerization of aniline, serve to enhance the electron mobility in the conjugated electrode due to its matrix structure. Metal oxide nanoparticles, such as gamma alumina γ-Al_2O_3, act as excellent filler materials for CPs due to their very high surface to volume ratio (Scott et al. 1987). Polyaniline conductive polymers were synthesized on gold (Au) electrodes using anodic oxidation techniques in the presence of γ-Al_2O_3 (Parvin et al. 2018). The differential pulse voltammograms (DPVs) of the modified PAn/γ-Al_2O_3/Au nanocomposite electrode at different concentrations of vitamin E exhibit linear relationship of anode peak current and vitamin E concentration. The DPVs are shown in Fig. 8.11.

8.2.5 Nanobiosensor for Vitamin A Detection

Vitamin A is one of the most essential vitamins required by the human body for maintaining proper vision and also a healthy immune system, and its regulated intake has been suggested for reducing the risks associated with immunological disorders especially in case of pregnant women and children (Lv 2017). Vitamin A detection has been carried out using HPLC, spectrophotometry, capillary electrophoresis techniques (Al-sulimany and Townshend 1973), but electrochemical sensing methods provide cost-effective as well as reliable detection of biomolecules

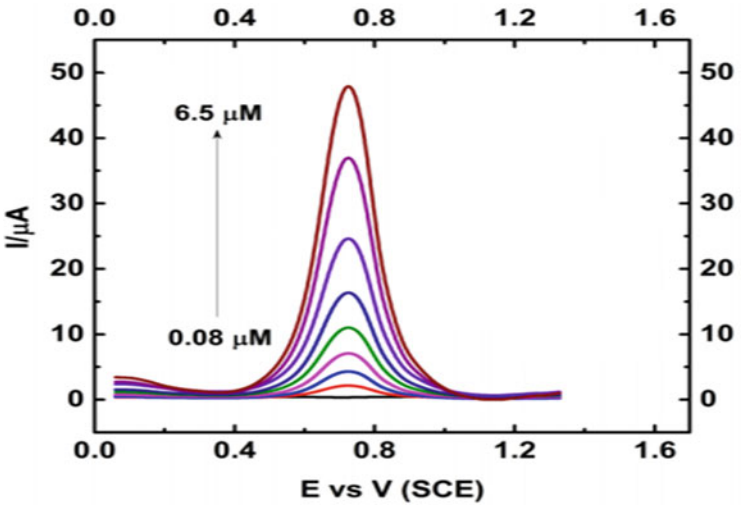

Fig. 8.11 DPV of PAn/γ-Al_2O_3/Au electrode at different vitamin E concentrations (Parvin et al. 2018)

making them suitable alternative for vitamin A detection (Wang et al. 2015). Ionic liquids (ILs) have found utility in electrode modification due to the increase in ionic conductivities after their addition (Mao et al. 2015; Khaleghi et al. 2016). Carbon paste based electrode modified with Pt:Co nanoparticles and binder IL (*n*-hexyl-3-methylimidazolium hexafluoro phosphate) were synthesized and cyclic voltammetry measurements were obtained in the presence of 0.3 mM concentration of vitamin A at pH 9.0. The results exhibited high oxidation peak currents when compared to unmodified CPE (Lv 2017). The CVs of different electrodes are shown in Fig. 8.12. The high oxidation currents are attributed to the high electrocatalytic performance of the nanoparticles used for modification of the CPE.

8.3 Challenges and Future Prospects

One of the biggest drawbacks of present day biosensors is their inability to simultaneously detect different kinds of vitamins due to their single target approach. With the ongoing developments in multifaceted nanoparticles, utilization of multimodal nanobiosensors for simultaneous detection of multiple types of vitamins with different solubilities will drastically improve the efficacy of the sensors in the field of diagnostics. Although nanotechnology has been found to improve the sensitivity of numerous biosensors like glucose biosensors, there has been limited application in the area of vitamin detection. Integration of nanobiosensors into wearables such as watches, wrist bands, epidermal patches, rings, etc., will allow real-time high precision monitoring of important analytes like uric acid, glucose, as well as

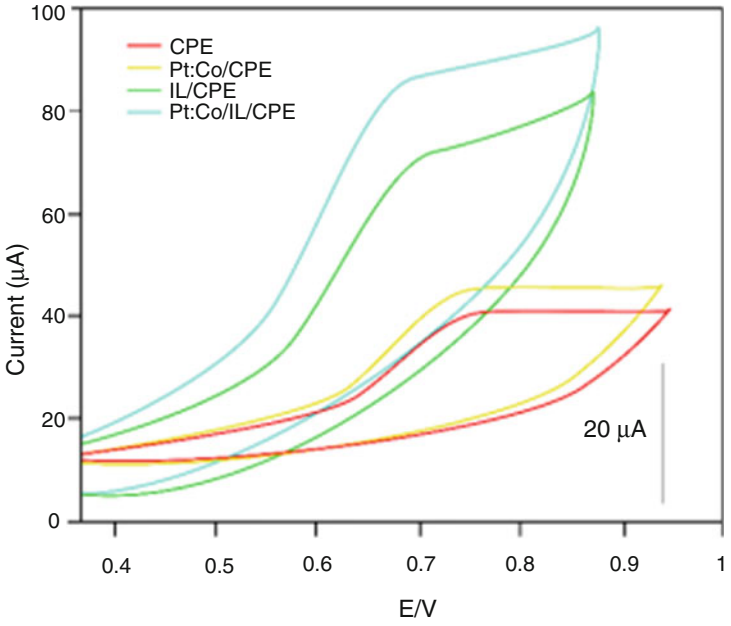

Fig. 8.12 CVs of unmodified CPE, Pt:Co/CPE, IL/CPE and Pt:Co/IL-modified CPE for 0.3 mM vitamin A concentration at pH 9.0 (Lv 2017)

essential vitamins with a greater degree of convenience for health-care monitoring in a noninvasive manner. Also, coupled with upcoming technologies like the "IoT" and "AI," POC nanobiosensors have immense potential for making groundbreaking progress in the areas of health-care management.

8.4 Conclusions

Nanobiosensors for point-of-care (POC) diagnostics applications were primarily developed for the rapid and cost-effective detection of disease causing biomarkers for the pharmaceutical industry. However, with the increase in number of diseases in the present times caused due to imbalance of essential minerals and vitamin in dietary intake of people, there is an increased necessity for the development of cost-effective and accurate nanobiosensors for chemical detection of vitamins. Nanoelectrodes functionalized with different nanoparticles, nucleic acids, and polymer compounds have shown promise for their utility in electrochemical sensing of analytes due to their high efficiency of electron charge transfer in redox reaction, which is evident from numerous cyclic voltammery studies carried out using nanocomposite modified and unmodified electrodes.

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Glossary

AI	Artificial intelligence
Asp	Aspartic acid
BNNT	Boron nitride nanotube
CPE	Carbon-paste electrode
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
ds-DNA	Double-stranded DNA
ELISA	Enzyme linked immune sorbent assay
FAD	Flavine adenine dinucleotide
FMN	Flavine mononucleotide
GCE	Glassy carbon electrode
HAP	Hydroxyapatite
HPLC	High performance liquid chromatography
ICP	Inherently conducting polymer
IL	Ionic liquid
IoT	Internet of things
ITO	Indium tin oxide
MWCNT	Multiwalled carbon nanotubes
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDA	Polydopamine
POC	Point-of-care
Pt:Co	Platinum cobalt
RGO	Reduced graphene oxide
RNA	Ribonucleic acid
SELEX	Systematic evolution of ligands by exponential enrichment
ZnO	Zinc oxide

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Chapter 9
Utility of Nanobiosensors as a Point-of-Care Diagnostics for Neurological Disorders: From Bench to Bedside

Amit N. Raju, Aliabbas A. Husain, and Rajpal S. Kashyap

9.1 Introduction

According to the World Health organisation report Neurological diseases, including neuropsychiatric diseases, cerebrovascular disease, neuroinfections contribute 6.3% of the global disease burden, and it was projected *that it will further increase* to 6.8% by 2030 (WHO 2006). Worldwide it remains the second leading cause of death (9.0 million [8.8–9.4]) and disabilities (Global Burden of Diseases 2019). Timely diagnosis of the disease is of paramount significance to the clinician for initiation of appropriate therapeutic interventions and reducing the associated mortality and morbidities with neurological disease.

Various radiological techniques, like Computed tomography (CT scan), Magnetic resonance imaging (MRI), Positron emission tomography (PET), and Single-photon emission computed tomography (SPECT), have remained the primary choice of many clinicians for the diagnosis of neurological disease. Although these investigations are useful for presumptive screening, most of the radiological findings are inconclusive and often have limitations in the diagnosis of disease with similar etiology and manifestation (Fred 2004). Moreover, most advanced radiological investigations like MRI are expensive and beyond the scope of most diagnostic facilities not only in low resource settings but also in good hospital setups. Moreover, some radiological investigations may have limited utility in patients with certain clinical conditions. Investigations like CT Scan utilize X-rays, which are not suitable for pregnant women, Although MRI does not use ionizing radiation to produce images, it is not suitable among patients with Epilepsy, having pacemaker's implantation, intracranial aneurysm clips, Cochlear implants, certain prosthetic devices, any other type of iron-based metal implants, and for pregnant ladies

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(Emmerson and Young 2021; Stecco et al. 2007). PET *and SPECT use a* radioactive isotope, or tracer is injected intravenously into the body, (Lu and Yuan 2015).

Over the period several biomarkers have been reported in blood and CSF for the diagnosis of a variety of neurological disorders (as shown in Table 9.1). These biomarkers can be estimated by conventional Immunoassay techniques technique like enzyme-linked immunosorbent assay (ELISA), Chemiluminescence immunoassay (CLIA), fluorescent Immunoassays (FIA), radioimmunoassay (RIA), and molecular techniques like polymerase chain reactions (PCR) (Meriggioli 2005; Jain 2013). Though conventional methods have potential, for specific, and sensitive diagnosis of neurological disease conditions, they suffer from a lack of utility to be used as a point-of-care test (POCT) application due to specific infrastructure requirement and depend upon skilled technician, lengthy protocol, and more importantly, longer time is needed to perform these assay, costly and due to difficulty in the automating these technique, and many time also require preprocessing of the samples such as concentration, purification, desalting, etc. (Bell and Kornguth 2008). The conventional techniques are not suitable in many situations when clinicians *need to make immediate decisions*, especially in neurological diseases like stroke, thrombosis, etc. While sometimes a single biomarker could not give the definitive diagnosis, there is a need to perform multiple assays. This increases the cost of the disease evaluation. Thus, there is a need for improved and rapid tools, for confirmative diagnosis and prognosis (monitoring) brain various neurological disorders.

9.2 Point-of-Care Diagnosis and Nanobiosensors

Point-of-care test (POCT) refers to the type of tests that can be done as point of care, usually at the patient bedside, and enables rapid diagnosis of disease in minutes rather than hours aiding clinicians, to improve treatment outcomes. For any diagnostic test with the utility to be used as POCT should fulfill seven characteristics, designated by WHO in "ASSURED" criteria which includes affordability, sensitivity, and specificity, user-friendly, rapid and robust, equipment-free and should be deliverable (Kosack Cara et al. 2017). With the advancement of the newer technologies, POCT technology has gained increased attention of clinicians owing to their rapidity and timely diagnosis and most importantly their utility to be used in low resource laboratory settings and field studies. The current global market of the POC diagnostic has been growing exponentially and is expected to reach USD 46.7 billion by 2024 from 28.5 billion in 2019 (https://www.marketsandmarkets.com). Earlier POC diagnostic tests only detect the presence of the specific analyte (biomarker) in the biological specimen (CSF, Serum, Urine, etc.) for establishing the diagnosis of disease. However, development of advanced biosensors technology, now the POC assay, can be performed quantitatively for the diagnosis of various diseases (Vashist 2017)

Table 9.1 Neurological diseases and their biomarker target for diagnosis and prognosis applications

Neurology disease	Disease type	Biomarker name	Marker type	Specimen for diagnosis
Alzheimer's disease (AD)	Degenerative	Tau protein, phospho-tau	Protein	CSF
		ApoE	Protein	Serum
		Aggregated β-amyloid peptide	Protein	CSF and serum
		APP mutations	Genetic variation	Blood
		Presenilin-1 and Presenilin-2 mutations	Genetic variation	Blood
Parkinson's disease (PD)	Degenerative disease	8-Hydroxydeoxyguanosine	Biochemical	Urine
		Dopamine releasing protein (DARP)		CSF
		α-Synuclein mutations	Genetic variation	Blood
		Parkin gene mutations	Genetic variation	Blood
		UCH-L1 mutations NR4A2 mutations	Genetic variation	Blood
Fragile X syndrome	Degenerative disease	X mental retardation gene 1 (FMR1)-CGG repeats	Genetic variation	Blood
Huntington disease (HD)	Degenerative disease	Huntingtin protein-CAG repeats	Genetic variation	Blood
Central nervous system lupus	Autoimmune disease	Antineuronal nuclear antibody b (ANAb)	Protein (immune marker)	Serum
		Cardiolipin	Lipid	Serum
		Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)	Protein	Serum
Multiple sclerosis (MS)	Autoimmune disease			
		Myelin basic protein	Protein	CSF, urine
		Oligoclonal IgG	Protein	CSF
		Glycoprotein	Protein	CSF

(continued)

Table 9.1 (continued)

Neurology disease	Disease type	Biomarker name	Marker type	Specimen for diagnosis
		Autoantibodies to the 20S proteasome	Protein (immune marker)	Serum, CSF
		Anti-hnRNP-B1 antibody (heterogeneous nuclear ribonucleoproteins antibody)	Protein (immune marker)	Serum, CSF
		HLA-DR mutations	Genetic variation	Blood
Guillain–Barré syndrome (GBS)	Autoimmune disease	Anti-GQ1b ganglioside antibody	Protein (immune marker)	Serum
Myasthenia gravis (MS)	Autoimmune disease	Autoantibodies to acetylcholine receptors (AchRs)	Protein (immune marker)	Serum, CSF
		Autoantibodies to muscle-specific receptor tyrosine kinase (MuSK)	Protein (immune marker)	Serum, CSF
Paraneoplastic syndromes of the CNS (PNS-CNS)	Autoimmune disease	ANA-Ma2 (for SCLC), ANA-1	Protein (immune marker), biochemical	Serum, CSF
		(anti-Hu), ANA-2 (anti-Ri), ANA-3	Protein (immune marker)	Serum, CSF
		Purkinje cell cytoplasmic Ab type 1 and type 2 (PCA-1, PCA-2), PCA-Tr	Protein (immune marker)	Serum, CSF
		Collapsin-response mediator protein Ab (CRMP5-IgG)	Protein (immune marker)	Serum, CSF
		Amphiphysin IgG	Protein (immune marker)	
		Anti-Rc antibody, for SCLC	Protein (immune marker)	Serum
		Progelatinase B/proMMP-9	Protein (immune marker)	Pleural fluids
Stroke	Cerebrovascular disease	Brain natriuretic peptide (BNP)	Protein	Serum, CSF
		Neuron specific enolase (NSE)	Protein	Serum, CSF
		Fibronectin (c-Fn)	Protein	Serum, CSF
		Oxidative stress marker purines	Oxidative stress marker	Serum, CSF
		S-100B	Protein	Serum, CSF

Pyridoxine-dependent seizures	Seizure disorders	Pipecolic acid	Biochemical	Serum, CSF
Tay–Sachs diseases	Tay–Sachs diseases	Beta-hexosaminidase (HEXA) gene	Genetic variation	Blood
Phenylketonuria diagnostic	Metabolic disorder	SNPs in phenylalanine hydroxylase (PAH) gene	Genetic variation	Blood
Glycogen storage diseases	Metabolic disorder	Associated membrane protein–adenosine monophosphate γ 2 (PRKAG2) gene	Genetic variation	Blood
		Lysosome-associated membrane protein 2 (LAMP2) gene	Genetic variation	Blood
Astrocytoma	Primary brain tumors	Vascular endothelial growth factor (VEGF)	Protein	CSF
Glioblastoma multiforme	Primary brain tumors	Cathepsin D	Protein	Serum
		Insulin-like growth factor 1 (IGF-1)	Protein	Serum
		Polysialic acid	Biochemical	CSF
		Neural cell adhesion molecule	Protein	CSF
		Antirecoverin	Protein	Serum, CSF
Medulloblastoma	Primary brain tumors	Survivin	Protein	CSF
Meningioma	Primary brain tumors	Polysialylated NCAM	Protein	CSF
Progressive multifocal leukoencephalopathy (PML)	CNS infection	Human polyomavirus (JC virus)	Virus	CSF
		Prion protein	Protein	CSF, urine
Neurocysticercosis (NCC)	CNS infection	HP10 Ag	Protein	CSF
Cerebral malaria	CNS infection	Plasmodium falciparum histidine-rich protein2 (PfHRP2)	Protein	CSF
Cryptococcal meningitis	CNS infection	CrAg	Protein	CSF

(continued)

Table 9.1 (continued)

Neurology disease	Disease type	Biomarker name	Marker type	Specimen for diagnosis
Lyme neuroborreliosis	CNS infection	Cerebrospinal fluid chemokine (C-X-C motif) ligand 13 (CXCL13)	Protein	CSF
Japanese encephalitis	CNS infection	E genes	Genetic marker	CSF

Note: Based on references—Bell and Kornguth (2008), Deng et al. (2015), Fleury et al. (2016), Pietikäinen et al. (2018), Liu et al. (2018), Wang et al. (2019), Kim et al. (2019)

9.2.1 Biosensors

Biosensors are devices used to detect and quantify the biological analyte (biomolecules) through biological receptor molecules like antibodies, DNAs, and enzymes (Rajpoot 2017). It is an extremely sensitive and specific technology. Owing to associated advantages, it has now been popularized in almost all fields of science like drug discovery, medicine, diagnostic, food safety, environmental monitoring, defense, etc. It has three main components: a high-affinity recognition probe to recognize the analyte and produce a signal, a signal transducer that converts chemical signal to electrical output, and a reader, which is a detector/output unit (Bhalla et al. 2016) as shown in Fig. 9.1. Based on the type of bioreceptor (Sensor) transducer used, it can be classified into various types. With the advancement in biomedical engineering, biosensors, and various POCT platforms like label based, label-free based, lab on a chip, nanomaterial-based, wearable, and wireless have been developed.

9.2.2 Nanobiosensor

The nanobiosensors are sensors that consist of nanoparticles (between 1 and 100 nm) as a transducer in biosensors (Malik et al. 2013). The use of nanoparticles significantly enhances the sensitivity of nanobiosensor for the detection of a very small amount of the analyte present in the biological specimen (Prasad 2014). Nanoparticle transducers used in various types of biosensors are given in Table 9.2. Nanobiosensors technology has been widely explored in various interdisciplinary fields including medical, environmental, and food Sciences (Chomoucka et al. 2012; Law et al. 2014).

Furthermore, the sensitivity (limit of detection) of nanobiosensors also depends on the type of detection system used in conjugation with the nanomaterial transducer. The detection system used in the nanobiosensor development includes electrochemical, electrical, optical, and mechanical detectors.

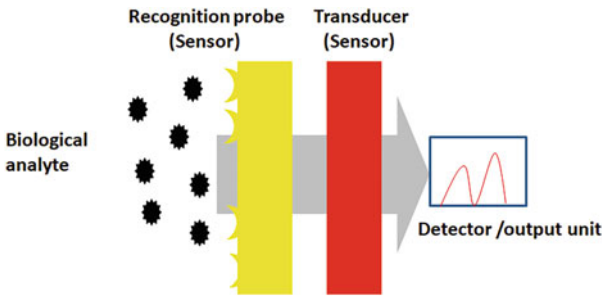

Fig. 9.1 Schematic view of the Biosensors

Table 9.2 Nanoparticles transducers used in various types of biosensors (Rajpoot 2017)

Biosensor type	Nanomaterial
DNA biosensor	Silver NPs
	Gold NPs and graphene
	Platinum nanotubes modified with poly-amidoamine
Enzymatic biosensor	Carboxygraphene
	Graphene nanoplatelet–titanate nanotube composite
	Graphene oxide and silver NPs
	Iron oxide–chitosan nanocomposite
Hydrogen per oxide sensor	Graphene oxide (ERGO)–silver NPs
	MnO_2
Immunosensor	Ferroferric oxide NPs
	Gold NPs
	Gold NPs/polyaniline nanofibers
	Graphene, platinum NPs
	PtCo alloy and graphene
	Magnetic beads
	Cu@Ag (Cu@Ag-CD) core–shell NPs

An electrical or electrochemical detector attached to the transducer measures the changes in the current or voltage using voltammetric, amperometric/coulometric, and impedance measurements when a recognition event takes place in the biosensor (Bard et al. 1985). Similarly, optical detection systems measure the changes in the optical activity either in the form of chemiluminescence, bioluminescence, fluorescence or reflectance, and Surface plasmon resonance (SPR) (Prasad 2014). Mechanical detection techniques are based on cantilever and quartz crystal microbalances (QCMs). Both detect changes in resonance frequency on the sensor surface as a result of analyte binding. The mechanical detection system is very advantageous as it is used for the development of the label-free nanobiosensor (Kubicek-Sutherland Jessica et al. 2017).

9.3 Novel Approaches for Development of Nanobiosensor-Based Diagnostics

Nanobiosensor-based POCT can be designed in a variety of the formats like microfluidics, microarrays, paper-based immunoassays, and optical-based sensors, which can be explored as the first steps in the diagnosis and prognosis of neurological diseases (Harpaz et al. 2017).

9.3.1 Lateral Flow Biosensors

The lateral flow assay (LFA) is a paper-based POCT device that rapidly detects the specific analyte (protein or nucleic acid) in biological fluids and food samples in the qualitative or semiquantitative way for detection of disease biomarker and food contamination. It is very user-friendly and can be used by clinicians, semiskilled technicians in various settings such as laboratories, clinics, and homes. It is a very cost-effective technique; thus, it is very ideal for low resource remote settings and robust field application (Quesada-González and Merkoçi 2015).

