# 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

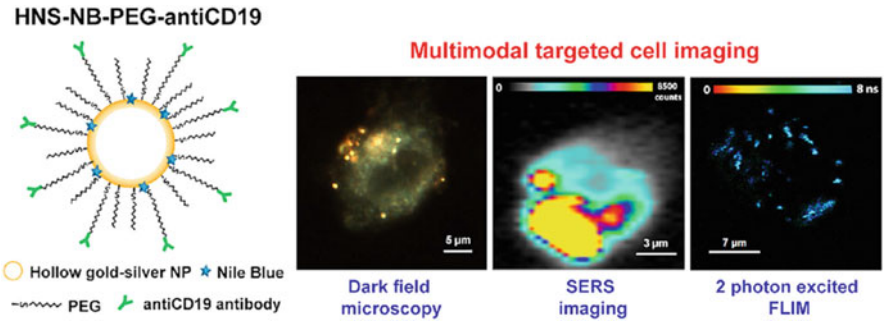


**Fig. 12.1** Novel system for the detection of cancer and imaging of cancer lymphoblast (Reprinted with permission from Nagy-Simon et al. 2017)

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

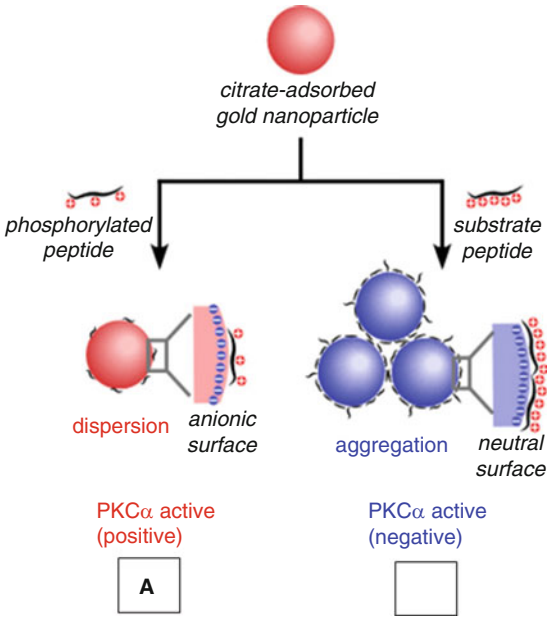


**Fig. 12.2** AuNP-based colorimetric assay for cancer detection ((**a**) The activated protein kinase C (PKC) will be higher that leads to the separation of AuNP and produces a red color and (**b**) The activated protein kinase C (PKC) will be lower that leads to the aggregation of AuNP and produces 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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