Chapter 3:

Nanomaterials based Biosensing: Methods and principle of detection

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Abstract

The food and medical sectors have to abide by the strict regulations of quality control and this requires strict monitoring of various types of chemicals and pathogens. These requirements have brought laurels to the field of biosensing to develop the most capable sensors to detect a target property instantly as well as precisely. Biosensors integrated with the nanotechnological approach makes them simple and tiny in size, which also provides a point-of-care platform. The last two decades have been devoted to developing different biosensors using nanoscale materials due to their sensitive and unique properties. Search is on, for the combination of materials for biosensors, which can make them multipurpose, inexpensive and eco-friendly. The molecules or analytes can be detected through different working principles of biosensors. Biosensing is a wide area and a number of biosensors exist with each having their own working principles and/or combination of two-three principles making them a hybrid sensor. This book chapter is aimed to revisit different working principles of biosensors in light of the growing use of various nanomaterials (nanoparticles, nanowire, and nanosheet) and various other materials that are in use in the development of biosensor.

Keywords: Biosensors, nanomaterials, receptor, nanoparticles, nanowires, nanosheet

List of Abbreviations

CNT	: Carbon nanotube
D	: Dimensional
DNA	: Deoxyribonucleic acid
FAD	: Flavinadenine dinucleotide
FET	: Field Effect Transistor
NPs	: Nanoparticles
RI	: Refractive Index
SPCE	: Screen Printed Carbon Electrode
SERS	: Surface-Enhanced Raman Scattering
SPR	: Surface Plasmon Resonance
SWCNTs	: Single Wall Carbon Nanotubes

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1 Introduction

Biosensing is an emerging research area that is being governed by the need to develop easy, simple, and inexpensive biosensors with instant and precise detection. Nanotechnology has brought tremendous capability to miniaturize biosensors and to make them more affordable. On the other hand, conventional methods of measurement are limited by the complexity of the process and requirement of skilled manpower and time to measure a particular change or property. A simple question one may ask, how does a sensor make life easy for humans? We can understand this through the utilization of biosensors in our daily life, such as a diabetic person can measure their glucose level instantly anywhere by herself/himself, pregnancy diagnostic kits can now more affordably be used without any prior experience.

One of the simplest biosensors is the body temperature measurement sensor, which most of the people uses in their life. There are many non-bio sensors in our daily life such as a clock or a watch to record time, a mobile phone used to receive the data and voice communication including the pedometer inside it, as well as a speedometer measuring the speed of the bike. Different sensors have different working principles but they all do the same thing *i.e.* measure the magnitude of a certain measurand such as a thermometer based on Hg (Mercury) measures thermal expansion, a non-contact thermometer based on infra-red radiations popularly used recently for fast monitoring of the body temperature caused by COVID-19, and a digital thermometer based on thermistor (thermocouple) principle. All three devices have the same goal to measure temperature, as shown in Figure *1*.