Basic lateral biosensor device (LFB) is made up of four parts: (1) Sample pad, (2) Conjugate pad, (3) Detection pad, and (4) Absorption pad. The sample pad consists of the cellulose material; when the sample is added to the sample pad, it flows through the conjugate pad, which consists of the glass fiber impregnated with label particles, that is, nanoparticles (NPs) and analyte-specific receptors. Analytes present in the sample bind with label particles and the bioreceptors (antibody, DNA or aptamers, etc.). Then the sample flows through the detection pad made up of nitrocellulose paper, where the binding reagent (detector) in the detection pad binds with the analyte in the test line. Absorption pad is also made of cellulose as it absorbs the excess sample (Lee et al. 2012). Sandwich and competitive assays can be developed of Lateral flow devices; similarly, multiplexing assays can be developed for detection of the two or more analytes at a time (Quesada-González and Merkoçi 2015). Schematic representation of the Lateral flow/Flow-through Biosensor is given in Fig. 9.2. The sensitivity and specificity of the LFBs depend upon the choice of the NPs used for labeling the different types of bioreceptors (Rajpoot 2017).

Earlier the use of the LFBs was limited to qualitative detection of the analyte in the sample however with the advancement of the technology now; the generated signal can be read with the help of the portable readers or directly using special software for Android, iOS, and Windows-based smartphones, etc. (Table 9.3). This software provides prompt and quantitative test results without any requirement of any costly equipment (Urusov Alexandr et al. 2019). The resultant technology enables the clinician to access the patient's results anywhere avoiding treatment delays (Fig. 9.3).

9.3.2 Optic Based Sensors

The optic-based biosensor is a compact analytical device having a biorecognition element integrated with a transducer system. It measures optical signal generated as a result of the interaction of the optical field with a biorecognition element. The optical signal received by the sensor is proportional to the concentration of the analyte recognized by the recognition element. It broadly works in two formats label free and label based. In the label-free mode, the signal generated is detected directly by the interaction of the analyzed material with the transducer, while in a label-based

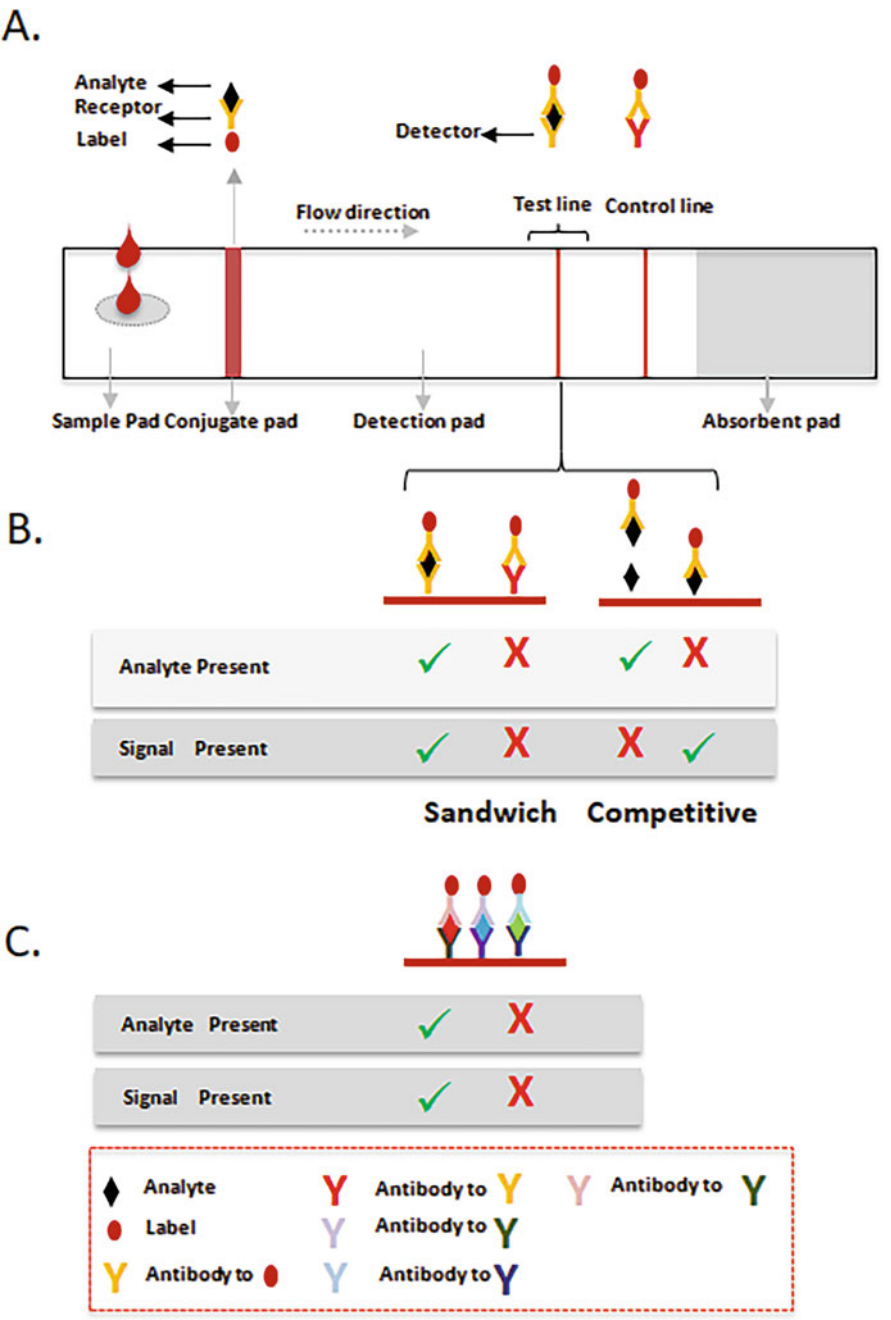

Fig. 9.2 Schematic representation of the nanoparticle-based Lateral flow device Fig. 9.1. Schematic representation of the lateral flow Nanobiosensors (**a**) Basic design, (**b**) Sandwich and competitive designs, and (**c**) Multiplexing designs

Table 9.3 List of portable lateral flow reader equipments and mobile-based applications for quantitative reading of the lateral flow assay result in test site

Reader/software	Company	Country	Reader/ software	Website
iPeak® PoC Lateral Flow Reader	IUL S.A.	Spain	Portable reader equipment	https://iul-instruments.com/
AppDx® Smartphone Reader	Abingdon Health	United Kingdom	Mobile phone application	https://www.abingdonhealth.com/
Immunochromato-Reader C10066 Immunochromato-reader C11787	Hamamatsu	Japan	Portable reader equipment	https://www.hamamatsu.com/
Portable POC reader HRDR-200	**Holomic LLC**	USA—California	Portable reader equipment	https://www.medicalexpo.com/
Quantum Blue®	BÜHLMANN Laboratories AG	Switzerland	Portable reader equipment	https://www.buhlmannlabs.ch/
Easy Reader	Vedalab	France	Portable reader equipment	vedalab.com
Novarum Smartphone Reader—Services	BBI Solutions	UK	Mobile phone application	https://bbisolutions.com/
AXXIN AX-2X	Axxin	Australia	Portable reader equipment	https://www.axxin.com/
BD Veritor System, BD Veritor Plus System	BD Company	United States	Portable reader equipment	https://bdveritor.bd.com/en-us/rapid-antigen-testing/covid-19
ESEQuant Lateral Flow System	QIAGEN	Germany	Portable reader equipment	www.qiagen.com
Cube-Reader	BioAssay Works	United States	Portable reader equipment	bioassayworks.com
Smartphone-based Lateral Flow Device Reader	Platform Kinetics Logo	United Kingdom	Mobile phone application	https://www.platformkinetics.com/lateralflow.html
cPoC Reader	Esterline	Germany	Portable reader equipment	esterline.com
LFA Reader	Dr. Fooke	Germany	Portable reader equipment	fooke-labs.de
Point-of-Care Diagnostic System	GenPrime	United States	Portable reader equipment	genprime.com

(continued)

Table 9.3 (continued)

Reader/software	Company	Country	Reader/ software	Website
Digital Strip Reader	Bio-AMD	United Kingdom	Portable reader equipment	bioamd.com
RDS-1500, RDS-2500	Detekt	United States	Portable reader equipment	idetekt.com
Lateral Flow Tester LFT 100	Hund Wetzlar	Germany	Portable reader equipment	hund.de
Reader HR201	Shenzhen Highcreation	China	Portable reader equipment	www.hkrgr.com
Vertu Lateral Flow Reader	VICAM	United States	Portable reader equipment	vicam.com
INCLIX; INCLIX™ S900	Sugentech	Korea	Portable reader equipment	sugentech.com
AgraVision™ LFD Reader	Romer Labs	Austria	Portable reader equipment	romerlabs.com
Infectious disease POC analyzer Magnia®	Magnasense Technologies Oy	Finland	Portable reader equipment	www.magnasense.com
Multiparametric POC analyzer SD MultiCare™	SD BIOSENSOR. INC	South Korea	Portable reader equipment	www.sdbiosensor.com
ReaScan	Reagena	Finland	Portable reader equipment	reagena.com
Alere™ DDS®2 Mobile Test System	Alere Toxicology	United Kingdom	Portable reader equipment	aleretoxicology.co.uk

optical sensor, a label is used for the generation of the colorimetric, fluorescent, or luminescent signal [e.g., handheld Glucometer]. A wide variety of biological materials, like antibodies, antigens, enzymes, receptors, nucleic acids, whole cells, and tissues, can be used as biorecognition elements in the optical-based biosensor. There are a variety of optical biosensors available, such as surface plasmon resonance (SPR)-based biosensors, evanescent wave fluorescence, bioluminescent optical fiber biosensors, interferometric, ellipsometric, and reflectometric interference spectroscopy, and surface-enhanced and Raman scattering biosensors (Damborský et al. 2016).

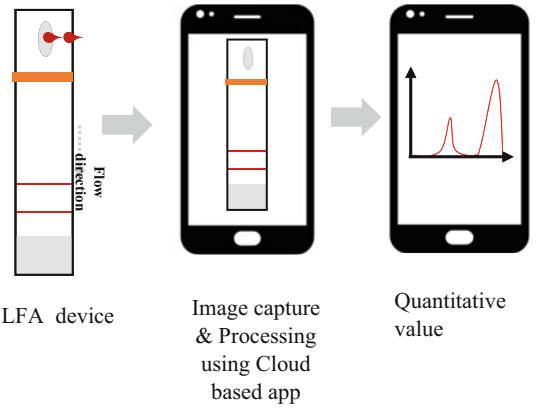

Fig. 9.3 Schematic representation of the software-based Quantitative analysis of the Lateral flow assay result

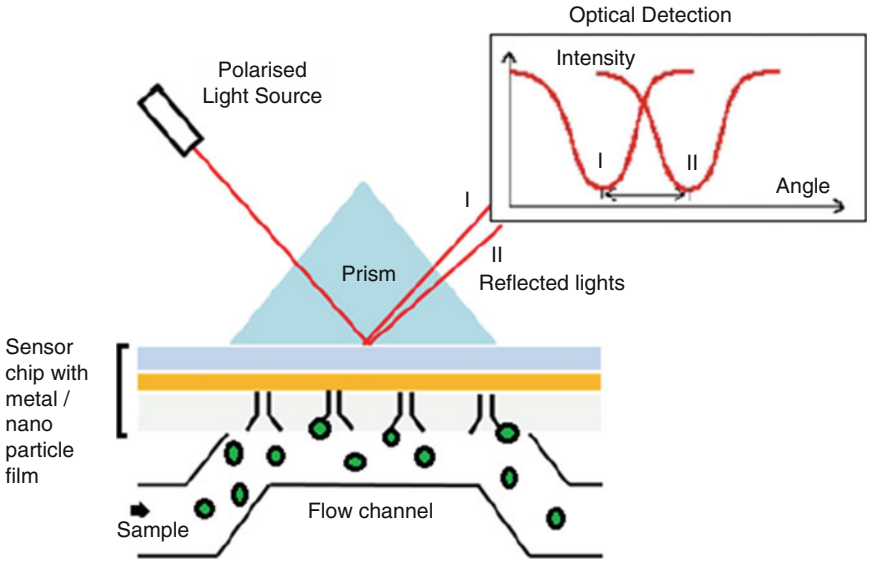

Fig. 9.4 Schematic representation of SPR-based biosensors

SPR-based biosensors are the most widely used optical biosensors that measure the change in the refractive index of the molecular on a surface that is directly proportional to the mass of the mass molecule (https://chenglab.ucr.edu/spr). In SPR biosensor, one of the molecules (ligand or analyte) is immobilized on the sensor surface (metal or metal nanoparticles surface). The optical detector then measures the intensity shift and presents the result in quantitative values (Fig. 9.4). SPR phenomenon enables detection of refractive index at the sensor surface, which is proportionate to the biomolecule concentration (Damborský et al. 2016).

9.3.3 *Microfluidics-Based Nanobiosensors*

Microfluidics is a liquid handling technology, which is used for controlling and manipulating fluids in extremely low volume [Microliter (10^{-6}) to picoliters (10^{-12})] with the help of networks of channels from tens to hundreds of micrometers engraved using soft photolithography, soft lithography, or nanoimprinting (https://www.fluigent.com/resources/microfluidic-expertise) (Nie et al. 2010). The integration of microfluidic devices and nanobiosensors technologies provides new opportunities toward the development of highly sensitive and specific assays, with the advantage of real-time and multiplexing detection, at a reduced cost (due to less reagent consumption) along with minimizing the waste production (Luka et al. 2015). Thus it is very suitable for the development of the point-of-care nanobiosensors assay.

There are three generations of microfluidics systems developed to further reduce the reagent consumption for the different biochemical applications. This includes a continuous microfluidics system, drop-based Microfluidics system, and digital microfluidic system (DMF) (Fig. 9.5). DMF is the most advanced droplet-based microfluidic system, which utilizes an extremely low volume of reagent consumption (Pollack et al. 2002). The technology has been successfully explored in

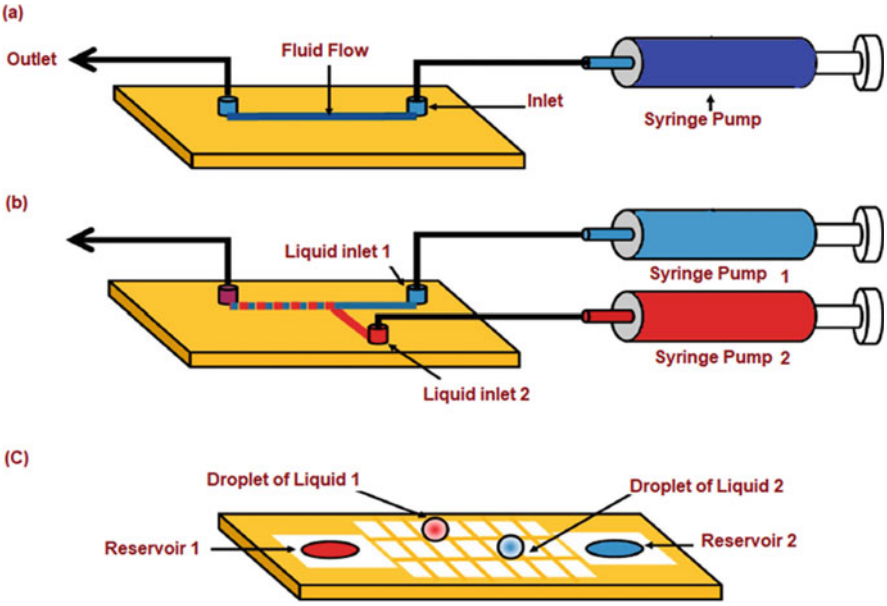

Fig. 9.5 Schematic presentation of the Microfluidics system (**a**) Continuous (**b**) Drop Based (**c**) Digital microfluidic system

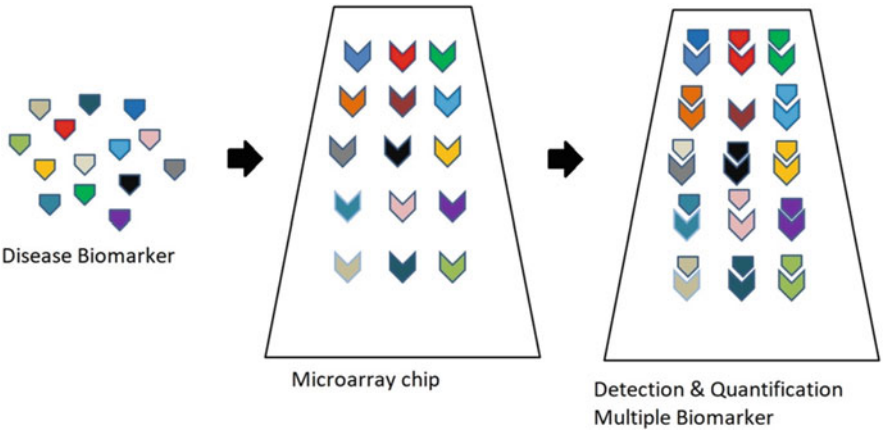

Fig. 9.6 The schematic representation of principle of microarray-based Nanobiosensors

biosensors for the quantification of many clinically important biomarkers like using enzymes, antibodies, and aptamers as a biorecognition element in the biosensors (Luka et al. 2015).

9.3.4 Microarray-Based Nanobiosensor

Microarray is a multiplex lab-on-a-chip that can be used for simultaneous analysis of the multiple analyte, in a very low volume of sample in a very cost-effective manner. In microarray, the recognition element (antibody or antigen/oligonucleotides probe) is used as an array of multiple spots in chip Hwang et al. (2017). The presence of a corresponding complementary analyte in the sample will result in a change in the optical activity, which is proportional to the analyte concentration (Fig. 9.6). Many microarrays-based platforms are commercially available for high-throughput screening of the protein, DNA, drug compounds, etc. for various applications (Lagraulet 2010; Ma and Horiuchi 2006). This technology can be explored for the POCT development for detection signatures of neurological diseases toward diagnostic and prognostic applications.

9.4 Nanobiosensors and Diagnostic Landscape of Neurodegenerative Diseases

In recent times, significant advances have been made in diagnosis of neurological diseases. Earlier, a definitive diagnosis of the neurological disease was done through an autopsy of the patient's brain after his death. Now with the help of clinical

examination, along with radiological imaging and biomarker technology, a definitive diagnosis of the neurological disease and its severity is possible (please see Table 9.1). The introduction of biosensors technology further increases the hope for the easy rapid diagnosis and monitoring of neurological cases. Researchers have successfully developed a nanobiosensor-based protocol sensitive quantification of many neurological diseases.

9.4.1 Nanobiosensor Technology in Diagnostic of Acute Stroke

In stroke, the blood supply to part of the brain is suddenly interrupted, if it continues for a longer time, the brain cells in the affected area die due to lack of oxygen and nutrients supply. Eighty to eighty-seven percent of stroke cases are ischemic type, while 13–20% was of hemorrhagic type. To date, only a single drug, that is, intravenous tissue-type plasminogen activator (*IV tPA*), is approved for the treatment of ischemic stroke. But the *IV tPA* can be given to the patient who reaches the hospital within the window period of 3 h (Arulprakash and Umaiorubahan 2018). Thus, most of the stroke cases in developing countries don't qualify for the *IV tPA* treatment due to delay in the diagnosis and thus have to be managed with alternative therapy. Thus, the early diagnosis and/or treatment of stroke is very critical.

Several biomarkers have been reported having the potential to be used as early diagnosis and prognosis of stroke patients (Harpaz et al. 2017). However, delays in diagnosis using conventional tools limit their use in clinical practice. With the integration of the nanobiosensor technology, POCT can be developed, which will enable the rapid estimation of stroke biomarkers at the bedside in the emergency department. Few nanobiosensorbased POCT developed for stroke and are very promising for acute stroke management can be explored in clinical practice for a favorable outcome.

Cohen et al. (2015) has developed quantitative detection of established acute stroke biomarkers of neuron-specific enolase (NSE) using the Tethered Enzyme Technology (TET). In brief, in Coupled enzyme pyruvate kinase (PK) and luciferase (Luc) were by Immobilization on SiO_2 NPs in a specific orientation and NSE, the level was detected by 3-step coupled reactions. Step 1 enolase/NSE in the sample catalyzes the conversion of 2-phosphoglycerate (2-PG) (Substrate for NSE) to phosphoenolpyruvate (PEP), Step 2—Immobilized PK, in turn, converts PEP and ADP to pyruvate and ATP, which is then used by Luc and generates a photon of light, which is directly proportional to the concentration of NSE. The result of the TET assay was also matched with the standard ELISA assay; however, the time required for the TET is only 10 min as compared with 3–5 h in ELISA-based assay. In addition, the TET assay was able to detect the subphysiological concentration ($<8.7 \pm 3.9$ ng/mL) of NSE. This could be a very useful tool as a POCT for stroke management.

Wang et al. (2019) developed a surface-enhanced Raman scattering (SERS) analysis technique based lateral flow assay for quantitative detection of another established marker for stroke known as glial specific protein S100-β in the plasma samples of the stroke patient. In this lateral flow assay, Gold nanoparticles (GNPs) were tagged with the Raman reporter 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). For quantitative measurement, the intensity of the developed test line was analyzed using a specialized XDR Raman microscope (Thermo Scientific, https://www.thermofisher.com) with a laser wavelength at 785 nm. The S100-β protein detected by this method is very low, (i.e., 1 pg/mL) concentration of S-100B, and was comparable with ELISA in a very short time. Owing to good sensitivity, selectivity, and stability, SERS technology could be extremely useful in the early diagnosis of disease with clinical application.

Sayan and Kotan (2016) developed an improved Lateral flow immunoassay (LFIA) for the detection of brain natriuretic peptide (BNP), using gold nanoparticles (GNPs). Plasma BNP levels have been reported to elevate in acute stroke patients and are associated with mortality and bad prognosis. Detection sensitivity of the assay in plasma samples is around 0.1 ng/mL within 10–15 min, presenting ~15-fold enhanced sensitivity compared with conventional latex-based lateral flow assay. Thus, the developed LFIA for BNP offers tremendous potential for POC test and personalized medicine for stroke patients.

9.4.2 Nanobiosensor Technology in Diagnostic of Alzheimer's Disease

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease and occurs mostly among older adults. A decrease in the clearance of myeloid beta plaques (Aβ) through the cerebrospinal fluid (CSF) sample is considered to be the important biomarker for the diagnosis of Alzheimer's disease (Niemantsverdriet et al. 2017). A peptide of 1–42 amino acids is the major component of the Aβ plaques. The CSF sample of the AD patients reflects a very low level of Aβ(1–42) <500 pg/mL (0.1 nM) as compared with normal individuals (controls), indicating its accumulation in the brain. At present, the diagnosis of AD mainly relies on the enzyme-linked immunosorbent assay (ELISA) based detection of the Aβ(1–42) in the CSF samples (Marksteiner et al. 2007; Irwin et al. 2012).

Kang et al. (2009) developed a vertically configured electrical detection system based on scanning tunneling microscopy (STM) to detect Ag-Ab binding events and evaluated the same for detection of immune complexes comprised of the model protein, beta-amyloid (1–42), corresponding antibody fragments, and gold (Au) nanoparticles-antibody conjugates. The developed STM detection system was reported to measure very low 10 fg/mL of beta-amyloid (1–42) concentration frequency. Cheng Xin et al. (2014) developed a dual detection biosensors platform using Electrochemical impedance spectroscopy (EIS) and localized surface plasmon

resonance (LSPR) on the same Au nanoparticle (AuNP)-modified indium tin oxide (ITO) coated glass surfaces for the detection of DNA hybridization related to a specific point mutation in apolipoprotein E gene (ApoE). ApoE is plasma lipoprotein. One or more mutations in the ApoE gene (located in chromosome 9) were reported to increase the risk of developing type-III hyperlipidemia, atherosclerosis, and AD. The developed biosensors can be further miniaturized for the rapid and sensitive and a high-throughput diagnostic device for detection of AD. Wu et al. (2014) has demonstrated electrochemical impedance spectroscopy (EIS) based nanobiosensor using gold nanoparticles (GNPs) as the sensing electrode for effective detection of Aβ(1–42). They have used an anodic aluminum oxide (AAO) layer with a nanohemisphere array as the substrate. A linear detection range between 1 pg/mL and 10 ng/mL of Aβ(1–42).

Recently, Carneiro et al. (2017) developed a label-free immunosensor for the quantification of Aβ(1–42), in the CSF samples. In this sensor, AuNPs are electrodeposited on the previously mercaptopropionic acid (MPA) modified gold electrode (MPA/Au electrode) followed by antibody mAb against Aβ(1–42) immobilization on the AuNPs surface with proper orientation. The binding of antibody mAb with the Aβ(1–42) in the samples is quantitatively measured using voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). The developed sensor has a very low detection limit, wide linear range, good accuracy, and reproducibility and gives results in 10 min and thus could be used for the early diagnosis of the AD patient and for studying the treatment response of AD patients.

Similarly, Brazaca et al. (2019) recently developed a paper-based LFIA for quantification of fetuin B and clusterin simultaneously in blood samples for rapid diagnosis of Alzheimer's. In brief, they immobilized the monoclonal antibody against fetuin B and clusterin antibody on gold nanoparticles (AuNPs) and deposited it on paper pads. When the sample is added to the paper-based device, it flows toward the selective antibody and AuNP-Ab get accumulate in the test zone causing the color change from white to pink, after 15 min pictures were taken, then, manually cropped specially designed MATLAB-based software which read the intensity of the color which corresponds to the Quantity of the fetuin B and clusterin in blood samples. The developed nanobiosensor has a potential for early-stage diagnosis of Alzheimer's disease and toward a better understanding of Alzheimer's developing mechanisms.

Zhao et al. (2019) developed a dual-quenching electrochemiluminescence (ECL) based on tris(2,2′-bipyridyl)ruthenium(II) [Ru(bpy)32+] as chromophores caged in three-dimensional (3D) zinc oxalate metal-organic frameworks [Ru(bpy)32+/zinc oxalate MOFs] for rapid excited-state energy transfer migration among Ru(bpy) and a second AuNP and NiFe-based nanocube MOFs were included as a dual quencher for sensitive detection of Aβ42. Developed 3D zinc oxalate MOFs were reported to detect a significantly low level of Aβ ranging from100 fg/mL to 50 ng/mL in CSF samples.

9.4.3 *Nanobiosensor Technology in Diagnostic of Parkinson's Disease (PD)*

Parkinson's disease is very common a progressive movement disorder that occurs due to the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) part of the brain. The presence of the protein clumps called alpha-synuclein (aSyn), or Lewy bodies, is the hallmark of the PD. The disease starts with a simple unnoticeable shiver in one hand, which progresses over time if not treated. Genetic changes in many genes and autoimmunity were reported to be the major causative factors (Aghili et al. 2018). CSF total aSyn levels were reported as the main biomarker for PD. Similarly, CSF Aβ42, CSF tau, and serum or plasma urate were also reported to be potential biomarkers in PD. But at present no biomarker is in clinical practice for the early and stage-specific diagnosis of PD has been reported, due to variability in the sensitivity, and specificity (Cova and Priori 2018). Many researchers have explored the advanced Nanobiosensors technology for the sensitive and rapid diagnosis of PD.

Kim et al. (2010) developed a glassy carbon electrode (GCE) using graphene. The developed electrode was able to detect dopamine in a linear range from 4 to 100 μM. Aghili et al. (2018) developed an electrochemical nanobiosensor for the detection of circulating biomarkers, miR-195 for early detection of PD. In this nanobiosensor, Exfoliated graphene oxide (EGO) and gold nanowires (GNWs) were used for modification of the surface of the screen-printed carbon electrode. Target miRNAs (miR-195) were detected using a specifically designed single-strand thiolated probe, which is detected using doxorubicin as an electrochemical indicator using pulse voltammeter. Developed sensors were reported to detect the target miR-195 with very high sensitivity with a detection limit of 2.9 fM. Karki et al. (2021) has documented various promising nanobiosensorbased detected systems for early ultrasensitive detection of Alzheimer's disease, which can be explored for noninvasive detection of AD. Similarly, this point-of-care test could be life-saving in the care of critical neurological diseases, especially in case of medical emergencies, and in low resource settings. However, this technology is yet to be explored or very limitedly explored in many neurological diseases like epilepsy, stroke, etc.

9.5 Translational Challenges of POC Nanobiosensors for Diagnosis of Neurological Disease

Nanobiosensor-based POCT offers an opportunity to the clinician to diagnose various neurological diseases at a very early stage so that preventive therapy can be started. This may add to the outcome of the Neuro patient.

As lateral flow assay-based, nanobiosensors assay being quick, user-friendly, portable, and easy to read and dispose of platforms does not require elaborate infrastructure facilities and expertise and does not demand, skilled technician to

perform the assay. It can be easily translated in the remote setup like villages and periurban areas lacking in the advanced infrastructure facility.

Similarly, it may also be useful in various public sector clinical settings where there is a considerable burden on the radiology department due to long waiting patient queues. The use of nanobiosensors as an adjunct to radio diagnosis will be helpful to manage a large number of patients. Quick screening of the patient with nanobiosensor-based LFA will rule out the unnecessary scan to be done.

Despite the many potential benefits of nanobiosensor technology, it has not been explored much in actual practice as they were limited to research and development level, no product has yet been commercialized for diagnosis of the neurological disease.

More advocacies and research are needed for using this POCT for the evaluation of the neurological disease, so that these techniques could reach the end-user, that is, clinician. Govt should also endorse these POCT, through newspaper, media, and in the Govt Hospital setting, particularly in the Primary Healthcare center and in the rural area, where the connectivity and resources are limited. Similarly, conventional lateral flow assay developed against many neurological diseases need to be upgraded by integration of nanobiosensor technology to increase the sensitivity of the existing assay, toward the POCT development for unexplored neurological disease. Further extensive research is also needed, to miniaturize advanced technology such as SPR LSPR, and Microarray-based sensors for making it cost-effective portable to be used for POC testing. Efforts are also needed for the development of the multiplex system so that more than one parameter can be assayed simultaneously to reach a correct conclusion about the complex multifactorial neurological disease. Further many potent biomarkers are yet to be explored/or very limited explored in Nanobiosensor technology-based diagnostic platforms, which may also be important as prognosis of diseases after therapy, or can serve as an adjunct marker.

The growing trend of advanced diagnostic development suggests that the next decade would be of nanobiotechnology-based biosensors, which will play an important role not only in diagnosis but also in linking diagnosis with treatment and development of personalized medicine.

9.6 Conclusions

POC is a very useful technology for low-resource settings. It could allow early diagnosis and the management of the neurological patient toward a better outcome. Another most demanding advantage of nanobiosensor-based diagnostics is that it reduces the biomedical waste generation. Careful planning and research are needed to bring the nanobiosensor-based POCT into clinical practice. The clinician should be aware of this advancement in the diagnostic field so that technology could be explored for the screening of neurological diseases. At the same time research focus should be to make the technology very cost-effective to be used on a routine basis. Extensive research is also needed for the development of the nanobiosensor-based

POCT assay for many neurological diseases for which such technology is not available and nanobiosensor-based POC confer change paradigm the clinical diagnosis in the coming future and will be helpful in the development of personalized medicine.

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Chapter 10
Point-of-Care Testing and Diagnostics for Sexually Transmitted Disease

Vinod Kumar and Prabhjot Kaur

Abbreviations

DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
FTA-ABS	Fluorescent treponemal antibody absorption
HSV-1 and -2	Herpes simplex virus HSV-1 and HSV-2
LFIA	Lateral flow immunoassay
LGV	Lymphogranuloma venereum
MHA-TP	Microhemagglutination assay for T Pallidum antibodies
NAAT	Nucleic acid amplification test
POCT	Point of care testing
RNA	Ribonucleic acid
RPR	Rapid plasma regain
STD	Sexual transmitted disease
STI	Sexual transmitted infection
VDRL	Venereal Disease Research Laboratory test

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10.1 Introduction

Point-of-care testing (POCT) is done at the site where patient encounters the health-care system first time. It provides instant and rapid actionable information about the patient health. This instant information is crucial in deciding patient heath management. The POCT in sexual transmitted disease is of immense importance in reducing the multiple clinic visits of patient, maintaining privacy, and facilitate the containment of infectious disease outbreaks. The available conventional diagnostic tests are confined to clinics and specialized laboratories, community clinics are not equipped with required facilities and trained persons to perform these tests (Speers et al. 2018; Van Der Pol et al. 2020). The commercially available tests are neither affordable nor accessible to many patients. Most of the individual with these infections does not even know about it and pass it to their partner. The only way to find out the infection is to undergo specific diagnostic test. The sexual contact between the uninfected and infected person is responsible for the transmission of STD/STIs. Their asymptomatic nature for a considerable time is a major reason for the spread and a challenge to prevent it. Other than the involvement of genital infections, STDs/STIs cause serious health-deteriorating problems like cervical cancer, pelvic inflammatory disease, pregnancy problems, liver disease, and infertility. Individuals with STD/STIs are more venerable to other infections like HIV (McCormack and Koons 2019; Markle et al. 2013).

A quick and at an exceedingly early-stage diagnosis is efficient in controlling the sexually transmitted infections claiming millions of deaths globally. About more than 30 different types of bacteria, viruses, and parasites are responsible for this transmission. The acute phase of STD/STI can be fatal and has significant health threat to high-risk patients (McCormack and Koons 2019). Aggressive screening and detection can potentially deter transmission and decrease STD/STI occurrence. The incidence of STD/STI is ethnic and racial based, poverty, unemployment and low education increases its incidence rate. In United States, half of the newly diagnosed STD/STI cases are of aged in between 15 and 24 year. Developed and self sustanable contries also unable to control the spread of curable STIs, despite the aggrasive screening of the potential STD/STI cases through ultrasenstive or specific detection assays. Only in USA about 20 million new infections occur each year. Approximately, more than one million of new cases of only curable bacterial STIs reported worldwide each day. About, half a million babies die each year only in sub-Saharan Africa alone because of congenital syphilis. Establishing a good quality of STD/STIs screening facilities is more difficult in limited resources setting(Markle et al. 2013; McCormack and Koons 2019; Unemo et al. 2017).

WHO is reporting more than one million STIs every day worldwide. An individual with STI (syphilis and herpes) is likely at a three-time higher risk to acquire HIV compared to the non-STI individual. The increased antimicrobial resistance is alarming according to WHO, the drug resistance to gonorrhea is suspected as a major threat to control STI spread (Bray et al. 2018; Korenromp et al. 2019; Rowley et al. 2019). Gonococcal has shown high resistance to quinolones, azithromycin, and

extended-spectrum cephalosporins. To screen and test susceptible infectious individuals immunoassays were introduced in the year 1917 (Kozel and Burnham-Marusich 2017). The addition of radioimmunoassay (RIA) in year 1960 and enzyme-linked immunoassay (ELISA) in year 1971 has increased the sensitivity of the detection (Yalow and Berson 1960; Kozel and Burnham-Marusich 2017). The automation of ELISA enables the high-throughput sample processing and makes it a dominant platform for laboratory analysis. However, these tests are highly complex and require trained personnel and specialized equipment.

The accuracy and constancy in diagnostics the immunoassays are being modified to perform rapid test at POC level. The first development was to include the use of capillary-based migration in cellulose acetate and coupling of antibodies to colloidal gold or latex particles. Presently there is potential use of lateral flow immunoassay (LFIA) platform, which is extremely versatile. To meet the STD-/STI-associated challenges, the practice of POCT is much needed (Moon et al. 2013; Kozel and Burnham-Marusich 2017; Speers et al. 2018).

10.2 Sexually Transmitted Disease and Infection (STD/STIs)

The infection transmitted or spared through sexual contact is majorly divided as curable and incurable infections. Curable infection included with syphilis, gonorrhoea, chlamydia, and trichomoniasis, while the incurable is of viral origin like HIV, Hepatitis B, Herpes simplex virus, and Human papillomavirus. A few STIs can also be spread through blood or blood products, while some can be transmitted from mother to the child during pregnancy and childbirth.

10.2.1 STD/STI Epidemiology

The four commonest and curable infections that account for approximately 357 million new cases globally every year are chlamydia (130.9 million), trichomoniasis (142.6 million), gonorrhea (78.3 million), and syphilis (5.6 million) cases. These infections are not fatal but significantly impact the healthcare burden or budget. However, the increase in the antimicrobial resistance in *Neisseria gonorrhoease* and *Mycoplasma genitalium* is alarming. WHO estimated an occurrence of new four curable STIs cases in year 2012 globally (Markle et al. 2013; McCormack and Koons 2019).

Estimated cases in million	Americas	African region	Eastern Mediterranean region	European region	South-East Asia region	Western Pacific region
Chlamydia	24.7	12	10.5	8.9	13.8	60.9
Gonorrhoea	11	11.4	4.5	4.7	11.4	35.2
Syphilis	0.9	1.8	0.5	0.4	0.9	1
Trichomoniasis	27.4	37.4	15.6	3.8	13.2	45.3

10.2.2 Disease Organisms and Laboratory Based Evaluation

10.2.2.1 Gonorrhea

Nucleic-acid-based hybridization, genetic amplification, and microscopy are the common methods used to detect gonorrhea. Among all these tests, nucleic acid amplification test (NAAT) is the gold standard and recommended. The culture of the infected person swab is also recommended to detect the drug resistance (Ng and Martin 2005). The major drawback of hybridization and amplification methods is the time consumption and required specialized facility that delayed the diagnosis.

10.2.2.2 Chlamydia

The recommended tests for the detection of chlamydia are NAAT, genetic probing, culture, and antigen detection. PCR has 95% sensitivity, while LCR sensitivity is around 85–98%. Culturing method has a specificity of 100% and sensitivity of about 60–90%. Available Enzyme immunoassay (EIA) had sensitivity and specificity of about 85% and 97% respectively, but this technique is useful only if screening volume is enough. This technique is also associated with high false positivity rate. Nucleic acid hybridization also needs a large amount of sample DNA, while its sensitivity is about 75–100% and specificity of 95% (Chernesky 2005). EIA test needs specific instruments to read the results. Often the new techniques, instruments, and diagnostic tests become company proprietary products that not only delay their delivery and availability but hamper the diagnosis. PCR and NAAT probing methods are of longer duration and require more time to deliver final results.

10.2.2.3 Trichomoniasis

The most detection method for trichomoniasis is (wet mount microscopy) microscopic evaluation of vaginal discharge using saline wet preparation. The sensitivity of this test is in 60–70%. Other adopted method is culture, which is time consuming, but accurate (Garber 2005). The existing technique the wet mount is very old and of poor sensitivity. Diagnosis through organism culturing is a time-consuming method

and has a high probability to miss the active stage of the disease. On the other hand, Trichomoniasis demands immediate testing, diagnosis, and resolution.

10.2.2.4 Syphilis

Treponemal and nontreponemal serological tests are the mainstays of syphilis diagnosis. The initial screening nontreponemal test includes VDRL and rapid plasma regain (RPR) is used. The specific treponemal test includes fluorescent treponemal antibody absorption (FTA-ABS) and microhemagglutination assay for T pallidum antibodies (MHA-TP). These are expected to provide confirmed diagnosis, however, these are unable to separate false positives. Dark field microscopy is typically used to detect spirochetes to test the chancre in primary syphilis (Ratnam 2005). The most used VDRL test is unreliable in late-stage syphilis and gives false-negative results if the syphilis infection is less than 3 months. There is high probability of getting false-negative results if an individual has Lyme disease, malaria, and tuberculosis.

10.2.2.5 Chancroid

Culturing is the gold standard for its diagnosis. Artificial media like gonococcal agar base with 2% bovine hemoglobin and 5% fetal calf serum are used for the detection cultures. PCR and indirect immunofluorescence are also used for the fast diagnosis. However, these tests are costly and need sophisticated setting. This test has the sensitivity of about 61–91% (Lewis 2000). As such there is no immediate confirmatory test available for Chancroid. The only definitive test available is isolation of *H. ducrey* on specific medium that requires expert technical and long time.

10.2.2.6 Herpes Simplex Virus

Tzanck smear or cytology of the scrapped infected lesion was used to detect HSV infection. This method is very insensitive and lacks specificity. Viral culture can also be used with low sensitivity. The PCR for HSV is most widely accepted technique for diagnosis. Further in combination with serological tests, it can be useful to differentiate between HSV-1 and HSV-2 infractions (Singh et al. 2005). The available HSV tests are 20% times misdiagnosed and reported to fail in diagnosing 25% of true positive cases. Hence, there is need to get advancements in HSV testing preferably the POCTs.

10.2.2.7 Lymphogranuloma Venereum (LGV)

NAAT and PCR are used with high sensitivity and specificity. However, this test is not offered at all the health-care centers. It is most recommended in the cases with

rectal or oral lesions. Serologic tests can also be used including immunofluorescence and complement fixation. These are the most commonly used tests for the diagnosis, but they can't distinguish chlamydia serotypes and recent or past infections (Ceovic and Gulin 2015). These tests could not discriminate between LGV and Non-LGV genovars, although no commercially FDA-approved test is available for LGV. Serological testing is very unspecific and generalized. POCTs can improve the diagnostic accuracy as well as speedup this process.

10.3 POCT and STD/STI

10.3.1 Basic of POCT

The development of POCT platform is to reduce the global health-care costs along with miniaturization, networking, storage, and multiplexing. POCT has the potential to perform in resource-limited settings. Current efforts are directed toward the translation of research into a POCT platform, which is affordable and cost effective. The development of such POCT platforms required a close association between the developer and end user, especially in resource limited settings. To attain low-cost diagnostics, simple processing, and qualitative analysis with higher sensitivity devices lateral flow (LF) platform is the first choice for POCTs (Srinivasan and Tung 2015; Kozel and Burnham-Marusich 2017). Because of its extreme versality, LF immunoassays (LFIAs) are the most widely used for the detection of antigen of high molecular weight, while low molecular weight antigen requires competitive format. PDMS, because of their excellent performance and easy prototype, provide a preferable platform for POCT devices (Unger et al. 2000). Only the low cost is not sufficient to meet the increased demand diagnostic devices, their performance, and accuracy is also important. A careful evaluation of their capability to perform analysis, collect data, and interpret is important. Present diagnostic setting also needs a recognized data management system, which helps to decide the available treatment options. The POCT data collection is of no use if it is not contributing to clinical decision making. Therefore to develop a diagnostic test based on the microfluidic/biological/electronic method, requires a complete understanding of the measurement technique, biomarker, fluidics, sample processing, and data collection (Srinivasan and Tung 2015; Kozel and Burnham-Marusich 2017).

10.3.2 Nano Sensors in POCT

10.3.2.1 Biosensors POCTs

A biorecognition element in combination with a transducer forms a biosensor. The biological sensing component of the biosensor can be an enzyme, microorganism,

RNA, DNA, and antibody and used for their classification (Wang et al. 1998; Ehrhart et al. 2008). Similarly, the transducer can be of piezoelectric, electrochemical, and optical, and used for the device classification. The biorecognition element of the biosensor interacts with the sample or analyse and senses its physicochemical changes including thermal, electrical, optical, and thermodynamic, which are further converted into electric signal by the transduce. Enzyme-based biosensor catalyzes the substrate and generates detectable product mostly commonly hydrogen peroxide (Wang et al. 2020). Nucleic acid biosensors rely on the strong base pair affinity, which further decide their sensitivity and specificity. The binding energy of the base pairs will be converted into electrical signals (Banerjee et al. 2013). Biosensor mounted with whole cell (known Cell based biosensors; CBBs) including bacteria, eukaryotic cells, or yeast, etc., which sense the analyte for produce a detectable signal (Jacobs et al. 2014; Banerjee et al. 2013; Wang et al. 2020).

10.3.2.2 Microfluidics Bases POCTs

Microfluidics is the most common platform used for the testing and diagnosis of POCTs (Price 2001; Kozel and Burnham-Marusich 2017). Because they perform efficiently in lesser samples, reagents, with easy handling. With the advanced in the synthesis of recombinant antibodies and their fragments has increased the sensitivity and specificity of POCTs in a small setup (Jacobs et al. 2014). Further advancement added is the use of aptamers, molecular imprints, and peptides (Zhou et al. 2014; Chen et al. 2010; Holliger and Hudson 2005). Their use has reduced the size of device without compromising its sensitivity and specificity of the tests. The use of chemiluminescence and antibodies however increases the cost of the test.