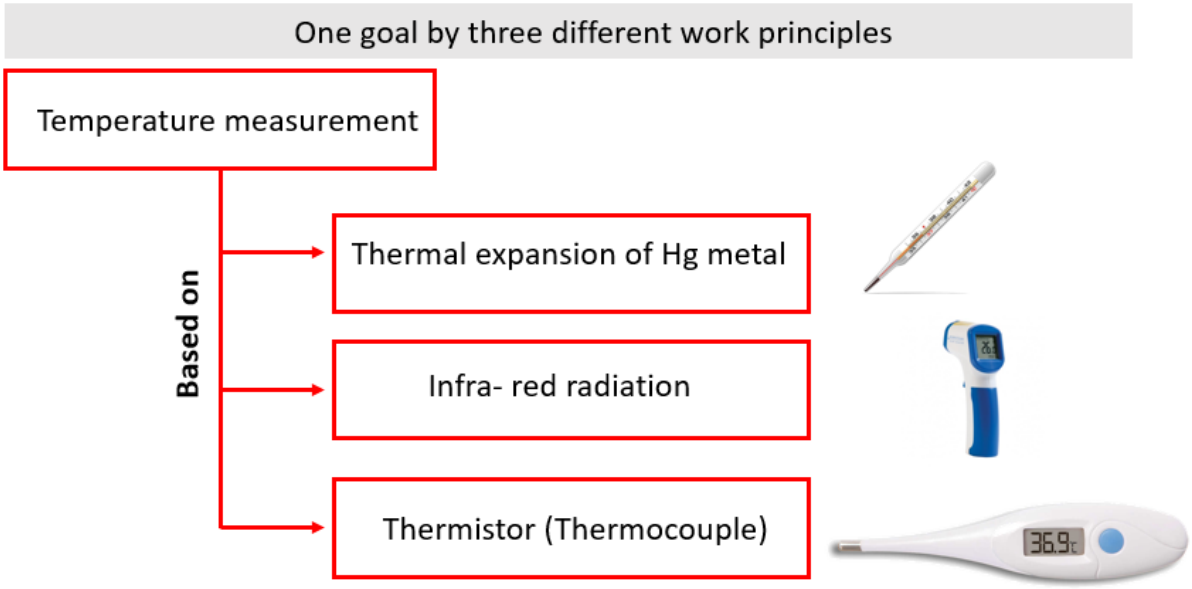

Figure 1: Temperature measurement sensor with three different working principles

Similarly, in food and medical sectors, many biosensors are based on leveraging different work principles such as estimation of glucose in the blood sample, cancer cell detection, bacteria detection, DNA identification, virus detection, detection of pesticides/chemicals/antibiotics, and biological toxin level detection in food and beverages. Biosensors are becoming an integral part of our life to raise awareness and consciousness at the early stages of a process. Biosensors either detect the target analytes or provides exact measurements. Different target analytes require different biosensor, and it can be associated with different working principles. Therefore, emerging nanoscale materials have been employed in combination with newer electronic circuits to develop easy to handle nanoplatforms for sensing various types of analytes. Nanoscale integrated biosensors can work as multiplexed biosensors, or one sensor can detect more than one analyte. However, the prime goal behind the development of biosensors is to improve the lower limit of detection.

Many biosensors are being used in medical and food sectors, having different work principles such as acoustic wave biosensors, electrochemical biosensors, fluorescent paper-based sensors,

electrogenerated chemiluminescence, colorimetric detection, Microbial fuel cell-based biosensor, etc. There are multiple biosensors, which are part of daily use. They can be categorized on a different basis such, as diverse principle-based, different sectors based, and different materials based, such as paper-based biosensors, electronic biosensors, hydrogel-based sensors, etc. This chapter is limited in scope to discuss various essential principles being used in the development of biosensors utilizing nanomaterials and categorization based on nanomaterials dimensions for example zero, one, and two-dimensional nanomaterials (nanoparticles, nanofibers, and nanosheets).

2 What makes sensors good?

The quality of a biosensor depends on some of its salient features such as selectivity, stability, reproducibility, sensitivity and linearity. Besides these properties, the sensor must be economical. The modern technology is integrated with the nanoscience/technology, which makes biosensors ultrasensitive, and they can detect even single biomolecule.

Fast detection: The quick response of a sensor has its importance, especially in point-of-care diagnostic. Also, during a life-threatening incident, the rapid response of the sensor is essential such as heartbeat monitoring, *in-vivo* monitoring, etc.

Selective detection: Selective detection of analytes by a biosensor is equally crucial because most of the biological samples (ex. blood) and food (ex. beverage) etc. are the pool of biochemicals. A biosensor needs to detect only the particular analytes, for which it is tuned, and it must be neutral to other interfering analytes. It is known as the selectivity of the biosensor.

Reproducibility: The reproducibility is the ability of a biosensor to produce similar or identical results on each time the measurement is repeated, or we can say there is a negligible change in the result to repeat the same sample multiple times.

Stability: The stability of a biosensor is an essential factor and indicated the consistency in results after a biosensor is deployed for a long time (storage/transportation). Better stability guarantees consistency, reproducible results and precision regardless of secondary factors such as the environmental conditions.

Accuracy of biosensor: The human body is prone to respond to slight chemical change. Therefore, a sensor must be accurate to quantify the measurement.

Minimum sample requirement: The sampling requirement for sensing is an important parameter and the smallest quantity requirement is the best especially in forensic science, where destructive testing and availability of samples could be very limited.

3 Methods for biosensors formulation

A biosensor is a device having three parts, as shown in Figure 2, the first part being the biological recognition system, which is highly specific towards the analytes and provides a quick response on interaction with analytes. The second part is a transducer, which takes the response from the recognition system, and converts it into an electric signal, which is calibrated with the standard value of the response. The third part deals with the amplification of the weak signals and displays on the controlling unit, although it is not necessary for all biosensors.