10.3.3 POCT in Monitoring STD/STI

10.3.3.1 Gonorrhea

Gonorrhea is the second most prevalent STI of bacterial origin that has immensely increased the STI burden because of its antimicrobial resistance. The available NAATs cannot be used at POCT because of high cost and time consuming. The available POCTs are IoCT, GeneXpert, and resistancePlus (Tucker et al. 2013; Gaydos and Melendez 2020; Speers et al. 2018; Van Der Pol et al. 2020). IoCT is a tabletop NAAT based test can be performed on vaginal swab or urine. It is a 4-step process and delivers the results within 30 min. The reported sensitivity and specificity are 100% and 99.9% respectively for women swab, while in male for urinary analysis the sensitivity is 97.3% and specificity is 100%. GexXpert is a real-time amplification-based tabletop test, which can be done on vaginal swab and urine. It takes 90 min to produce the results in a 4-step procedure. This test is preferably used in pooled samples including pharyngeal, rectal, and urine (Chen et al. 2010). While

ResistancePlus is plexPCR tabletop test providing result after 4 step procedures in 50 min. It is first molecular test for the simultaneous detection of infection and ciprofloxacin susceptibility. This technique used the high-level multiplexing including gonorrhea (*opa* and *porA*) and *gyrA* gene. Its recorded sensitivity is 100% and specificity is 99.1%. The ciprofloxacin susceptibility detection is done at 96% sensitivity and 100% specificity (Ebeyan et al. 2019; Goldenberg et al. 2012). Gene Xpert assay has been used successfully in Australia as POC. A prospective prototype recombinase polymerase amplification (RPA) has been developed by TwistDx diagnostic UK. Its specificity is about 100% and provide results in 15 min (Harding-Esch et al. 2019). Along with this the other prototype is Novel Dx platform. It is an inexpensive microfluidic diagnostic molecular assay with self-contained test specific cartridge. This device produces the results within 25 min (Meyer and Buder 2020).

10.3.3.2 Chlamydia

Presently available POC of Chlamydia detection incudes GeneXpert, aQcare Chlamydia TRF kit, ACON Plate CT Rapid Test, Chlamydia Rapid Test, ACON Duo, QuickVue Chlamydia Rapid Test, AUFC, and Biorapid Chlamydia Ag test (Hurly et al. 2014; Herbst de Cortina et al. 2016; Goldenberg et al. 2012). Out of these testes GeneXpert is FDA approved and displays >97% specificity and sensitivity (Tabrizi et al. 2016). Next to this is aQcare Chlamydia TRF kit and AUFC kit, their performance is acceptable with sensitivity and specificity of about 90%. aQcare Chlamydia TRF kit is a LFIA platform based POCT kit used to detect the Chlamydia antibody in the sample (Ham et al. 2015). The AUFC (rapid automated urine flow cytometry) test detect the chlamydia tagged specific fluorescent polymethine dye in the urine. Other available tests performance is below average, their specificity and specific is <75% and <50% respectively (Herbst de Cortina et al. 2016).

10.3.3.3 Trichomoniasis

The POCTs which are used for the detection of Trichomoniasis includes acridine orange staining, real-time PCR, Affirm VP III, and OSOM trichomonas rapid detection test kit (Herbst de Cortina et al. 2016). For the routine and fast diagnosis OSOM rapid and Affirm VP III tests are being used. OSOM test results in 10 min, while Affirm VP III delivers it in 45 min. APTIMA test, based on NAAT is used for the detection of *Trichomanias vaginalis*, with specificity of 87–100% and sensitivity of about 97–100%. The OSOM test is based on colour immunochromatographic dipstick technology. The sensitivity and specificity of this test is 83.3% and 98% (Hegazy et al. 2012). While Affirm VP III used direct hybridization has sensitivity of about 90–100%. This can equally detect the Trichomoniasis in urine and swab samples (Brown et al. 2004).

10.3.3.4 Syphilis

In resource limited settings the detection of treponema pallidum antigen or anticardiolipin antibodies are the preferable techniques. The POCT for syphilis are divided into treponemal and non-treponemal tests. Treponemal test is of two type immunochromatographic and agglutinant. While non-treponemal is useful in detecting active infection. The combined detection of treponemal and non-treponemal is call duo detection. Chemobio DPP is the one such test kit, which produce the results in 10–15 min. VisiTect Syphilis detection kit provides the sensitivity of 85.1% and specificity of 96.5% (Pham et al. 2020). The Determine TP diagnostic kit detection sensitivity is about 90% and specificity of 94.1% (Lien et al. 2000). While SD bio line diagnostic kit and SyphiCheck WB kit had sensitivity and specificity of 87.1%, 95.8% and 94.5%, 99.1%, respectively (Jafari et al. 2013).

10.3.3.5 Chancroid

Diagnostics of Chancroid is based on the detection of *Haemophilus ducreyi* the causative agent. PCR, antigen detection (immunofluorescence), non-adsorption EIA, adsorption EIA and Lipo-oligosaccharide EIA are the POCTs for diagnosing Chancroid. The sensitivity and specificity of PCR-based detection is about 83–96% and 100% (West et al. 1995; Lewis 2000). Antigen detection test is done with the use of monoclonal antibody raised against 29 kDa outer membrane proteins. The detection sensitivity and specificity of this test is 93–100% and 63–74%, respectively. If compared to the culture its sensitivity is about 100% and specificity is 74%, while compared to PCR the sensitivity and specificity is of 89% and 81%, respectively (Ahmed et al. 1995). Enzyme immunoassays (EIA), dot immunobinding, agglutination, complement fixation, and precipitation are the Serological based detection tests. The sensitivity of these test lies in between 55% and 83% and specificity of 49–71% (Alfa et al. 1992). LOS EIA detection test has the sensitivity 96% and 97% compared to culture, while compared to PCR the highest sensitivity is 74%, and specificity 97% (Ahmed et al. 1995; West et al. 1995). Matrix assisted laser desorption/ionisation mass spectrometry (MALDI/TOF-MS) is another technique which provide the results within 10 min based on protein profiling (Haag et al. 1998). The present POCT available need further modification in order meet the gold standard specificity and sensitivity. At present the recorded sensitivity and specificity is too low for the available POCTs.

10.3.3.6 Herpes Simplex Virus

There are few available diagnostic kits for HSV infection. Onsite HSV-2 IgG/IgM is a LFIA kit, use glycoprotein G2 to differentiate between HSV-1 and HSV-2 (Saville et al. 2000). This kit delivers the results in 10 min and requires only 10 μL of sample.

H-DiaCMV is used to detect the CMV herpesvirus family virus. It is real-time PCR based kit, which measures the load of virus in the blood. BioTracer HSV-2 detection kit can detect the virus in serum, plasma, and blood, and produce the results in 10–20 min. The sensitivity of this kit is 97.2% and specificity is 97.6%. ARIES is a PCR-based POCT, which can differentiate between HSV-1 and HSV-2. This kit is used for the fast detection of virus in the skin lesions. HSV OligoGen is a combined PCR and immunochromatographic based detection kit. The amplified HSV DNA hybridized to the specific probe to display results as a coloured band (Laderman et al. 2008; Singh et al. 2005; Saville et al. 2000).

10.4 Present Challenges and Future of POCT in STD/STI Spread and Diagnosis

The unrecognized STD/STI burden has been increasing globally. High cost, lack of laboratory usage, and social stigma have accelerated the spread. The available and practicing laboratory tests are costly, time-consuming, and insufficient for screening a larger population. Only the symptomatic individuals are tested for the presence of infection, while the prevalence of asymptotic individuals will be quite large. But their screening is challenging even in developed countries. A paradigmatic shift in the basic laboratory diagnostic and introduction of POC or rapid testing has revolutionized STD/STI diagnostics. However, these POCTs or rapid tests are still at the preliminary stage of their development and need vital advancements. POCTs have increased the self-dependency of patients but did not decrease the importance of the clinic. Because the end results of these tests are not user practical. There is still a need for formal training and expertise for their smooth conduction and result analyses. Most of the available POCTs are either nucleic acid amplification or immunoassay based that need a well-equipped laboratory and experts. The sensitivity and specificity of the available POCTs lack behind the gold standard tests. This demands a combined effort from physicist, biologist, and chemist to design the futuristic POCTs. In STD/STI infections where the number of infections causing organisms are high the POCTs testing become critical. Further the structural complexity of proteins and genes of the organisms are the major obstacles to achieve standard specificity and sensitivity. Most of the infections shares similar clinical phenotypes and require one by one exclusion based of their testing. This has a significant impact on the time constrain treatment measures. This demands for an aggressive multiplexing and identification of the causative agents. In current scenario, the multiplex diagnostic is restricted to established health-care systems and requires a well-trained personal. Other aspect associated with STD/STI is the drug resistance and considered a global public health emergency. *Neisseria gonorrhoeae* cause gonorrhoea shares top five threats of urgent antibiotic resistance along with *Clostridioides difficile* and carbapenem-resistant Enterobacteriaceae. According to current guidelines, a prevalence of $>5\%$ for an antibiotic excludes it from empiric

therapy and at same time choosing next antibiotic is crucial. Development of POCTs for drug resistance screening is a tough job when these are unavailable for routine testing. For a POCT to detect resistance, first we need to identify the genetic marker that predict it, and at present only few of them are known. Secondly, the results should be fast, till date the fastest available test takes about 90 min to predict the resistance of gonorrhoea.

10.5 Conclusions

Present diagnostic tests used to screen and diagnose the STD or STI are extensively costly, time-consuming, and require a trained individual along with a specific well-equipped laboratory. Personal hesitation and social stigma prolong these infections and evolved the infected individual as a potential spreader into the community. Unfortunately, the multiple drug resistance of the STD or STI causative microbes is the biggest challenge of present and future to tackle with. POCTs can play a tremendous role in controlling and managing the STD or STI both at home and clinic. Early diagnosis not only helps in getting accurate treatment but also works as preventing measures. Advancements to user-friendly POCTs devices will encourage the self-testing and screening for STD/STI infections. This will be a progressive but ascertain the infection and its spread.

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Chapter 11
Nanobiosensor-Based Microfluidic Point-of-Care Platforms: Fabrication, Characterization, and Applications

Nimisha Roy, Sonal Jaiswal, Amar Dhwaj, Deepti Verma, and Amit Prabhakar

11.1 Introduction

The war against COVID-19 is far from over, imposing immense restrictions on regular patient care. The need for innovation in the patient care paradigm has been felt strongly across the globe. With hospitals running out of beds for patients during the exponential surge of COVID-19 positives, the focus has shifted to providing diagnosis and treatment near the patient bedside, more precisely point-of-care diagnostics and treatment (Rezaei et al. 2021). Even if the patient is quarantined, point-of-care devices can help in patient monitoring efficiently. Apart from the pandemic, many diseases like diabetes, hypertension, cancer, asthma, etc., require continuous patient monitoring. Since most of the patients with these ailments are elderly, visiting the doctor in person is impossible. In such scenarios, a monitoring device that could measure and analyze the vital parameters of the body is of paramount importance. With point-of-care devices, this goal can easily be achieved. Even in scenarios like war or a natural calamity, these devices would be of immense importance.

When we talk of point-of-care diagnosis, what do we mean? What does point-of-care stand for, and why is it relevant in the current scenario? This chapter deals with all these questions in detail, along with some examples to illustrate the devices already in various stages of conceptualization, clinical trials, and commercialization.

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11.1.1 Point-of-Care: Definition, Concept, and Evolution

Point-of-care refers to the medical diagnostic assays performed near the patient. PoCs are simple, time-saving alternatives to conventional central laboratory testing where the patient has to be driven to the diagnostic center. In cases where the patient needs to be quarantined, these devices would prove to be a game-changer. We all are familiar with glucometer and pregnancy testing kits. These two are classic examples of point-of-care devices that approximate test results based on detections of biomolecules by biosensors. The commonly used pregnancy kit senses the presence of the Human Chorionic Gonadotropin (HCG) hormone in the subject's urine, which in turn is indicative of embryo formation. The glucometer testing is based on the quantification of electrons generated upon a series of reactions catalyzed by the enzyme glucose oxidase producing gluconic acid and ferrocyanide. Glucose oxidase is present on the lancet of the glucometer. Figure 11.1 describes the basic concept of the point-of-care detection and diagnosis paradigm. The patient is at the comfort of his home, he simply uses a POC device to measure his major physiological parameters whose data can be sent to the hospital for analysis (via the internet), and the doctor decides the subsequent course of action. Recently, POC devices interfaced with internet-of-things (IoT) have emerged as an effective mode of remote patient monitoring (Nasajpour et al. 2020).

An ideal POC device should be simple, safe, easy to use, robust in the storage of reagents, and its result must be concordant with an established laboratory method (Price et al. 2010).

Biosensors are the crux of POCs as they sense the analyte in a biological sample. In 1962, Clark and Lyon popularized the term "biosensor" when a *glucose oxidase*–based amperometric sensor was launched into the market (Heineman and Jensen 2006). A biosensor is a device that translates a biological response into a quantifiable

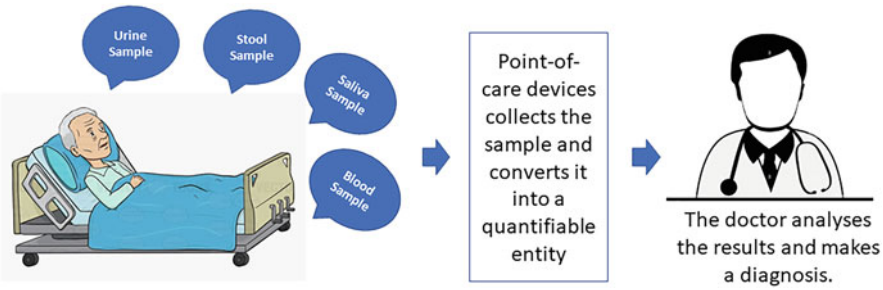

Fig. 11.1 The basic concept of point-of-care detection and diagnosis

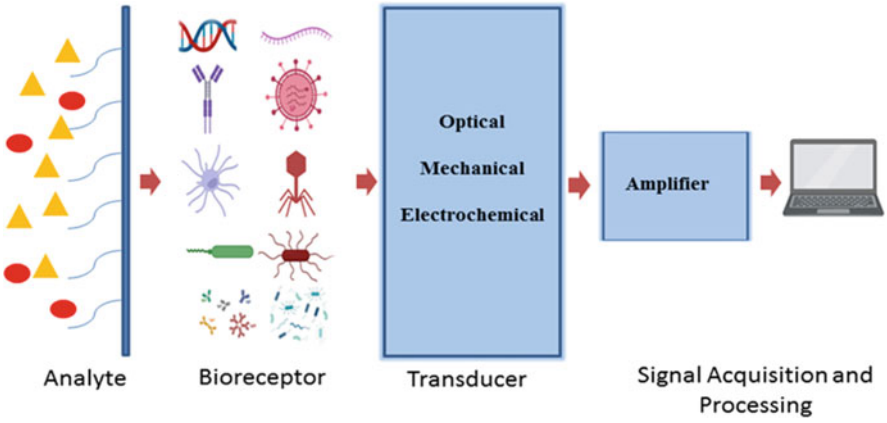

Fig. 11.2 Schematic of a biosensor describing all the components: bioreceptor, transducer, and signal processing unit

electrical signal and consists of three units: a biorecognition element or bioreceptor, a transducer, and a signal processing unit (Fig. 11.2) (Bhalla et al. 2016; Khan and Rao 2020; Goode et al. 2015).

The functioning of the biosensor starts with the analyte-bioreceptor interaction. A biochemical reaction takes place due to the binding of the analyte leading to the creation of a signal. The signal processing unit, basically an operational amplifier, takes this signal as an input and amplifies it. Signal amplifiers are electronic circuits that convert the signal into quantifiable or amplified form and are also known as *Signal Processing systems/Units*. Several signal amplification approaches have been explored in the case of electrochemical nanobiosensor to process the signal, such as ELISA-based (Akama et al. 2016) and colorimetric detections (Zhao et al. 2020). On the other hand, nanomaterial-based signal amplification gives the nanobiosensor catalytic properties and utmost sensitivity and selectivity; nanomaterial-based signal amplification depends on their high surface area. Not long ago, some nanomaterial with catalytic behavior was introduced into an electrochemical nanobiosensor design, which catalyzed the substrates into reaction product to increase the electron transfer for signal amplification or processing, and thus, nanomaterial-based signal amplifiers have excellent potential to improve the sensitivity and specificity—some examples of nanomaterials as signal amplifiers are photoactive materials in the case of photoelectrochemical nanobiosensor.

This signal is further converted into an electrical signal and sequentially converted into a quantifiable parameter. Based on fluctuation in this parameter, the result is displayed on a monitor in the form of a graph, color, or number. The nanobiosensor devices based on functional nanomaterials have potential applications in point-of-care diagnosis, therapeutic systems, disease identification, clinical and medical diagnosis, and industries. Still, when it comes to accuracy, high sensitivity, selectivity, fast response, good durability in biological media, along

Table 11.1 Various biosensors are used for viral detection (Zhao et al. 2020)

Target virus	Biosensor
Rotavirus	Carbon nanotubes, quantum dots, graphene and its derivative (FETs), and silicon nanowire tube
Zika virus	A paper-based cell-free biosensor based on CRISPR technology, electrochemical and optical biosensor
Influenza virus	Electrochemical biosensors Impedance spectroscopy-based biosensor
Dengue virus	Electrochemical
Kaposi's sarcoma-associated herpesvirus (KSHV)	DNA-functionalized AgNPs and AuNPs
H1N1 virus	AuNP-based Lab-on-chip
Tamiflu-resistant virus	AuNP-based Lab-on-chip
Hepatitis C viruses (HCV), Cyprinid herpesvirus-3 (CyHV-3) virus, Bovine viral diarrhea virus (BVDV), Cucumber green mottle mosaic virus (CGMMV), Maize chlorotic mottle virus (MCMV), and Spring viraemia of carp virus (SVCV)	Unmodified AuNPs
Norovirus	Electrochemical and optical (Surface plasmon resonance-based) biosensor
Ebola	Electrochemical and optical biosensor

with supporting media like buffer, are all critical aspects that must be wisely considered.

To this aim, nanomaterials can be used as a transducer to transform the signals emanating from the interaction of the analyte with the respective bioreceptor into measurable signals. They can also be used as a bioaffinity platform for the entrapment of biomolecules like nucleic acids (RNA or DNA), antigens, enzymes, antibodies, Abzymes (antibodies with enzymatic activities), microorganisms, cells, etc. that selectively bind with the analyte. A crucial implementation of nanomaterials is to strengthen the stability and sensitivity of the microfluidic platforms. The other applications include assays for recognizing pathogenic agents, nucleic acids, and the discovery of biomarkers associated with a particular disease like cancer, where early detection of the disease can save the patient's life. As opposed to the traditional analytical devices, nanomaterial-based biosensors for point-of-care applications have a high accuracy in lesser times and long-term monitoring of clinical biomarkers, growth factors, and biomolecules like amino acids, glucose, lactose, etc.

Due to their sensitivity and quick response time, biosensors are fully capable of providing an alternative that is both a rapid and reliable solution for clinical diagnosis, real-time detection, and continuous monitoring. Biosensors have come a long way as far as detecting viral species is concerned (Table 11.1). These biosensors can be effectively utilized after certain modifications for rapid coronavirus detection. The sensitivity and selectivity of a biosensor can be enhanced by performing surface

modifications to improve the biosensing capacity of the nanobiosensors by tagging specific agents. The various nanomaterials with their differing sensitivity and unique properties can be used according to medical and clinical analysis needs. Some advantageous features are low cost, miniaturization, easy fabrication, online monitoring, and simultaneous sensing are further enhancements in electrochemical biosensors.

According to the standard definition, "Nanotechnology is the science of design, characterization, production, and application of structures, devices, and systems by regulating the shape and size of a nanometer-scale." In 1959, the renowned physicist and Nobel laureate Richard Feynman put forth the idea of "nanotechnology" in his celebrated Caltech lecture titled "There's plenty of room at the bottom." Feynman presented the vision of printing the complete encyclopedia on the tip of a pin. However, in 1974, the first one to use and define the term "Nanotechnology" was Norio Taniguchi, a Japanese scientist, who said that "nanotechnology mainly deals with the processing of separation, consolidation, and deformation of materials by one atom or one molecule" (Silva 2004).

Microfluidics refers to the manipulation of fluids in the microlevel dimension channel. Nanotechnology has positively influenced biomedical research with its potential applications in intelligent miniaturized devices capable of performing at the micro or even smaller scale. Such microfluidic devices are called "micrototal analysis systems" (μ-TAS). These systems are advanced microfluidic platforms where the entire process of sampling, sample transport, chemical reactions, and subsequent detection is performed on a single chip. Microfluidics has numerous applications in biology, synthetic chemistry, analytical chemistry, and biotechnology. Microfluidics enables the control of a small volume of fluid in a precise manner, and this is important for sensing of biological molecules in a sample for qualitative and quantitative analysis in an aspect of PoC, which ensures the fast detection, diagnosis of disease, and monitoring of model with minimal time requirement so that patient can start treatment at an early stage of the disease. With regards to this, there is a DNA detection device in the microchannel on the principle of hybridization between two complementary strands of DNA by fixing the fluorescently labelled/radio-labelled DNA probes on the surface of the channel and then allowing the fluid (analyte) to move and interact with their immobilized probes; thus, DNA present in sample binds with their complementary strand via Watson and crick base pairing and the rate of interaction between analyte and probe can be enhanced by applying some electric field.

With the advent of nanotechnology, sensors underwent massive revamping. Nanomaterials began being tested as promising biosensing elements, thus giving rise to the birth of a new class of sensors called nanobiosensors. Nanobiosensor is a "*self-contained analytical system based on the nanomaterial that incorporates transducer to detect minute concentration or activity of any kind of biomolecules in the sample*." Due to the dependency of the physical and chemical properties on the nano-size, these are superior for diagnostics and therapeutics application with specific and selective detection and suitable for drug profiling. For instance, let us consider typical living organisms made up of several cells that are typical of

5–20 mm, however, cells part is even smaller—are proteins with the standard size of 5 nm, which can be compared with small synthetic nanoparticles, and this gives an estimate of using nanoparticles as a tiny probe to allow us to analyze the cellular processes without any interference. Nanobiosensor devices/platforms have broad series of usages in clinical and medical diagnosis, biotechnology, environmental and health monitoring practice, and food industries and are the most emerging applications of nanomaterials and outcome of analytical technology.

Nanomaterial-based microfluidics biosensing devices improve the analytical functions and fabrication with nanomaterial of microfluidics channel causes proper miniaturization of the device and this miniaturization could be beneficial for point-of-care diagnostic devices; point-of-care system consists of the analysis of biological analytes like blood gas, protein, glucose, and infectious microbes. Microarray is a microfluidics point of care device in which different types unlabelled probes are immobilized on the surface of microchannels, then run the other analyte of additional source labelled analytes simultaneously to compare the expression profile of genes whether the foreign genes from a single person (patient) or the same genes from two individuals, that is, healthy and diseased patient. The merits of microfluidics POC devices include low sample volume requirement, minimally or entirely noninvasive method of sample collection, the necessity of a smaller number of reagents, simplicity, short duration of analysis, multiple analyte detection, and easily comprehendible results (Li 2008).

Better health quality management is crucial for good health, and this is possible only by making timely decisions based on fast diagnostic and competent data evaluation. Thus, developing diagnostic devices for personalized healthcare such as POC devices is imperative. Nanotechnology has opened new fascinating possibilities for new analytical assays in various fields. One of them is biosensing, which mainly includes nanobiosensor and microfluidic-based analytical platforms. An ideal point-of-care device is portable and assures reliability. Nanomaterial comprises of nanoparticles that are less than 100 nm in dimension and as an early diagnosis is essential both for identification of disease and therapeutics nanomaterials possesses functional and attractive properties, which can be applied for a wide range of sensing system.

Table 11.2 briefly describes various commercially available or FDA-approved PoC devices being used for diseases such as acute coronary syndrome (Chan et al. 2013), heart failure, and HIV. These devices are simple, easy to use, portable, and use serum, whole blood, or plasma as the specimen.

Table 11.2 Some of the approved microfluidic PoC devices

Commercially available/FDA approved POC	Manufacturer	Disease targeted	Specimen used	Working principle
Triage® System	Alere, Inc.	Acute coronary syndrome	EDTA whole blood, plasma, or finger prick	Portable two-site fluorescence lateral flow
i-STAT® Analyzer	Abbott Technologies, Inc.	Heart failure	EDTA whole blood or plasma	Handheld two-site Immune enzymometric assay with electrochemical detection
Cardiac reader™ or Cobas®h 232 system	Roche diagnostics	Heart failure	Heparinized whole Blood or finger prick	Portable or handheld Two-site gold-labelled Lateral-flow Immunoassay
Clearview HIV1/2 STAT-PAK		Human immunodeficiency virus	Whole blood/ serum/plasma	Gold-labeled lateral-flow immunoassay
Multi spot HIV-1/2		Human immunodeficiency virus	Serum/plasma	Fluorescence lateral-flow immunoassay
Reveal® G3 HIV-1		Human immunodeficiency virus	Serum/plasma	Gold-labeled flow-through immunoassay

11.2 Noble Metal Nanoparticles as a Sensing Element in Point-of-Care

At the nanoscale, matter does not obey Newtonian physics; instead, nanoscale is governed by quantum physical phenomena like size effect, quantum confinement, and density of states (DOS). As quantum effects dominate, nanoparticles' optical, electrical, and magnetic properties are affected drastically. Nanostructuring alters the electron distribution, chemical properties, and magnetic properties compared to the bulk metals. The finite-size effect and surface effects define the magnetic behavior of particles at the nanoscale, giving rise to unique attributes (Akbarzadeh et al. 2012).

Nanoparticles of noble metals such as gold, silver, and platinum possess unique properties like small size, nontoxicity, high surface-to-volume ratio, self-assembly, tunable optical properties, and ease of functionalization/conjugation with antibodies, nucleic acids, nucleotides, and peptides. These properties can be changed precisely, even in real time, to a very great extent by varying the thermo-kinetic parameters during the synthesis. This same property is the crux of a nanoparticle's immense utility in several fields.

11.2.1 AgNPs and AuNPs as Nanoprobes for Detection of Cancerous Cells

AuNPs find application in Surface-enhanced Raman Spectroscopy (SERS). Raman Spectra is a molecule's vibrational fingerprint as the spectra are generated from molecular vibrations. In SERS, the signals are amplified by nanoparticles around the target tissue. Since AuNPs interact strongly with visible light, SERS is substantially increased. AgNPs have been successfully used in various studies to detect abnormalities depicting the onset of cancerous tumor formation. AgNPs possess the property of strong absorbance and scattering of light in the wavelength regions of surface plasmon resonance. In a study done by Jun et al., AgNPs having a magnetic core were coated with silica as the stabilizing agent. These coated nanoparticles were then successfully used to target breast cancer cells (SKBR3) and leukemia (SP2/O) cell lines owing to the strong Surface-enhanced Raman Spectroscopy (SERS) signals emitted by the nanoparticles (Jun et al. 2010).

AuNPs usually accumulate in a specific area and scatter the light subjected to them. This has been utilized in phase contrast microscopy, photoacoustic imaging, and dark-field microscopy. In TEM, AuNPs are being used successfully as labels for ultrastructures owing to their high molecular weight. There is no denying that early-onset diagnosis can be a boon for cancer patients. The strong optical scattering of AuNPs can help in detecting the overexpressed markers at the very onset of cancer. Since the AuNPs are biocompatible, they can be safely conjugated with antibodies to detect overexpressed antigens in cancers with the risk of toxicity. Their small size makes the detection of the precise location of the cell abnormality possible.

11.3 Lab-on-Chip Based Biosensing

Strong utilization of microfluidics comes from the emergence of miniaturized chip formats known as Lab-on-Chip (LoC) devices. These devices are microsystems capable of performing the entire function of a biological/chemical laboratory on a single-chip platform. An LoC performs the desired function with the help of the integration of active or passive components such as filters, mixers, valves, etc., in a microfluidic system. Some factors must be considered while constructing a microfluidics device, and these are the choice of materials, the dimension of the device, and fluid control devices (pump, valves, and mixer). Microfluidic devices can be manufactured from several materials such as glass, silicon, polymers, and gels. The pregnancy test kit is a classic example of a microfluidic biosensor based on lateral flow.

Estevez et al. suggested that lab-on-chip technology integrated with optics will be of huge relevance in future medical emergencies like a pandemic we are currently facing. Their study provided an extensive glimpse of the photonics biosensor technologies that could be interfaced with lab-on-chip devices. Some examples

include microring resonators, photonic crystals, interferometers, etc. Although many challenges need to be addressed, the potential of lab-on-chip MEMS-based devices has enormous advantages owing to low reagent requirement, high sensitivity, rapid prototyping, and ease of scaling up the production (Estevez et al. 2012).

11.3.1 Microfluidic ELISA Chip

ELISA (96-well plate) carries the disadvantage of a long assay time of 6 h with a complicated procedure that requires large sample volumes (~100 μL) of the reagents. As a result, ELISA's applications in rapid real-time clinical diagnosis and prognosis of some fast-developing diseases are restricted. Tan et al. developed a microfluidic ELISA device based on a capillary glass array. Utilizing the high surface-to-volume ratio of the capillary and the rapid chemiluminescent photo-imaging method with a commercial camera, the capillary-based ELISA device significantly reduced the sample volume to 20 μL and shortened the total assay time to as short as 16 min (including detection time), which represent an approximately tenfold and fivefold reduction in assay time and sample volume, respectively, in comparison with the traditional plate-based method. Furthermore, through the double exposure method, the detection dynamic range was increased ten times over the traditional well-based ELISA (Tan et al. 2017). The developed device can be broadly used in the rapid biochemical analysis for biomedicine and research/development laboratories. Based on this study, a portable, precise, and cost-effective microfluidic-based COVID-19 antibody testing platform has been developed by the startup, Optofluidic Bioassay. This lab-on-chip platform is the first microfluidic approach to the gold standard testing protocol of ELISA but is faster and requires lesser sample volumes. This device is currently being validated for use on COVID-19 antibodies.

11.3.2 Fabrication of Microfluidic POCs

Over the years, many considerable developments have taken place in the area of microfluidic device fabrication techniques. Staring with photolithography, today, we have sophisticated techniques like 3D printing. These developments have paved the way for the mass production of these devices. In the following section, we give a brief about some of the common fabrication methods of microfluidic-based POCs.

11.3.2.1 Traditional Silicon-Based Microfluidic Devices

Since the beginning of microfabrication, silicon wafer has been used as the substrate for casting the master of microfluidic devices. Techniques like photolithography, Etching, thin-film deposition, LIGA (Lithography, Electroplating, and Molding),

and thin film deposition are some standard procedures for the fabrication of MEMS devices. Photolithography is perhaps the most elegant technique to develop microdevices. It involves the fabrication of a photoresist master and subsequent casting of the microdevices by replica molding. Etching involves the removal of the substrate material not protected by the photoresist. It can either be isotropic or anisotropic depending on whether the rate of etching is the same or different in all the directions. The thin-film deposition method makes use of creating and depositing a thin layer on the silicon substrate. These thin-film coatings might act as masking layers or sacrificial layers for structure development on the substrate.

11.3.2.2 Glass-/Polymer-Based Microfluidic Devices

In recent years, there has been an increased interest in using glass/polymer substrate in place of silicon wafers. The main reason behind this is some toxic aspects associated with the chemicals used in the RCA cleaning of the wafers. Soft-microfabrication, microcontact printing, and micromolding are some of the techniques used in the fabrication of glass-/polymer-based microfluidic devices. Soft microfabrication is an extension of the traditional photolithography in order to utilize a diverse range of glass- and polymer-based materials for device fabrication.

11.3.2.3 3D Printing

In recent years, 3D printing has become a novel and time-saving way of fabricating microfluidic devices. It is an additive manufacturing technique facilitating the rapid fabrication of complex biomedical devices that are usually difficult to design using conventional methodologies such as machining or photolithography. Currently, microfluidic devices are being produced in large numbers using this cost-effective technology. 3D printers can use a wide range of materials such as filament for printing. Even bioprinting has been made possible in whole tissues and can be printed using bioink. These bioinks are complex mixtures of cells and ECM components. The advent of 3D printing has marked a major milestone in the evolution of microfluidic-based biosensor fabrication (Prabhakar et al. 2021).

11.4 Applications of Nanobiosensors

From the last few years, nanobiosensors have been developed to function as signal amplifiers, Bioreceptor labels, Mediators, and support nanomaterial modifiers (Fig. 11.3).

Nanobiosensor ensures the fast detection of sample and facilitating a better diagnosis, monitoring, and management at an early stage of disease and thus early treatment as there are some problems with some conventional method of blood test

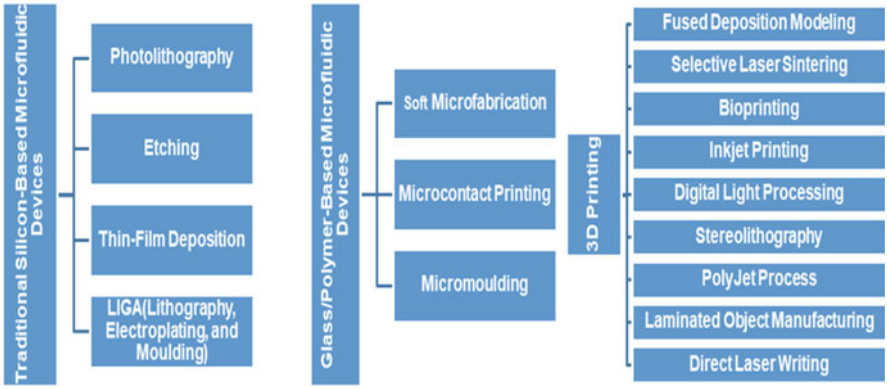

Fig. 11.3 Various types of microfluidic device fabrication techniques (Lei 2014; Scott and Ali 2021; Prabhakar et al. 2021). Traditional silicon-based microfluidic devices are fabricated using techniques like photolithography, etching, thin film deposition, or LIGA. LIGA is an acronym for Lithography, electroplating, and molding. In recent years, glass- and polymer-based microfluidic devices have also gained popularity due to their low cost. These are generally prepared using the technique of soft microfabrication, microcontact printing, and micromolding. Soft lithography is an extension of conventional photolithography, which caters to a wide variety of materials. Finally, the most advanced method that has solved the problem of scaling up production is 3D printing. There are various methods to 3D print these devices. The main ones are fused deposition modeling (FDM), Selective Laser Sintering (SLS), bioprinting, inkjet printing, Digital Light Processing (DLP), Stereolithography, Poly Jet Process, Laminated Object Manufacturing (LOM), and Direct Laser Writing

for early detection of biomarkers, and these are low yield sensitivities because of low concentration of analytes in the circulatory system of patient at an early stage of disease and large time needed for analysis and nanobiosensors can resolve these challenges, as effective detection nanobiosensors detect the biomarkers at 100 times lower concentration in the sample. The nanomaterials can also be used in drug administration, cancer therapy, or other diseases.

11.4.1 Second-Generation Biosensors

Mediators are situated in between the transducer and affinity platform to generate an enhanced response in a biosensor. The biosensor devices, which consist of mediators, are known as "Second-Generation Biosensors." These *second-generation biosensors function to mediate the product of reaction/process occurring at the bioaffinity platform to the transducer efficiently*. Indeed, the unique properties of nanomaterials have led to their widely used biosensor as a mediator also. Nanomaterial as mediator is an electric connection between the biomolecules (bioreceptor and/or analyte), and electrode surface reflects the crucial parameter in the biological or electrochemical sensors at a smaller scale (Hayat et al. 2016). In

Table 11.3 Application of nanoparticles as mediators and their specific functions

Sl. no.	Nanoparticles used as mediators	Function
1.	Silver nanoparticles in combination with pyrolytic graphite electrode	Bridge to wire the electron transfer
2.	Platinum modified electrodes (Pt-nanoparticles) with electrocatalytic activity with two branched carbosilane polymers	It measures the NADH from +0.3 V and uses alcohol dehydrogenase (ADH) to protect against poisoning electrodes (Ghalkhani et al.)
3.	Gold nanoparticles	Increases the rate of electron transfer and thus mediates the reaction product from bioreceptor to transducer surface (Liu and Ju)
4.	Nanoparticle MXene-Ti_3C_2 developed by the etching process of Al from Ti_3AlC_2 to immobile bioreceptor (e.g., Hb)	MXeneTi_3C_2 accelerates the electron transfer from Hb protein and thus detects the H_2O_2 with an extremely low limit of detection

today's trend, the conventional mediators have been replaced by metallic nanomaterial-based mediators, because nanomaterial holds tremendous potential to impact the domain of biomedical research, nanomaterials with extremely small sizes and appropriate surface modifications allow the interaction with target with high efficacy and thus mediate the reaction product to the varieties of transducers depending on what kind of biosensor is operating. The conductive feature of nanoparticles increases the rate of reaction or electron transfer between biomolecules to the surface of the transducer.

The metallic nanoparticles that serve as electron or wire transfer have been used recently instead of conventional mediators in electrochemical nanobiosensors. In contrast, several non-metallic nanoparticles like-oxide-based nanoparticles can also improve electron transfer; silver particles also possess this electron transfer activity due to their electric conductive nature. Some of the nanoparticles being used as mediators are mentioned in Table 11.3.

Some spectroscopic based studies have shown analysis that MXene-Ti_3C_2 has good enzyme immobilization with compatibility for proteins and has excellent bioactivity and stability, in this case (play a major role in food, pharmaceutical, and environmental assays), detection is important and can be measured at low applied potential by using peroxidase. The fixation of proteins (or bioreceptor) on the surface of MXeneTi_3C_2 increases the sensitivity of the device for H_2O_2 detection. In some amperometric assays, this mediator operates in combination with peroxidase and causes a further increase in the sensitivity and specificity, but it is costly and requires more time; hence, co-adsorption of horseradish peroxidase and thionine on an electrode surface has been exploited as an H_2O_2 sensor to simplify the analysis system (Zhan et al. 2014). A very important aspect of the nanobiosensor based microfluidic devices is the limit of detection of the device for a particular biomarker or analyte. Table 11.4 mentions the limit of detection for some of the devices being used for disease biomarker detection.

Table 11.4 The limit of detection for some of the devices being used for disease biomarker detection

Target	Microfluidic device type	Limit of detection (LOD)	References
Breast cancer biomarker	Microfluidic immunoassay device	60 μU/mL	de Oliveira et al. (2017)
Levodopa/Parkinson's disease	Microneedle sensor array	0.25–0.5 μM	Goud et al. (2019)
Papillomavirus	Microfluidic interdigitated electrodes	10.5–60.2 pM	Soares et al. (2018)
Lactate/glucose	Microchip device A photolithographic wearable microfluidic device	50 μM	Martín et al. (2017)
Human immunodeficiency virus and hepatitis C	Paper-based microfluidic device	300–750 pg/mL	Zhao and Liu (2016)

Several signal enhancement techniques were demonstrated to have effective detection performance of nanobiosensor and these nanomaterial-based signal amplifications rely on their high specificity (Mohammadi et al. 2017). The course of action, to pattern nanomaterial-based nanobiosensor for target molecules (NAs/Protein) where nanoparticles are used for signal amplification where it generates current with high intensity, to understand the principle of nanomaterial-based signal amplifiers let consider an example—when analyte-specific probe immobilized on the support and add sample/analyte and then add simultaneously labelled nanoparticle and deposited nanoparticles to the fixed probes consequently 'both generate a current of the same potential but different intensity from which amplified form can be easily evaluated by looking at the potential of current (measurable due intensity generated by labelling) which reflect the signal processing'. Thus, with the help of labelling, we can check the enhancement in signal amplification due to the use of nanoparticles as signal amplifiers.

Nanomaterial for electrode materials for detection platform is gold nanoparticles (AuNPs), Carbon nanotubes (CNTs), which provide a large surface area to fix the DNA molecules to enhance senses, Graphene (G), Polymer nanoparticles, and their composite nanoparticles. Some bifunctional nanoparticles may have a symbiotic effect of both catalytic activity and compatibility and conductivity to improve the signal transduction and amplification, which lowers the limitations. There are some advanced nanoparticle-based signal amplifiers developed in which nanoparticles signal amplifiers conjugated with other electrochemical metals to provide high throughput analytical devices for sensitive detection of even small size analytes (DNA, microRNA assays). The nanoparticles base signal amplifiers plan with microfluidics immunosensor can be used for measurement of biomarkers of diseases or cancer in a sample. There is a variant of CNT called multi-walled carbon nanotube (MWCNT) which has a large surface area with several functional groups and electrical properties, a very sensitive detection limit of 0.5 pM can be achieved.

Another use of nanomaterials in the electrochemical biosensors is the labelling of biomolecules like antigen, antibodies, and nucleic acids (RNA and DNA). This is a crucial role in nanotechnology in the development of devices; carbon nanomaterials were used as label supports in nanobiosensors beside conjugation with labels are also modified with the detection of antibodies or antigen these conjugates provide functionalization such as more labels and electrochemical signal amplification. Tang et al. combined MWCNT with nano-silica and HRP and used them as labels of anti-staphylococcal Enterotoxin B antibodies in sandwich pattern immunosensor to control the capillary action and to manipulate the sample fluid in the device, and the result was confirmed by ELISA. By using carbon nanomaterials as immobilizers for bioreceptors labelling the competitive type of immunoassay has also been developed (Tang et al. 2013).

Metal nanoparticles can also be used in both immunosensor (basically protein analytic devices) and DNA sensors, but the mass transfer problem associated with it can be resolved by using an external magnetic field with the use of magnetic nanoparticles (magnetic beads) to the biomolecules, and it also plays the role as supporter and separation tools in microfluidic devices. Bound to biomolecules, magnetic nanoparticles can be exploited for labelling of biomolecules or any structures or microorganism in an immunoassay in which magnetic field generated by target labelled with magnetic nanoparticles in continuity can be sensed by a magnetometer, Upon binding of analyte or disease-causing agents with antibodies is the basis of tests when antibodies labelled with magnetic nanoparticles gives a signal on exposure to a magnetic field generated by the labelled target and free antibodies can be dispersed in all direction by producing no net magnetic signal.

Carbon nanotubes (CNTs) have extensively been used for PoC testing with substantial success. Point-of-care tests based on the CNT are of three types: CNT-based lateral flow, CNT-based printed electrode, and CNT-based Lab-on-Chip. Having a large surface area, CNT develops an increased current density and offers more space for immobilization of biomolecules (probes), thus leading to more and more stability and sensitivity of detectors. The electrical properties of CNT-based platforms depend on the chirality of nanomaterial, for example, in the case of CNT, number of carbon layers and flaws and functionalization must be kept in mind while using these point-of-care devices for better analysis.

11.4.2 Nanobiosensor Based Point-of-Care Devices for Various Disease Detection and Diagnosis

In this section, we will give a brief example of some of the most promising nanobiosensors based microfluidic point-of-care devices developed for analyzing body fluids using different working principles. Some of the diseases and biomarkers for which the microfluidic PoC devices have been employed so far are discussed in the following section. These include

1. Detection of urinary tract infections
2. Parkinson's disease monitoring
3. Detection for metabolites in sweat
4. Nucleic acid amplification
5. Bacterial quantification in blood plasma

11.4.2.1 Detection of Urinary Tract Infections

Urine analysis is done to detect a number of diseases associated with the Urinary tract, Kidney, diabetes and to confirm pregnancy as well. Figure 11.4 describes the schematic of the paper-based colorimetric immunoassays, but instead of using desktop scanners, the smartphone with a high-resolution camera is used to resolve the problems related to lack of portability and sensitivity toward ambient light, which provide rapid and accurate optical detection of the sample (urine or blood) with fewer pieces of equipment (Jalal et al. 2017).