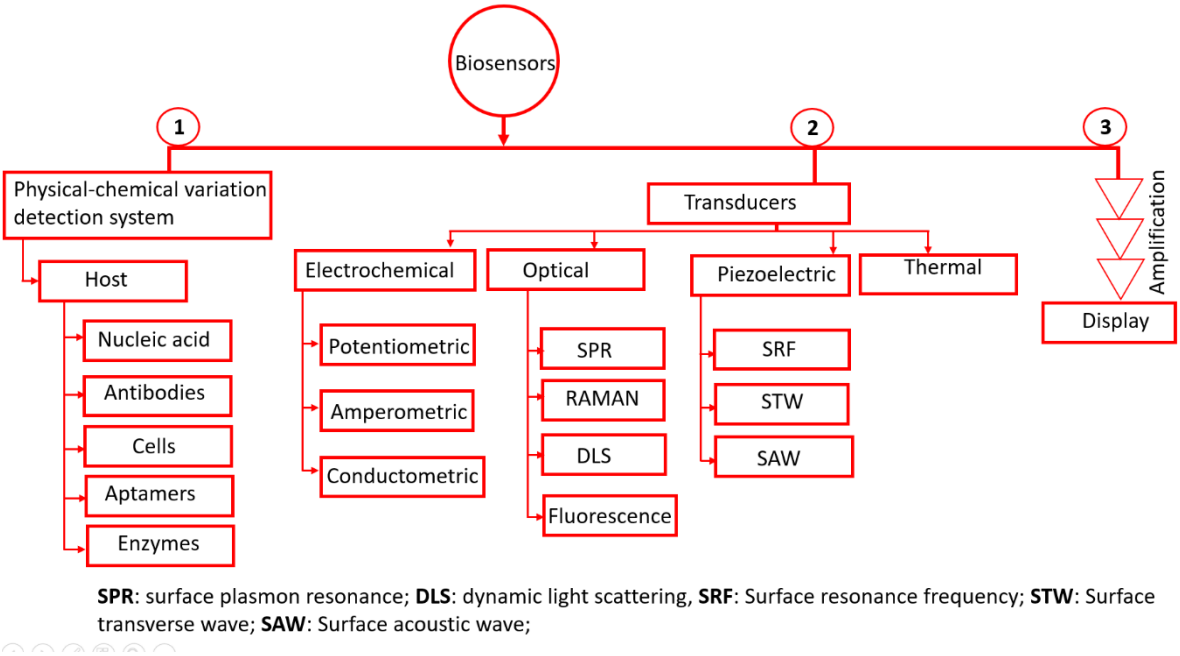

Figure 2: Classification of biosensor parts and working principles

Recently developed biosensors are based on various types of nanomaterials and possess advantage due to their high surface energy to volume ratio, and delivers response on minor changes over the surface such as metallic nanoparticle change their surface plasmon response on binding with other molecules, nanowires change their frequency of vibration on binding with another biomolecule, fluorescence efficiency variation in nanosheets on the landing of analytes, etc., as shown in Figure *3*.

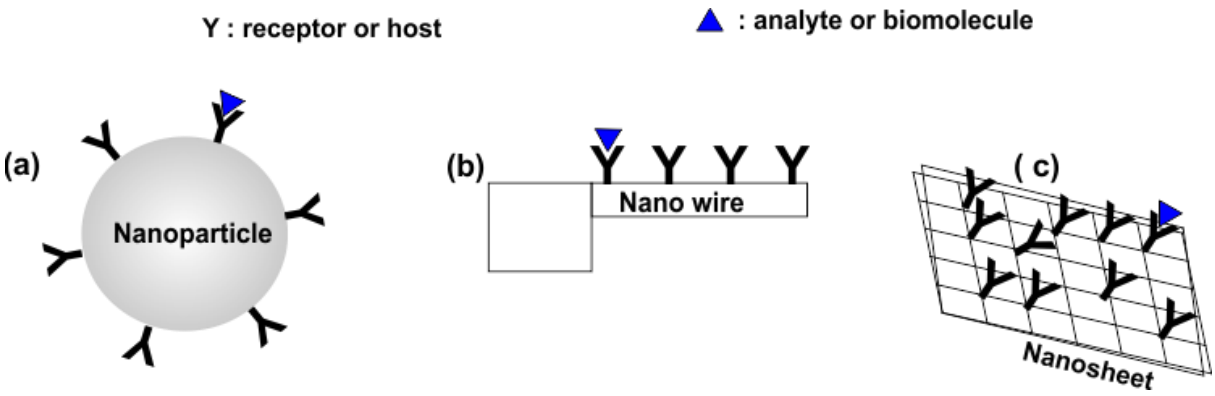

Figure 3:Schematic of functionalization with receptor **(a)** nanoparticles 0D **(b)** nanowire 1D **(c)** nanosheet 2D

4 Working Principle

The following sections discuss the various working principles for the different biosensors based on various nanomaterials (i.e. 0D, 1D, and, 2D). These working principles may be used individually or in combination to achieve optimum results.