The major advantage of using a paper-plastic hybrid microfluidic device for colorimetric assessment is that it provides control in the reaction volume eliminating any temporal variations. The microfluidic device is illustrated in Fig. 11.4. A finger-pressed type of PDMS (poly(dimethylsiloxane)) pump is used to fill the predefined volume reaction chamber with the sample (urine in this case), which reacts with an array of paper-based reagent test pads embedded across the plastic microchannel (Fig. 11.4a, b). An imaging box including PDMS light diffuser is used to distribute the light uniformly on the sample and improve device accuracy. The colorimetric

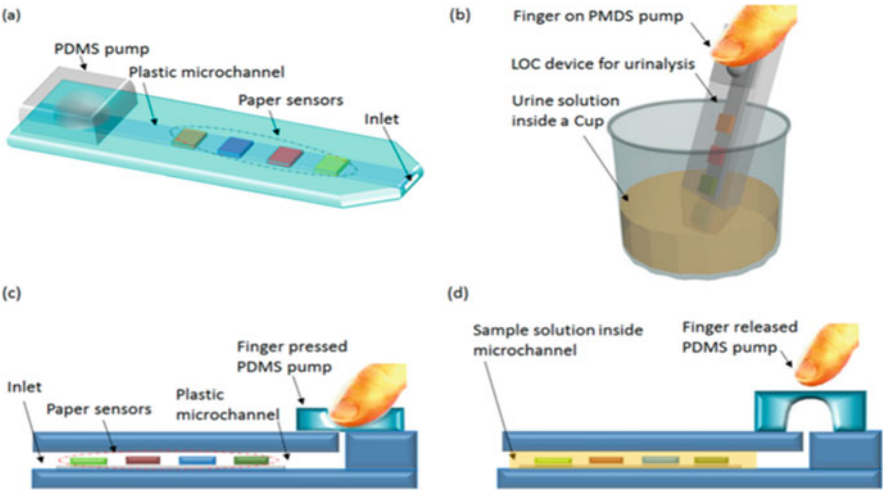

Fig. 11.4 Schematic of the paper-based colorimetric immunoassays (**a**) representation of device microstructure; (**b**) Procedure of sample loading; (**c**) Device during the pressing of PDMS pump; (**d**) Sample loading occurs after the pressing of PDMS pump

change of the reagent pads upon reacting with the analytes is sensed by a smartphone camera, as shown by pictures in Fig. 11.4c, d.

This innovative microfluidic device, using a paper-polymer combination, has a single channel integrated with a micropump to use to get better control in reaction volume. A PDMS (polydimethylsiloxane) pump is used by pressing it with a finger to influx the predefined reaction chamber with a sample (in this case, urine) which flows and reacts with an array of paper-based reagents fixed across the plastic microchannel lines. This PDMS diffuser is used to spread the light all over the sample evenly and thus enhance the precision. As the reaction between the analyte and embedded test reagents occurs, it fluoresces the light of high/varying intensity, and the change in fluorescent upon the reaction in the channel is observed by a smartphone camera.

11.4.2.2 Parkinson's Disease Monitoring

Another example of a reliable microfluidic system with rapid and accurate measurement is "L-Dopa Sensor." It senses the drug L-dopa and its bio-catalyzed product, that is, Dopamine, and it consists of microneedle sensors with transducers (in this case carbon paste) with WE1 and WE2 and RE without carbon paste in Fig. 11.5. A L-Dopa is a drug to treat the symptoms of Parkinson's Disease, which catalyzed into an active component in the body, that is, Dopamine: a neurotransmitter that controls the motor nervous system and fullfills the biological need. L-sensor activity is decided by modified electrodes with nanoscale chemicals/particles, for example, CNT-chitosan glassy carbon electrodes (GCE), CNT-chitosan screen printed electrodes (SPCE), 3D graphene foam, TiO_2 nanofiber graphene oxide, etc. Microneedles in the sensor are used because its less invasive, less time consuming, and have transdermal sensing. In the case of L-Dopa Sensor, there are mainly three microneedles out of these two packed with carbon paste; an electrode transducer—RE with Ag wire transmit data to smart device, that is, computer screen, WE1 contain unmodified carbon paste voltammetric electrode detects the L-Dopa concentration with square wave voltammetry (SWV) shown in Fig. 11.5b and WE2 contain carbon paste amperometric electrodes modified with enzyme tyrosinase (TYR) detect the tyrosinase-based conversion of drug L-Dopa into product, that is, Dopaquinone as in Fig. 11.5e and thus biologically active compound: Dopamine. In the case of nonenzymatic detection via microneedle WE1 sensor, rapid square wave scan leads to the "direct oxidation of L-Dopa" and thus transmits the current signal through a transducer on screen from where the concentration of compound can be calculated. In the case of enzymatic detection, enzymes integrated into carbon paste amperometric microneedle WE2 catalyzes the reaction of L-Dopa to dopaquinone in the interstitial fluid of PD patients and thus gives the current signals on screen; the current intensity is proportional to the concentration (Goud et al. 2019).

In this sensor, the Ag-wire serves as a "reference electrode" in two-electrode sensing without any counter electrode. Both these WE2 and WE1 generate current

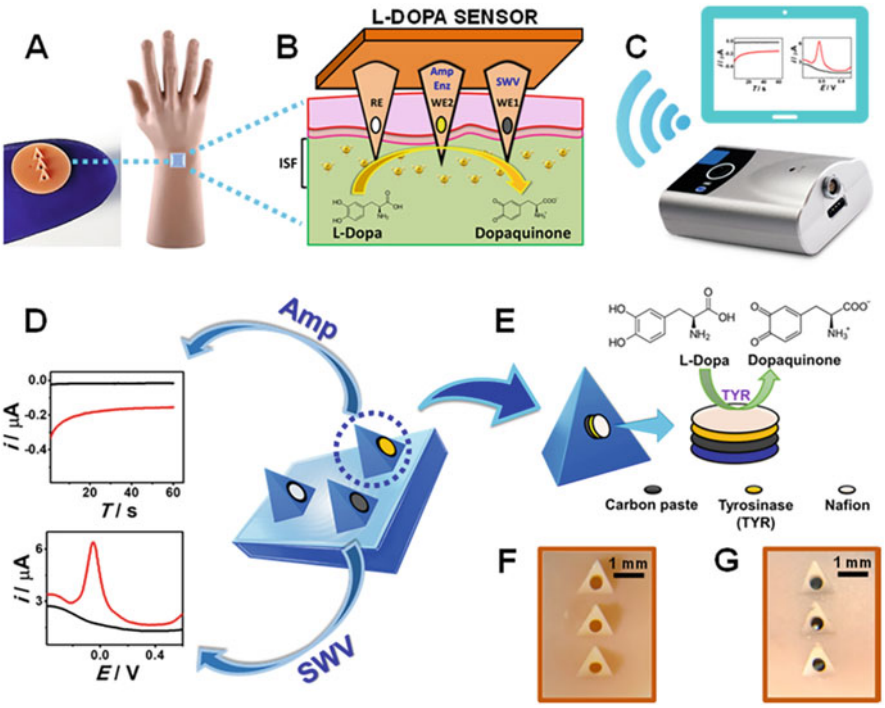

Fig. 11.5 Description of the microneedle sensor array for L-Dopa detection: (**a**) microneedle sensor in a mannequin hand and (**b**) the L-Dopa sensor array. (**c**) Handheld wireless electron analyzer for wireless data transmission. (**d**) Illustrative representation of the sensor array principle. (**e**) CP microneedle electrode, with the corresponding reagent layers: tyrosinase and Nafion. (**f**, **g**) Optical images of microneedles before and after assembling with CP (Goud et al. 2019)

signals AMP and SWV, respectively, mentioned in Fig. 11.5d offered high sensitivity and selectivity toward detection of L-Dopa as well as dopamine concentration and facilitated the drug administration to patients in less time. Hence, microfluidic-based sensors achieve the detection of two independent processes (Goud et al. 2019).

11.4.2.3 Detection for Metabolites in Sweat

Figure 11.6 shows the schematic and functioning of the microfluidic device for metabolic detection and sweat sampling. Figure 11.6a represents the different layers of the device, Fig. 11.6b illustrates the working principle of the device, and Fig. 11.6c shows the device prototype being used for sampling and analysis.

This noninvasive device is fabricated using a hybrid approach of lithography and screen printing for real-time monitoring of glucose and lactate levels in the sweat by utilizing amperometric biosensing with the help of an immobilized oxidase enzyme (Martín et al. 2017).

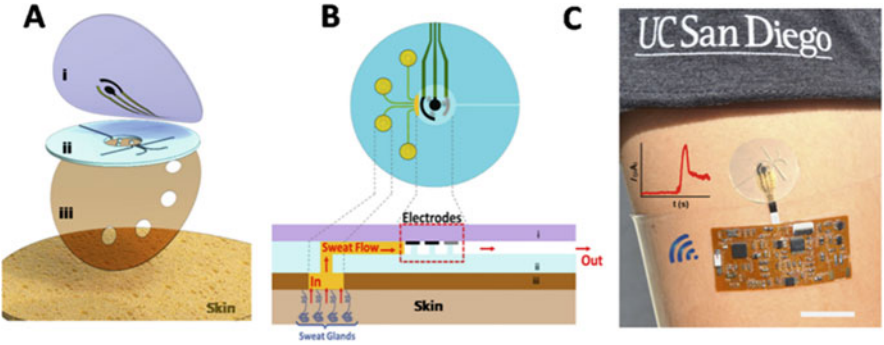

Fig. 11.6 Schematic and functioning of the microfluidic device for metabolic detection and sweat sampling. (**a**) Representing the different layers of the device. (**b**) Illustrates the working principle of the device. (**c**) Shows the device prototype being used for sampling and analysis

11.4.2.4 Self-Powered Integrated Microfluidic Point-of-Care Low Cost Enabling (SIMPLE) Chip for Nucleic Acid Amplification

It elucidates a microfluidic system based on nucleic acid amplification technique to allow diagnosis of disease. From blood samples with rapidity and accuracy is described herein. It offers a low cost, less time-consuming, and quantitative as well as qualitative Nucleic acid detection/amplification as compared to conventional PCR. This advanced device is designed to trim the entire process, starting from sample input to quantitative nucleic acid output (Fig. 11.7). Some of the key steps while designing this device were: prepatterning of the reagent amplification initiator magnesium acetate so that reaction can run directly on-chip only when the other reagents, that is, probes, primers, nucleotides, and enzymes, enter the well to avoid ambiguity. This patterning has been done by "Digital Microfluidic Patterning," which consists of four steps: Digitization (integration of MgOAc with the stencil and degassing to remove bubbles), Drying (to concentrate the reagents toward apex structure by capillary tension), Stencil peeling off and alignment and bonding of PDMS on the top of the layer containing pattern for the SIMPLE chip- and fluorescein is added to only aid the visualization, second it uses automating sample preparation, because isothermal polymerase can amplify the NA in complex mixture of blood sample; however, RBCs (hemoglobin) and blood cells can also interfere with readouts thus "Digital Plasma Separation" which prepares the sample for RPA/digital amplification by simultaneously enabling autonomous plasma separation and Autonomous sample compartmentalization and thus It enables us to on-site NA detection directly from blood sample without typical separation machine, third by designing it in the way to remove the necessity of peripheral pumps, connectors, power sources and control equipment best of many pumping system is "Vacuum Battery System" consisting of two parts one is—simply punched void which stores vacuum and another part is a lung like vacuum component to allow the air diffusion through thin gas permeable silicone (PDMS), fourth lower the instruments and

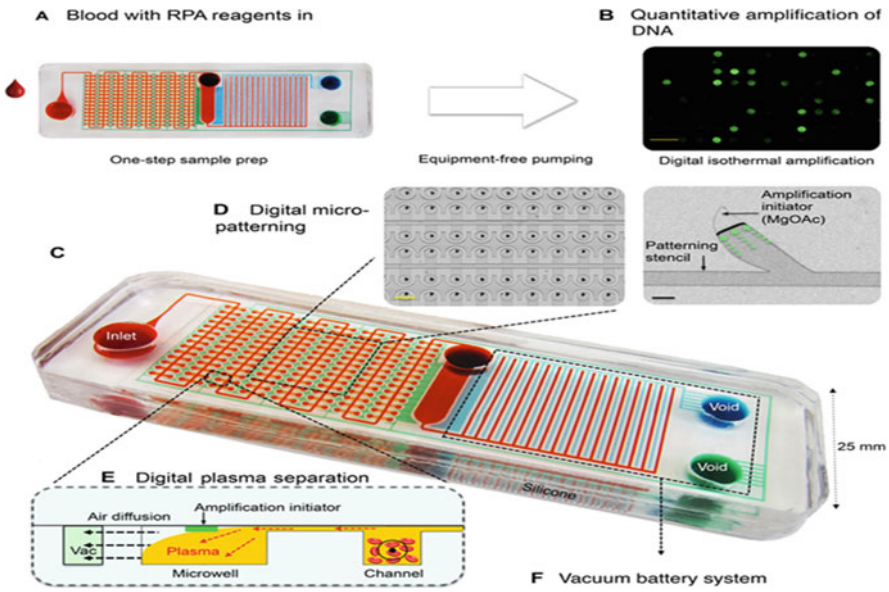

Fig. 11.7 The schematic of the SIMPLE chip for nucleic acid testing. (**a**) The simple operation protocol requires minimal handling without the need of a power source/external pumps. The user simply drops blood/amplification reagent mix into the inlet and then the chip performs automatic sample preparation. (**b**) The chip is then incubated on a reusable heat pack, and endpoint isothermal digital amplification of nucleic acid is done (RPA). Scale bar, 2 mm. (**c**) Dye-loaded chip for visualization of microchannels. Red shows fluidic channels—blue shows the main vacuum battery system. Green shows the auxiliary vacuum battery system. (**d**) Digital microfluidic patterning enables reagent patterning with common laboratory equipment. (**e**) Side view of the digital plasma separation design, which removes blood cells via sedimentation and skims plasma into dead-end wells for digital amplification. (**f**) The vacuum battery system frees the chip from external pumps or power sources for pumping. The vacuum is pre-stored in the large "battery" voids. Fluid is pumped by slowly releasing the prestored vacuum potential via air diffusion through lung-like structures

expertise by choosing new NA amplification technique: "Isothermal Amplification" with isothermal polymerase enzyme which can amplify at nearly constant temp., we use 'Recombinase polymerase amplification (RPA)' because of its robust in plasma sample than PCR or loop mediated isothermal. Figure 11.7 shows the device design and prototype developed by Yeh et al. The schematic, working, and output graph have also been shown in Fig. 11.7 (Yeh et al. 2017). Amplification (LMIP) recombinase activity enables this enzyme to hold on reagent nearby so that reaction takes place independent of temperature so that's how amplification can be done at room temp., too, fifth "Digital Amplification" to lower the device complexity and which can real-time quantification of NA concentration can be done only by endpoint readouts. Thus no need for imaging shuttles in conventional PCR and here, fluorophore quencher probes are used to allow the generation of detectable light signals upon binding with complementary sequences. And this is how rapid

quantitative and qualitative nucleic acid detection of a patient sample can be done on small chips at low cost with high specificity and sensitivity (Yeh et al. 2017).

11.4.2.5 Bacterial Quantification in Blood Plasma

Dey et al. designed a microarray device for bacterial quantification in blood plasma for Systemic Inflammatory Response Syndrome (SIRS). This device provides a label-free approach to quantify bacteria (*E. coli*) in the blood plasma of a subject using a biofunctionalized nanoplasmonic substrate (Fig. 11.8). The basic principle of this device is plasmonic interferometry. The device can perform one-step sample handling for label-free sample handling. The crux of the device is the bioprinted microarrays. The turnaround time of the device is 40 min.

Microfluidic systems consisting of a PDMS chip integrated with an optical system has also been used to quantify real-time platelet aggregation in patients with Arteriovenous thrombosis (AT). AT is a cardiovascular condition in which the platelets aggregate to occlude the blood flow (Li et al. 2012). The observation made using this platform led to a critical conclusion that a high shear rate of blood flow accelerates the process of platelet aggregation, leading to thrombosis.

A microfluidic rapid detection test (RDT) strip chip can not only accelerate malaria detection but can also help save thousands of lives. One such RDT strip chip detects the proteins derived from the blood of malaria parasites in a microfluidic

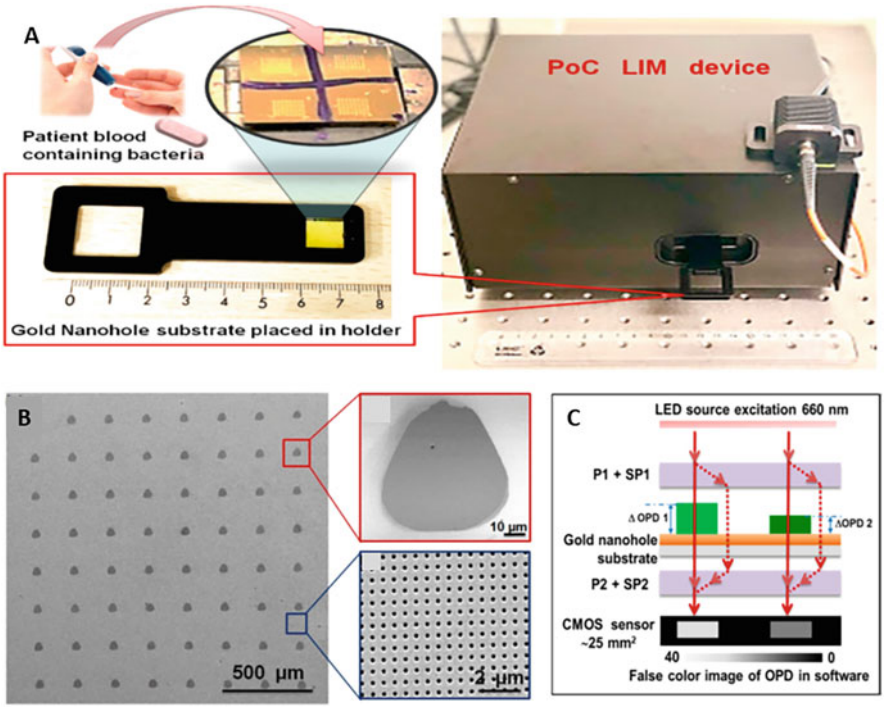

Fig. 11.8 (**a**) Device size and prototype, (**b**) SEM images of the 8 × 8 microarray of protein G inside the device (**c**) Different layers of the device (Dey et al. 2018)

format. This chip enables the generation of a series of visible lines to indicate the presence of specific antigens in the blood that is clearly visible to the naked eye when the antibody is accumulated at the test line (Bell et al. 2006)

Detection of malignancies in the case of all the cancers will be able to enhance the chance of patient survival and reduce the financial burden that a patient faces for opting for the treatments when cancer is diagnosed at a later stage. Circulating nucleic acids like the miRNAs serve as an indicator for chronic pathologies like lung cancer. Circulating cell-free miRNAs are reported to be closely associated with the clinical outcome of lung cancer patients. Quantum dot-based nanobiosensors coupled with FRET can be used to detect these miRNAs. miRNAs have a small size, are unstable, and present in low concentrations (Singh et al. 2018).

11.5 Challenges and Future Work

At a small scale, nanobiosensors are being successfully utilized in clinical diagnosis and patient monitoring. The application of nanobiosensor-based PoC devices is still limited owing to various factors like lack of dedicated equipment for large-scale production of the devices. This in turn increases the cost of production per device, leading to an increase in the price of such devices. Once a proper infrastructure for mass production is in place, these devices would be more affordable and readily available. Rapid prototyping and additive manufacturing modalities are increasingly being optimized to fill these gaps between labs to market. Researchers are also focusing on how to increase the ease of multiplicity, reproducibility, sensitivity, and selectivity of these PoC devices. In addition, nanomaterial-based biosensors will undoubtedly find applications in more diversified fields like environment monitoring, food sciences, and material sciences.

Microfluidics holds the potential to revamp the health-care paradigm by providing innovative and simple clinical diagnostic tools, which are not only fast but require a minute volume of the sample to provide accurate point-of-care bioanalysis. Since the patient's bedside generates the information, a lot of time is saved in the facilitation of therapeutic interventions (Pagaduan et al. 2015).

μTAS has been used successfully to analyze different biomolecules like fatty acids, carbohydrates, nucleic acids, and proteins. Depending upon the nature of the analyte and other reagents to be used in a particular test, the materials for fabricating the microfluidic device can be customized. Interfacing with different detection systems also expands the versatility and sensitivity of microfluidic devices for various biomarkers. Finally, the fabrication of these microdevices has become relatively simple due to enormous improvements in fabrication protocols and 3D printing. These devices make it easy to design layouts that can be adapted to suit various tests/reactions with minimum changes.

Integration of multiple processes in a single microfluidic device offers automated sample analysis applicable to clinical laboratories and point-of-care diagnostics. Recognition of this technology's benefits undoubtedly played a vital role in

developing commercial microchip electrophoresis platforms provided by companies such as Agilent, BioRad, and Bio-Techne. However, these commercial systems are still expensive and have benchtops rather than the point of care. Furthermore, validation of assays for clinically relevant biomarkers is needed for this technology to be widely used in clinical laboratories or point-of-care diagnostics.

Currently, the research is focused on effectively modifying the surface properties of the polymeric substrate used in a microfluidic device to exercise control over the physical phenomena that affect interactions inside the microfluidic channels. This would help generate results that are both reproducible as well as predictable. An important aspect that needs to be characterized is the effect of the device layouts, channel dimensions, and structural designs on fluid dynamics and assays. Advances in fabrication methods can further decrease the channel dimensions and ease the integration of processes and electronics needed for automated analysis. Miniaturization and improved packaging of detection systems, such as optical and electrochemical, along with data analysis techniques, would move a step closer to a complete lab-on-a-chip system for in-field applications. New additive manufacturing techniques like 3D printing need to be implemented. Bulk manufacturing will help to scale up the production of these devices, lower the cost, and lead to rapid production of these sensitive devices (Pagaduan et al. 2015).

An amalgamation of nanobiosensors with microfluidics can help realize the vision of the PoC-based remote health-care paradigm, which is cost effective and straightforward to use and sufficiently lowers the excessive pressure on hospitals and doctors in situations such as a pandemic or epidemic, even in remote rural and tribal areas where health-care facilities are not yet developed.

11.6 Conclusions

Microfluidics and nanotechnology are two of the most promising technologies to have emerged in this century. Some of its exemplary qualities of microfluidics are its ease of multiplexing, high-throughput screening, cost effectiveness, and low reagent consumption. Combining these properties with the infinite powers of nanotechnology, microfluidics, and biosensing, researchers have revolutionized the point-of-care detection and diagnostic paradigm to a very great extent. The biomarkers for detecting abnormalities can easily be traced using microfluidic systems augmented with nanoparticle-based biosensors. Carbon nanotubes (CNTs), quantum dots, cantilevers, magnetic nanoparticles, noble metal nanoparticles (silver, gold, and platinum), dendrimers, etc., are commonly used nanomaterials in biosensing. Among these nanoparticles, colloidal gold nanoparticles have been used most extensively with considerable success. In this chapter, we tried to present a brief compilation of the basic concepts, state-of-the-art developments, and applications in the domain of nanobiosensors based on microfluidic point-of-care devices (Khan and Rao 2020). With rapid prototyping consistently flourishing, the development of the nanobiosensor-based microfluidic devices into a commercial product would not

take too long. The primary aim of scientific research and development has always been the well-being of society. Point-of-care diagnostics, monitoring, and therapy have become even more crucial in the present time when the world is looking forward to a world more immune to a pandemic such as the one we are facing now.

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Chapter 12
Nanobiosensor: Advancement in Disease Diagnostic

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12.1 Introduction

Biosensor is a system that can identify various biological components as biomarkers in different physiological conditions, which can further extrapolate their applicability in extensive applications like disease diagnosis, agricultural issues, and food processing areas. The components, which make biosensors superior in applications, are *bioreceptor* and *transducer*, and mainly, both are either coupled or amalgamated within a single system (Velasco-Garcia and Mottram 2003; Vigneshvar et al. 2016). The coupling of analyte and bioreceptors plays an important role in the sensing ability of biosensors. The bioreceptor should be attached to a stable support system, and both the components, the analyte and the bioreceptor should have affinity for each other. Subsequently, different characteristics will change followed by the stimuli generation, and this stimuli detection will be transducer aided. Hence, the strength of the induced signal will reflect the concentration of an analyte in the sample (Power and Yalcinkaya 1997). Further, the recognition system has a greater impact on biosensing as it influences *selectivity* and *specificity* (X. Zhang et al. 2009).

Example
Glucose biosensors are universally accepted and popular amidst health-care professionals for the intermittent detection of blood glucose level in mostly diabetic patients to maintain desired glucose level.

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The journey of biosensors began in the nineteenth century when an oxygen probe was constructed for *blood gas analysis*, and this work was carried out by Prof. Leland C. C. Further, he extrapolates his research to widen the scope of an analyte, which leads to the development of a *glucose oxidase* enzyme system as a biosensor for the determination of oxygen coupled with glucose (Clark and Lyons 1962). Eventually, this concept was utilized by S. Updike, who successfully developed a biosensor for the detection of glucose (Updike and Hicks 1967). The rate of biosensor development was sky-high in the late nineteenth century. This statement is more justifiable with the following illustrations: DNA sequence-based biosensors have grabbed attention in numerous fields, like clinical and forensic research. In addition to that, Ag-Ab reactions, enzyme-substrate reactions, label-free analysis of human body fluid for diagnosis, and heavy metal detection reflect the prominence of biosensors (Ziegler and Göpel 1998).

In this modern era, many diseases are fatal demanding a timely treatment. This means that the disease can be treated only when diagnosed at an appropriate time or in the early stages. At present, a single disease condition can reflect various signs and symptoms, which can be considered as the main obstacle in the path of accurate detection of disease conditions, leading to a delayed recognition or fallacious of the actual disease condition. To detect that in a precise manner, some components, like nucleic acid, peptides, proteins, and cells, can be helpful and are also well known to be biomarkers. All these biomarkers have a strong relationship with the body's physiological processes, so even a minute change in physiological process in a disease condition can reflect in the change in the presence of a biomarker. Thus, biomarker detection will be helpful for doctors to start treatment for particular disease conditions in an early phase. In the above context, it is crucial to get the results at a faster rate, and a point-of-care device or biosensor can serve the purpose (G. J. Zhang and Ning 2012). Because of superiorities like being less expensive, quick detection, high-performance detection, good specificity, less laborious, and no instrument requirement for point-of-care device, it is dominating over conventional methods in diagnosis (Sanvicens et al. 2009).

Because of its severity, cancer has emerged as a threatening disease condition, a major reason for global mortality nowadays. According to several research reports, it is found that approximately 18 million people worldwide are suffering from cancer, out of which half of the population succumbs to death, and that the number of deaths surged to nearly ten million in 2018 due to late detection and improper treatment (Bray et al. 2018; Sun et al. 2018). If we speak about the types of cancer based on gender, it is observed that prostate cancer is the most prevalent type of cancer in men, whereas breast cancer is the most common in women. Currently, widely accepted methods for cancer detection are tissue sampling, computed tomography, magnetic resonance imaging, ultrasound, and optical imaging. However, these methods are incapable of detecting cancer in its early stages or distinguishing between benign and malignant tumors. Biosensor comes into the picture and draws the attention of health-care professionals due to its cost-effectiveness, simplified nature, ease of movement, and compliance with patients and doctors (Shandilya et al. 2019). Further, the application of a biosensor in cancer diagnosis will assist in accurate

detection, which ultimately alleviates the mortality rate from cancer (Hsieh et al. 2016). In this research, various biosensors like electrochemical test strips, and lateral flow assays have been examined (Campbell et al. 2018). However, the biosensor is not able to detect the lower concentration in body fluids in the primary stage of cancer. Hence, to enhance the sensitivity of detection, nanotechnology has entered into the area of the biosensor.

Nanotechnology has emerged as a new approach to overcome the limitations of existing biosensors. Their properties, like efficient electron transfer, enhanced catalytic properties, and applicability in biomolecule labelling and adsorption, make them most appropriate for biosensing applications (Noah and Ndangili 2019). When nanotechnology amalgamates with biosensors, then it will pave the way for the development of nanobiosensors, which serve as a modern diagnostic approach. In the context of the above nanoparticles having a crucial role in the further development of nanobiosensors for several applications (Sanvicens et al. 2009). The advantages like quick response, high sensitivity, flexible design, maximum surface area as having less size in nanometers, and cost-effectiveness make nanoparticles ideal in nanobiosensor development (Doria et al. 2012). In aspects of an enzymatic reaction and an electrochemical reaction, the nanosize will help to accelerate the reaction faster than existing biosensors. The majority of nanoparticles employed for the development of nanobiosensors for diagnosis are metallic nanoparticles, which include gold, silver, and iron oxide nanoparticles. Sharifi et al. have demonstrated the application of nanoparticles in the diagnosis of the disease condition (Sharifi et al. 2019).

Example
Application of Nanobiosensor consisting of nanoparticle and secondary antibody tagged with enzyme known as horse radish peroxidase for the detection of prostate-specific antigen, which is the biomarker for prostate cancer.

12.2 Nanotechnology in Biosensors

In this modern era, nanotechnology has emerged as a potential solution to overcome the various limitations in current sensing applications. The advantage of nanomaterial is that it alters the sensing capabilities of the existing microsystem and provides a greater surface area. The nanoparticles that are dominating these fields are gold nanoparticles, silver nanoparticles, and magnetic nanoparticles. The two primary evaluation parameters, like sensitivity and specificity, can be improved by the unique properties of nanoparticles, like various physical, chemical, optical, and magnetic properties. Furthermore, the two most important factors, like the size in the various nanometer ranges and the compositions in the reaction, will decide the development and establishment of the unique properties, for instance,

superparamagnetism in magnetic nanoparticles (Doria et al. 2012; X. Zhang et al. 2009).

Nanoparticles
Gold nanoparticles, Silver nanoparticles, Magnetic nanoparticles, Quantum dots, Graphene oxide nanoparticles.

12.2.1 Types of Nanoparticles

12.2.1.1 Gold Nanoparticles (AuNPs)

In the world of nanotechnology, gold nanoparticles are found to be superior to other nanoparticles, especially owing to their physicochemical properties, which make them unique among all. The gold nanoparticles display a surface plasmon resonance (SPR) effect. This property will help with label-free detection and real-time monitoring. When an electromagnetic wave is coupled with an electron in a metal, then it reflects the phenomenon of the SPR effect (Herizchi et al. 2016). Additionally, photothermal conversion, photochemical conversion, surface-enhanced Raman spectroscopy, and surface-enhanced fluorescence are also responses in extension to SPR. The SPR can be influenced by various parameters like shape, size, charges present on the surface, and the dielectric constant of the medium where they are present (X. Zhang et al. 2009). Hence, applications like in vivo and in vitro diagnosis, and imaging can be made possible because of the peculiar properties of gold nanoparticles. In another aspect, it also reflects easy coupling, biocompatibility, site targeting, delivery, catalytic activity, and biological activity. Besides, high X-ray absorption and radioactivity are widely used properties in the detection and treatment of cancer (X. Bai et al. 2020).

Definitions Plasmon—Collective oscillation of conduction electrode due to the presence of light.

Plasmon resonance—When incident light frequency is resonant with Collective oscillation of conduction electrode, then absorption band occurs.

Surface plasmon resonance—It occurs frequently on the surface of metal, because the disturbance of incident electromagnetic wave on metal abruptly decreases with the depth of the metal.

Localized surface plasmon resonance—When SPR limited to the small volume that means nanoparticles can be comparable with incident light wavelength.

Similar to a coin having two sides, gold nanoparticles also reflect the two most important properties: the extinction coefficient and the local electromagnetic field. The electromagnetic field near the nanoparticles plays an important role in the application part. It is true that when the LSPR phenomenon happens, the extinction coefficient will increase approximately 1000 times in intensity as compared to the

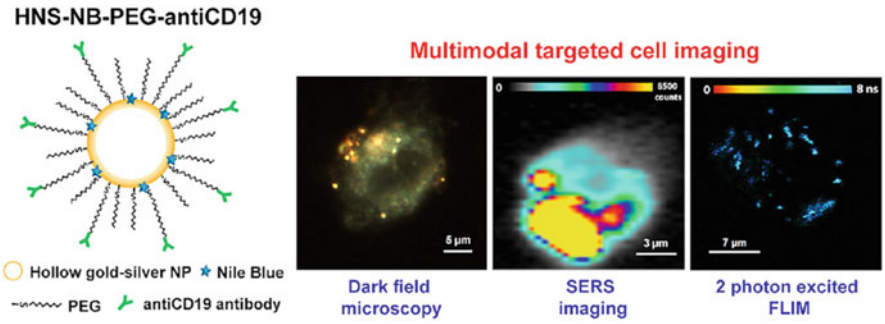

Fig. 12.1 Novel system for the detection of cancer and imaging of cancer lymphoblast (Reprinted with permission from Nagy-Simon et al. 2017)

other ordinary molecules (De Puig et al. 2015). This concept can be extrapolated to establish applications like colorimetric assays for cancer detection and photothermal and photodynamic therapy in cancer treatment. It is found that there was a rise in or enhancement in the electromagnetic field in terms of magnitude field near nanoparticles due to the occurrence of SPR (Moskovits 1985).

In context to the above, the AuNP is a member of the SERS (Surface-enhanced Raman spectroscopy) family and hence applicable in multiple applications like imaging of tumors, even small sizes can be determined, detection of tumor cell markers, and monitoring of the tumor cells. Although SERS nanoparticles are becoming more important in medical diagnosis, some critical issues must still be addressed, such as materials with high SERS properties, good biocompatibility, nontoxicity, and protection from the external environment. In one experiment, Nagy-Simon et al. (2017) developed a novel system for the detection of cancer, where they used gold-silver nanosphere, Nile blue dye as an SERS agent, PEG polymer, and antiCD-19 antibody (Fig. 12.1). Furthermore, they also demonstrated the uptake of this nanosystem was through an active way and achieved effective incorporation of an antibody-conjugated nanosystem as a contrast agent. Besides, the investigation was done and compared based on surface-enhanced Raman spectroscopy, dark field, and two-photon excited fluorescence lifetime imaging microscopy (Nagy-Simon et al. 2017).

Shiota et al. (2018) have developed a unique nanosystem using AuNPs that has a horse-bean shape to evaluate the metabolite level to detect cancer. The two metabolites, which helped them distinguish between normal and cancer cells, were hypotaurine and glutathione (Shiota et al. 2018). Feng et al. (2017) have successfully established a noninvasive method for the screening of cancer where they have used SERS spectra of purified modified urinary nucleosides. Nasopharyngeal and esophageal cancers have been detected by the researchers in their research work. They have also promised that this type of detection technology can be reliable and that its application can be extrapolated in clinical trials as it is label free and has good sensitivity and specificity (Feng et al. 2017). Chakraborty et al. (2020) introduced the phenomenon that AuNP is also able to exhibit the apoptotic pathway, which can

be beneficial in the treatment of osteosarcoma. This effect will be nanoparticle size driven; hence, the size range of 40–60 nm will have the potential to produce reactive oxygen species (ROS) that further destroy the membrane of mitochondria (Chakraborty et al. 2020).

The fluorescence is also contributed well as a physical property of AuNP in the detection of various diseases. This phenomenon has two sides, fluorescence enhancement and fluorescence quenching, and these can also be considered as a consequence of the process of fluorescence development. When some light energy is supplied to the molecule of fluorescent nature, then it promotes from the ground state to an excited state. Subsequently, after vibrational relaxation, this comes down to the initial excited state followed by landing in the ground state. And in between this process, two types of decay come into the picture: the radiative decay rate, which further emerged as fluorescence, and nonradiative decay rate as thermal radiation. In this entire process, fluorescence quenching is the Fluorescence resonance energy transfer (FRET) driven. Hence, when the distance between AuNP and the fluorescence molecule is less than 5 nm, then it gives birth to the fluorescence quenching, whereas more than 5 nm gives birth to the fluorescence signal. To get the interaction between them will require the attachment of various linkers, which can further reflect the interaction. On account of this phenomenon of molecule interaction, various disease diagnoses can be done very efficiently. This concept was firstly used by researchers in 2006, when they employed a fluoroimmunoassay that depends on the quenching of AuNP (Ao et al. 2006). In 2019, Kotcherlakota et al. (2019) demonstrated fluorescence-based cell imaging in diagnosis, but they used a natural fluorescent agent from the plant instead of synthetic dyes (Kotcherlakota et al. 2019). Recently, Jara-Guajardo et al. (2020) have detected Alzheimer's disease using fluorescence enhancement by the finding of β-amyloid (Jara-Guajardo et al. 2020).

Another property of AuNP is colorimetric biosensing capability has also been grabs attention in biomedical applications. In the traditional colorimetric analysis, it is quite difficult to determine the minute sample because of less specificity and sensitivity. The high molar absorption coefficient is the crucial factor that leads the colorimetric detection. This unique biosensing assay is completely based on the state of analyte influenced by AuNP either aggregated free and this must be indicated by the transformation of color from red to blue and purple to grey. Hence, while detecting tumor-related proteins, nucleic acids, and cytokines will be the broader categories of analytes for assay. This approach of cancer detection was firstly applied by Kang et al. (2010) where they have demonstrated the aggregation of AuNP as a consequence of charge. In their experiment, they have used a cancer biomarker that is protein kinase Cα (PKCα) and substrate for this marker having a positive charge and AuNP with a negative charge (Fig. 12.2). In the presence of cancer cell samples, phosphorylation occurs and leads to an increase in anionic charge and separation of AuNP, whereas in a normal cell sample, AuNP shows aggregation due to the inhibition of phosphorylation (J. Kang et al. 2010).

Further, it is not only limited to the applicability of protein biomarkers for cancer detection, Ramanathan et al. (2019) have studied and proved genomic DNA can also be another way for colorimetric detection. They have established the method for

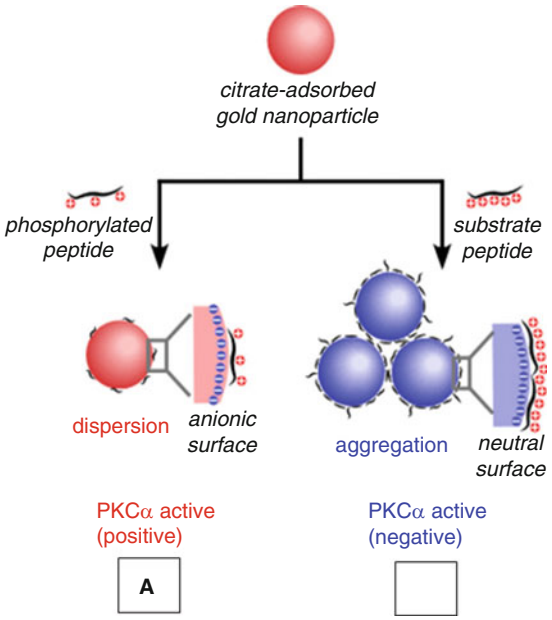

Fig. 12.2 AuNP-based colorimetric assay for cancer detection ((**a**) The activated protein kinase C (PKC) will be higher that leads to the separation of AuNP and produces a red color and (**b**) The activated protein kinase C (PKC) will be lower that leads to the aggregation of AuNP and produces a blue color (Reprinted with permission from J. Kang et al. 2010))

detecting non-small cell lung cancer, in which the mutation in early growth factor receptor was determined using this approach (Ramanathan et al. 2019). The applicability of AuNP colorimetric assay is not only limited to cancer detection but also extrapolated to other disease conditions like inflammatory diseases. António et al. (2020) have carried out an experiment where C-reactive protein (CRP) was determined as a biomarker in inflammatory conditions. In detail, they have attached citrate-capped AuNP with an aptamer sequence having binding affinity to CRP. While reaction has taken place between CRP and aptamer, AuNP gets opened and aggregated and shown blue color, whereas in the absence of CRP, AuNP will remain intact, and no aggregation will form consequently will show red color (António et al. 2020). These colorimetric assays can be useful even in very minute concentrations because signals can be enhanced using polymerase chain reactions. Hence, it is the most appropriate method for point-of-care diagnostic applications.

12.2.1.2 Iron Oxide Nanoparticles (IONP)

In this modern age, various nanoparticles are involved and contribute to diagnosing and treating cancer and infectious diseases. Iron oxide nanoparticle has emerged as a novel and unique magnetic nanoparticle one as it has a strong magnetic property. Because of its excellent magnetic property, IONP contributes to several biomedical applications like diagnosis, imaging, and treatment (Magro et al. 2017). Further, unique physical properties, biocompatibility, and stability are also additional benefits of IONP. This nanoparticle is employed in various medical applications like drug

delivery, gene delivery, biosensing, magnetic resonance imaging (MRI), contrast amplification, biophotonics, cancer diagnosis, and tissue engineering (S. Liu et al. 2020).

In nanotechnology, the interaction between the nanoparticles and the desired site, either cell or any tissues, plays an important role in diagnosis and treatment. Furthermore, the distribution of the nanoparticles throughout the body may be affected by various parameters like particle surface charge, size, surface properties including hydrophobicity, hydrophilicity, and porosity (Feliu et al. 2016). Hence, it is obvious thing that nanoparticles must have good stability to interact appropriately with the required site. To achieve this objective, surface modification will be required, which will impact several properties of nanoparticles like the size, water solubility, interactions with cells and tissues, and ultimately biodistribution (Sperling and Parak 2010).

In context to above, it is well known that magnetic nanoparticles can form large particles due to the interaction with each other, because of their hydrophobic surface. Hence, to improve stability and biocompatibility surface functionalization is a must. The introduction of an organic molecule to the surface of IONP leads to the formation of numerous functional groups like amino, carboxy, and hydroxyl groups. These functional groups can be employed to amalgamate with the antibodies, enzymes, and DNA.

The preliminary approach toward prevention of nanoparticle agglomeration is to functionalize the surface of these nanoparticles using polymers such as polyethylene glycol (PEG), polyethyleneimine (PEI), polyvinyl alcohol (PVA), polydopamine (PDA), dextran, chitosan, and starch (Zhu et al. 2018). The presence of weak forces surrounding the nanoparticles forces them to come closer to form bigger nanoparticles. Anbarasu et al. (2015) have successfully done the surface modification IONP with PEG. Hence, the two most important properties, magnetization and superparamagnetism, exhibited by the IONP can be further applicable in the MRI and biosensors (Anbarasu et al. 2015). In another experiment, Salah and Ayesh (2020) have employed PVA to modify the surface and found that the polymeric membrane is flexible, which can be utilized for the construction of flexible electronic devices (Salah and Ayesh 2020). In another aspect, various biomolecules like proteins, monoclonal antibodies, and polypeptides can be employed for the IONP surface functionalization. Because of this approach, IONP can be target specific to be more potent in actual applications and it will also enhance the biocompatibility and distribution in the body. Esmaili et al. (2021) have found that free IONPs are prone to oxidation; hence, they performed an experiment where they have used casein for coating of IONP to enhance the biocompatibility and reduce the oxidation of IONP. They also found a good anticancer activity against breast and prostate cancers (Esmaili et al. 2021). Tiefenauer et al. (1993) have utilized a monoclonal antibody, which is having the strongest affinity to bind to carcinoembryonic antigen for surface functionalization, further this was used as a tumor-specific contrast agent for MRI (Tiefenauer et al. 1993).

Silica is an important inorganic molecule, non-toxic in nature, appropriate for functionalization and it forms various cross-linking bonds that can protect the IONP.

Because of cross-linking bonds, further multiple groups can be added and well biocompatible (D. Chen et al. 2016). Ta et al. (2016) synthesized IONP and coated it with silica to increase the biocompatibility for further bioconjugation. Later, they have also attached multiple biomolecules on the surface of IONP for future applications (Ta et al. 2016). Lu et al. (2017) produced silica-coated IONP and due to the superiority of nanoparticles further applied it in the field of biomedicine (C. H. Lu et al. 2017). Further, to prevent the oxidation of IONP it is required to provide a metallic envelope. The important property of IONP is that the saturation of magnetization will be influenced by the use of metals but will vary and depend on the type of metal used. In another aspect, various metal oxides also can assist to achieve and maintain crucial properties like magnetism, and luminescence in targeted drug delivery. Hence, surface functionalization with appropriate metal oxides can serve the purpose (G. Liu et al. 2013b). Carbon coating is another method for surface functionalization that demands attention. This coating will be helpful to retain electrical conductivity, magnetic property, the electromagnetic field for the application in the diagnosis, and targeted drug delivery in the treatment of cancer (Tulebayeva et al. 2018).

12.2.1.3 Graphene

In the world of nanotechnology, along with other nanoparticles, graphene has also emerged as a versatile player in biomedical engineering. Graphene is the strongest material that has good mechanical strength, among others. The chemistry of graphene, for instance, its hybridization state, serves to gain and retain the highest mechanical strength. Besides mechanical strength, optical properties, large surface area, ripples, surface-enhanced Raman spectroscopy (SERS), fluorescence quenching ability, and biocompatibility make graphene a better player in disease diagnosis (Syama and Mohanan 2019). However, the main obstacle in the way of application of graphene is its hydrophobicity (J. Liu et al. 2013a). To resolve the issue about hydrophobicity, researchers performed further modification in the chemistry of graphene and found that graphene oxide (GO) is the best derivative and having good hydrophilicity, hence GO emerged as a unique combatant in biomedical engineering to fight with hydrophilicity (Lerf et al. 2006). Importantly, graphene and graphene oxide have different structures although showing chemical similarity (Buchsteiner et al. 2006).

The fluorescence quenching property of graphene will drive the development of graphene biosensors. For instance, GO will be tagged with fluorescent-labeled ssDNA and show the quenching. However, when *ssDNA* comes in contact with a complementary sequence, it releases the GO and reflects the fluorescence. The graphene-based materials can be employed for the development of fluorescence resonance energy transfer (FRET), field-effect transistor (FET), and DNA detection biosensors (Shen et al. 2012). For instance, Kwon et al. (2012) developed a FET-type aptasensor for the detection of vascular endothelial growth factor (VEGF) as a cancer biomarker. They have used nitrogen-coated graphene for further

immobilization of antivascular endothelial growth factor RNA aptamer (Kwon et al. 2012). Furthermore, biosensors can assist in the detection of dopamine, epinephrine, and norepinephrine, also metabolites or elements involved in human metabolism like uric acid which can contribute to pathological research work (Suvarnaphaet and Pechprasarn 2017). GO biosensors are not only limited to the detection of harmful metal ions, hormones, proteins, and fungi toxins but also detecting the numerous enzyme DNA helicase, thrombin, trypsin, and metalloproteinase that are involved in various processes (Chung et al. 2013).

In another study, the researcher has used a graphene biosensor that reflects high specificity and sensitivity for detecting *E. coli*. The glucose-induced metabolic activities are also determined using a graphene biosensor (Chem et al. 2011). In the field of bacterial infection of *Gram-negative bacteria*, it is found that some membrane component is known as lipopolysaccharide secreted in the human body consequently, other inflammatory cytokines are released that leads to sepsis formation and sometimes complete organ damage. To determine bacterial endotoxin, Limulus Amebocyte Lysate (LAL) test is available; however, the higher sensitivity toward the pH and temperature, other experimental conditions, and sample preparation make limited use of this test. To overcome this hurdle, researcher employed nanotechnology for efficient detection using various nanosystems like Tetramethylrhodamine dye-labeled LPS-binding peptide-GO complex, electrochemical aptasensor like LPSbinding aptamer (LBA)-conjugated Au@Fe_3O_4 magnetic beads-complementary DNA 1 probe, and magnetocatalytic graphene quantum dot (QGD)-based Janus micromotors (L. Bai et al. 2014; Jurado-Sánchez et al. 2017; Lim et al. 2015).

Graphene shows excellent quenching ability, due to which it arises as artificial nanomaterials for live-cell imaging and intracellular analysis. For instance, graphene oxide nanosheets (GO-nS) are conjugated with aptamer and further applied for live-cell imaging. Further, GO combined with aptamer and carboxyfluorescein for imaging of live cells to determine the ATP and GTP (Y. Wang et al. 2010). In another study, graphene nanosheets were conjugated with the dye Cy7 for in vivo imaging (Yang et al. 2010). The application is not only limited to live-cell imaging but also extended to the MRI, in which a combination of iron oxide nanoparticles and GO sheets is employed as a contrast agent. This type of contrast agent will increase the application window because of its biocompatibility (W. Chen et al. 2011).

12.2.1.4 Quantum Dots

The quantum dots (QDs) are known as semiconductor nanoparticles, and it was first described in 1981 by Ekimov and Onushenko, followed by their first application in biological imaging in 1998. Nowadays, QD is involved in the biomedical field for diagnostic and therapeutic applications and the development of photodetectors and photovoltaic devices (Matea et al. 2017). The application of QD in the biomedical field is due to its optical and electronic properties, and these properties can be

modified through controlling size. Further, two more properties, like exceptional photostability and stable photoluminescence, make them unique and dominant over materials (Alaghmandfard et al. 2021). Imaging plays a crucial role in cancer theranostics where conventional dyes fail due to less NIR emission possibility. QD draws attention in imaging due to its excellent optical properties, high quantum yield, size-dependent light emission, and outstanding chemical and photostability (Matea et al. 2017). Compared with normal dye, QD shows broad excitation spectra, large stoke shift, and narrow emission spectra. In another aspect, normal dyes are susceptible to photo-bleaching, which affects the quantum yield. Additionally, QD emitting in the NIR has significantly enlarged fluoresce potential in biomedicine due to lower tissue absorption and low autofluorescence (Wagner et al. 2019).

W. Zhang et al. (2016) have developed a sensor platform to detect the disease biomarker α-fetoprotein (AFP) in human serum samples (W. H. Zhang et al. 2016). They have synthesized the QD of 12 nm and then modified the surface of QD by dopamine. This QD is used for redox-mediated indirect fluorescence immunoassay (RMFIA). Eventually, AFP concentration was determined based on the degree of fluorescence quenching of the QDs during sandwich RMFIA. They have quantified the lowest concentration in serum, that is, 10 pM. M. Johari-Ahar et al. (2015) have developed nanostructures immunosensor (MPA|AuNP@SiO_2|QD|mAb) to detect ovarian cancer antigen CA-125. The base of this Nanobiosensor was a gold electrode modified with mercaptopropionic acid (MPA). Further, this electrode was coupled with CdSe quantum dots (QDs), silica-coated gold nanoparticles (AuNP@SiO_2), and anti-CA-125 monoclonal antibody (mAb). They reported a LOD of 0.0016 U/mL, which indicated the high sensitivity of immunosensor.

12.3 Diagnostic Applications of Nanobiosensors

12.3.1 Cancer

Globally, about 18 million new cancer cases are reported every year, out of which 9 million cases end up in death. Despite all the developments in medical sciences, cancer remains the major reason for high global mortality rate compared to other diseases (Parkin 2001). The late detection of cancer stage in conventional techniques and improper treatment leads to a higher number of deaths. Hence, the early detection of cancer using a more sensitive and specific method is an important criterion to treat the patient efficiently. The detection of cancer by the antigen-antibody reaction will not serve the purpose as only one antigen can be presented by several organs. For instance, the expression of HER2 reflects the three different types of cancer breast, ovarian, and gastric cancer, only the level of expression varies. Hence, instead of antibodies, more advanced aptamer probes come into the picture for appropriate recognition of antigens in cancer. However, some factors like binding affinity, signal strength, and sensitivity limit the application of aptamers alone. Because, one aptamer will not be able to produce sufficient strength of signals

with a low level of protein in the early stages of cancers (Shamah et al. 2008). Further, in the early stage of cancer blood consisting a low level of protein biomarker, which can be difficult to detect with conventional biosensing techniques. Hence, to detect such minute quantity in blood more sensitive and specific biosensing system is required. To overcome these limitations, nanoparticles will assist in binding more aptamers to a single nanoparticle, enhancing the strength of the signal for cancer detection in the early stages (X. Chen et al. 2009).

W. Lu et al. (2010) demonstrated a rapid and highly sensitive colorimetric method for breast cancer detection using gold nanoparticles combined with aptamer. As a result, they have shown that this assay can detect the protein at an even lower level with higher sensitivity (W. Lu et al. 2010). It is not only limited to the detection of protein in the sample but also extended to the determination of mutation in DNA of a cancer patient. Lee et al. performed a colorimetric assay of gold nanoparticles for the determination of mutation in epidermal growth factor receptor (EGFR) in non-small cell lung cancer. They have proved that gold nanoparticles will perform aggregation and also change the color in presence of mutated DNA, whereas in the absence of mutation, no change in color and no aggregation was observed (Lee et al. 2010). It is also possible to modify the surface of nanoparticles for better activity and efficiency. Yola (2021) developed an electrochemical biosensor for the detection of breast cancer marker by surface modification of gold nanoparticles.

12.3.2 Infectious Diseases

Worldwide, besides cancer-like threatening conditions, numerous infectious diseases, for instance, tuberculosis, cholera, influenza, and fungal infections, are also in a race (Lin et al. 2013; Sojinrin et al. 2017). As per the WHO statistics, annually, about ten million die out of infectious disease, among which majority of the population are infants or children under 5 years. Furthermore, some of the infection causes sudden death and some causes long-running infection and also the permanent damage to the human due to the inadequate knowledge of infectious disease, which leads to late diagnosis and improper treatment. Further, infectious diseases are characterized by the rapid spread of disease, fast mutation rate, and occurrence in remote areas. To conquer these challenges, modern techniques must have peculiar characteristics like rapid, inexpensive, miniaturized, precise, specific, and sensitive. In this world of nanotechnology, it is possible to find out the origin of mutation, the nature of virulence protein, and other abnormal proteins of nanoranged pathogens (Lin et al. 2013). Furthermore, with the help of nanoparticles, either molecular marker or direct pathogen can be determined. Initially, Storhoff et al. demonstrated the application of gold nanoparticles and colorimetric assay for the detection of methicillin-resistant *Staphylococcus aureus*. They have developed a colorimetric assay using the unique property of gold nanoparticles where DNA sequences were conjugated on gold nanoparticles and this DNA sequence was complementary with the pathogen DNA sequence. Consequently, the color will be changed depending

upon the reaction between the gold probe and pathogen DNA. Further, the aggregation will happen in presence of pathogen genetic components, which ultimately leads to the color change from red to purple, whereas no change in color was observed in absence of the pathogen. These results also tell about the specificity of the gold probe toward the efficient detection of the pathogen in the sample (Storhoff et al. 2004).

One of the fatal infectious diseases is tuberculosis, caused by *Mycobacterium tuberculosis*. Because of its severity, early diagnosis and timely treatment are crucial. The existing detection methods are like smear microscopy, sputum sample analysis has less sensitivity and time consuming, which can extend the onset of treatment. Hussain et al. developed a simple, inexpensive, less time-consuming, reliable, and accurate assay with the help of gold nanoparticles, which replaced the conventional method like real-time polymerase chain reaction (RT-PCR). The gold nanoparticles were coated with complementary to target DNA sequence, forming the complex with the pathogen and indicating the positive results (Hussain et al. 2013). In one experiment, Sharma et al. (2015) proved the unique potential of iron oxide nanoparticles in detecting cholera caused by *Vibrio cholerae*. They had used IONP and immobilized monoclonal antibodies with an affinity toward the pathogen, and this detection was electrochemically assisted. They concluded that this method was highly sensitive, could sense or detect even minute concentrations like 0.4 ng, and showed a good reproducibility index (Sharma et al. 2015).

12.3.3 Malaria

Malaria is caused by the parasites *Plasmodium falciparum* (*Pf*), and it is one of the major causes of death worldwide. It is difficult to diagnose and treat malaria based on common symptoms like fever, chills, illness, headache, and flu, because these symptoms can also resemble some other disease. In malaria patients, the lactate dehydrogenase (LDH) concentration was very low even at some femtomolar level in red blood cells (RBCs) and the parasites in the saliva. Conventionally, microscopic analysis and polymerase chain reaction (PCR) were employed for the detection of malarial biomarkers; however, they are limited in real-time applications owing to time consumption, a requirement of skilled personnel, etc. This urges the need for development of rapid, inexpensive, patient-compliant, and simple detection technique to expedite the diagnosis (Minopoli et al. 2021). To overcome this, researchers developed a rapid test for the malaria diagnosis, also known as the immunochromatographic test. This test is now commercialized and used to detect the parasite antigen, lactate dehydrogenase, and various proteins (Hawkes and Kain 2007; Minopoli et al. 2021). However, in this method, some limitations are found like initial RBC disruption is required to enhance the concentration of markers and the sensitivity also gets affected by environmental conditions. Hence, the involvement of nanoparticles in the detection is crucial to avoid such distractions (Guirgis et al. 2012; Minopoli et al. 2021).

Minopoli et al. (2021) established a method for blood protein detection of the parasite using gold nanoparticles in conjugation with antibodies and aptamers. Initially, gold nanoparticles were coated with antibodies followed by the immobilization of fluorescently labeled aptamers for an appropriate detection scheme using the sandwich phenomenon. To demonstrate the sensitivity, they detect the *Pf*LDH in the whole blood sample and found that this biosensor is capable to detect even a small quantity like 0.3 ng/mL. This method overcomes the limitations like pretreatment of blood samples and the effect of environmental factors (Minopoli et al. 2021). Guirgis et al. (2012) developed a biosensor for the detection of antigen in infected blood, in which they have used gold nanoparticles and the well-known phenomenon of fluorescence quenching. The gold nanoparticles were coated with antiheat shock protein-70 (HSP-70) so that during the reaction between the spiked sample and AuNP, fluorescence will change due to the binding of fluorophore-HSP-70 and AuNP. The fluorescence quenching will be driven by the binding of parasite protein and AuNP (Guirgis et al. 2012). Jeon et al. had developed a colorimetric aptasensor biosensor for the detection of LDH of two different species known as *Plasmodium vivax* and *Plasmodium falciparum* and also states that LDH is the marker in the detection. In addition, cationic polymers have also been used along with gold nanoparticles for assistance in color detection. This aptasensor shows the color change when reacts with LDH and transforms from red to blue as it depends upon the concentration of LDH in a sample (Jeon et al. 2013).

12.3.4 Viruses

The viruses are nanosized pathogens that act as a small bomb that can damage the human health. The viral infection can either be prevented or cured with innate immunity, because once it enters the human immune system, it rapidly multiplies using host energy as a source (Draz and Shafiee 2018). Unlike bacteria and fungi, it is very difficult to isolate the virus from infected samples and perform analysis such as simple light microscopy due to the nanosize of virus particles and their nuclei components. An electron microscope can serve the purpose to some extent in research and routine clinical diagnosis but some constraints like cost, time, and safety concerns limit the applications (Yin et al. 2017). Over the years, multiple serological- and molecular-based detection methods were developed and played an important role in viral detection. Despite rapid development in both methods, some limitations like reliability, accuracy, cross-reactivity, sensitivity, specificity, reproducibility, and genetic variability of viruses limit their applications in virology (Ratcliff et al. 2007). Because of the numerous properties of nanomaterials, for instance, optical, electronic, mechanical, and magnetic, it serves as a better platform for virology. This era begins in 1997 when Zehbe et al. (1997) developed a method for detection of the human papillomavirus (HPV) in case of cervical cancer. They have used gold nanoparticles and streptavidin complex along with silver acetate for staining for easy detection (Zehbe et al. 1997).

Y. Liu et al. (2015) have developed a biosensor for the detection of influenza A virus (IAV) in a single step approach with high accuracy, stability, and specificity. The biosensor was made up of gold nanoparticles and coating of a monoclonal antibody specific to the virus also known as a monoclonal anti-hemagglutinin antibody (mAb). They concluded that the reaction occurred, because gold nanoparticles covered the viral surface area and did not form any cross-linking between them (Y. Liu et al. 2015). To widen the scope of gold nanoparticles and to combat challenges like viral diversity and faster mutation rate in virology, the functionalization of nanoparticles will be another approach. Zheng et al. (2017) have developed glycan-functionalized gold nanoparticles for influenza virus detection; however, glycan functionalization will help gold nanoparticles to detect and differentiate fourteen different types of virus strain in the colorimetric procedure (Zheng et al. 2017). In addition to gold nanoparticles, IONP also contributes to virology. Thanh et al. developed a biosensor to detect hepatitis B surface antigen (HBsAg) using IONP. IONP was combined with biotinylated anti-HBsAg to detect the HBsAg and this method proved to be sensitive and much specific (Thanh et al. 2019).

Recently, the outbreak of SARS-COV2 infection has challenged the entire healthcare sector toward rapid and accurate detection of the virus. Several affinity-based nanobiosensors (through antibody/DNA) have been developed and reported recently (Pradhan et al. 2021). Recently, Surface-enhanced Raman spectroscopy integrated with novel nanostructures has resulted in rapid identification of either the whole virus or specific spike protein. For this, several types of novel nanostructures have been reported such as silver nanoparticles, gold nanostars, carbon nanotubes, graphene oxide-based quantum dots, etc. (Bardhan et al. 2021; Jia et al. 2021; A. Pramanik et al. 2021). These smart sensors have been reported to detect the viral load within a very short span of time (as low as 7 min) compared to the conventional RT-PCR (Zavyalova et al. 2021).

12.3.5 Diabetes

Worldwide millions of people suffering from diabetes, which leads to the development of other serious conditions like *loss of vision*, *cardiac diseases*, *and kidney diseases* (Cash and Clark 2010; He et al. 2021). Further, it is not possible to treat diabetes completely with existing treatment, but with continuous surveillance over blood glucose levels, other severe complications can be avoided or prevented. To maintain the glucose level people sensor strips for glucose estimation where the small blood sample is required from patients which can be isolated or withdrawn by simple finger prick method (Cash and Clark 2010). Since long only glucose measurement has been done using electrochemical enzymatic measurement with minimum laboratory requisite, this method furnishes rapid and accurate results for glucose determination (J. Wang 2008). However, some restraints of conventional methods are well known like patient compliance during blood sampling, variation

between sampling time points. In the case of remote villages, it is also difficult to set up a laboratory for such experiments along with technically trained personnel (He et al. 2021). In context to above, new products come in the market to monitor and evaluate the blood glucose level continuously, which helps to maintain the glucose level. Sometimes it is difficult to determine the minute quantity in blood, hence conventional methods lacking the sensitivity and specificity in the accurate detection. Nanotechnology research plays a crucial role in the development of nanosensors that can overcome these limitations and provide a better platform for future diagnosis. Nanomaterials will furnish a higher surface area, enhancing the catalytic properties, and also giving nanosized sensors (He et al. 2021).

To develop such a nanosystem for regular monitoring, several nanomaterials contributed like graphene, carbon nanotubes, nanofibers, and quantum dots due to which important parameters like sensitivity, specificity, and response time can be improved (Noah and Ndangili 2019). X. Kang et al. (2009) first time have developed graphene-chitosan electrode-based nanosensor for glucose estimation where they have immobilized glucose oxidase. This electrochemical biosensor provides the best environment to the enzyme for long-term stability and by retaining good sensitivity. They also demonstrated the sensitivity and found that this nanosensor showed higher sensitivity than other nanomaterials (X. Kang et al. 2009). Rossi et al. employed iron oxide nanoparticles for the immobilization of enzyme glucose oxidase for estimation of glucose. They have demonstrated the immobilization of an enzyme onto a nanoparticle with the help of an amino group can more efficiently bind an enzyme than physical adsorption. This nanosystem is stable for 3 months when stored at 4 °C by retaining its activity (Rossi et al. 2004). J. Chen et al. have developed AuNPs decorated Ni MOF/Ni/NiO nanocomposite for serum glucose estimation.

12.4 Challenges Encountered and Their Troubleshooting

Although there is tremendous progress in developing novel nanobiosensors, there always remains a major challenges in translation and commercialization of the laboratory-scale research into a viable prototype for clinical use. One major hurdle is the regulatory policies associated with the technology transfer investment in most of the countries. However, they have become increasingly welcoming via workshops and funded proof-of-concept grants. Challenges also arise from the differential hands-on skills with respect to the human resources. Modern sensing system offers benefits in terms of flexibility in usage for nonexpert users using nanotechnology by scaling down complex analytical devices in clinical settings. Although nanotechnology seems promising for the future years, there are certain challenges that still remain to be addressed. Recognition and safety handling issue associated to nanomaterial toxicity and effects on human health is a requisite. Upon overcoming these challenges, healthcare would reach another dimension with respect to the disease diagnosis, detection, and prevention at much earlier stages. Side effects associated with nonspecific drugs could be avoided due to the identification of

exact target using biosensors. On the whole, healthcare in terms of monitoring and detection would become a much easier and inexpensive task irrespective of availability of resources, skills, medicines, etc. With the increasing promise in nanobionsensor research, there is an incredible scope for further research in designing new strategies for novel sensing, which would be rapid, sensitive, specific, and personalized (Diagnosis et al. 2016; Juanola-Feliu et al. 2012; P. K. D. Pramanik et al. 2020).

12.5 Conclusion

Currently, most of the diseases including cancer, microbial infections, diabetes, etc. are fatal to humans, causing an imbalance in the health-care system. One of the major reasons for this can be attributed to the delay in detection and subsequent treatment. The two approaches like early diagnosis and timely treatment of these diseases can resolve that issue and will also help in the management of the health-care system. With the assistance of existing diagnosis methods, the disease can be detected but only when biomarkers are present at higher concentrations in blood, serum, or any other body fluid and unable to detect the lower concentration in the early stages of the disease. Nanomaterials intervene in the development of biosensors to enhance the detection limit at even less concentration. Several nanomaterials like gold nanoparticles, iron oxide nanoparticles, and graphene can serve the purpose by providing peculiar properties like SPR effect, optical activity, conductivity, physical properties, and electrical, magnetic, and electromagnetic property, which contribute to the development of efficient Nanobiosensors with higher sensitivity, specificity, stability, shelf-life, response time, less expensive, patient complaint, portable, and rapid in action. Till now many researchers have already developed such Nanobiosensors for early detection of cancer, infectious disease, bioimaging, and diabetes, and this trend is still in progress at a higher speed for further development and all these instances are embedded in our review. Additionally, we have presented few recent publications to get the updated knowledge on Nanobiosensor development. Despite all the advantages, still, some areas like scale-up, safety, toxicity, and entanglement in signal detection require attention and further research to translate these lab scale nanosensing devices to clinical applications.

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