4.1 Nanoparticles (0D materials)

The materials with its all three dimensions less than 100 nm are considered as nanoparticles. They have unique and sensitive properties suitable for developing the biosensors such as high surface energy to bind with other molecules, surface plasmon resonance, etc. The nanoparticles-based biosensor is further divided into two categories: Label-based biosensor and Label-free biosensor. In the former technique, nanoparticles functionalized with the specific receptor (enzyme, fluorescence, antibody, etc.) directly bind to the analytes. On the other hand, in the Label-free technique, the nanoparticles directly interact with the analytes to give the response.

4.1.1 Surface plasmon resonance-based

The surface plasmon resonance (SPR) is a versatile character of the metallic nanoparticles. This phenomenon is recognized by the collective oscillation of the valance electrons when incident

electromagnetic radiation oscillation matches the surface electrons, and it appears as an absorption band, the schematic of oscillation shown in Figure *4* [1]. The position of the absorption band is very sensitive to the dielectric constant of the surrounding medium, nanoparticle shape, size, and ligands interactions (increase or decrease surface electron density). The shifting of the band towards lower wavelength (high energy) is referred to as a blue shift and shifting of the band towards higher wavelength (lower energy) is referred to as redshift. The shift of SPR band by more than 2 nm in the visible range (~500 nm wavelength) can be recorded easily. On the change of SPR band position (by analytes-nanoparticles coupling), the reaction jar reflects the change in colour, which can identify through the naked eyes, as shown in Figure *5* [2]. Slight change of refractive index (RI) around nanoparticle's surroundings can be detected as a signal, and this phenomenon is being utilized to develop many different types of biosensors. Similarly, a change in the size of nanoparticle also alters the surface plasmon band and can be used for the development of the sensor. The spherical gold nanoparticles suspension having an average diameter of 10 nm appears as red colour (SPR band at 520 nm). On the other hand, silver nanoparticles suspension having the same size appears as yellow colour (SPR band at 395 nm)[3,4]. Therefore, the core-shell nanoparticles (Au nanoparticles with Ag surface) optical properties can be tuned to the cyan-green region by altering the Ag layer thickness. Also, these nanoparticles are surface modified using engineered antibody-conjugated, which are prone to bind to harmful biotoxin staphylococcal enterotoxin A (SEA)[3,5]. It is a small protein mostly found in staphylococcal food poisoning[6]. Therefore, a solution assay containing these nanoparticles functionalized with engineered conjugate antibody works as a biosensor; the assay changes their colour on binding with biotoxin SEA, which may be observed through naked eyes as shown in Figure *5*.

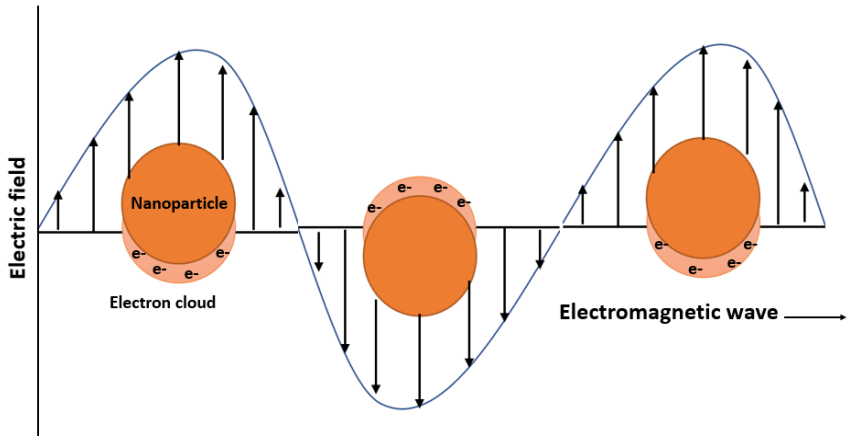

Figure 4: Surface plasmon Resonance on nanoparticles

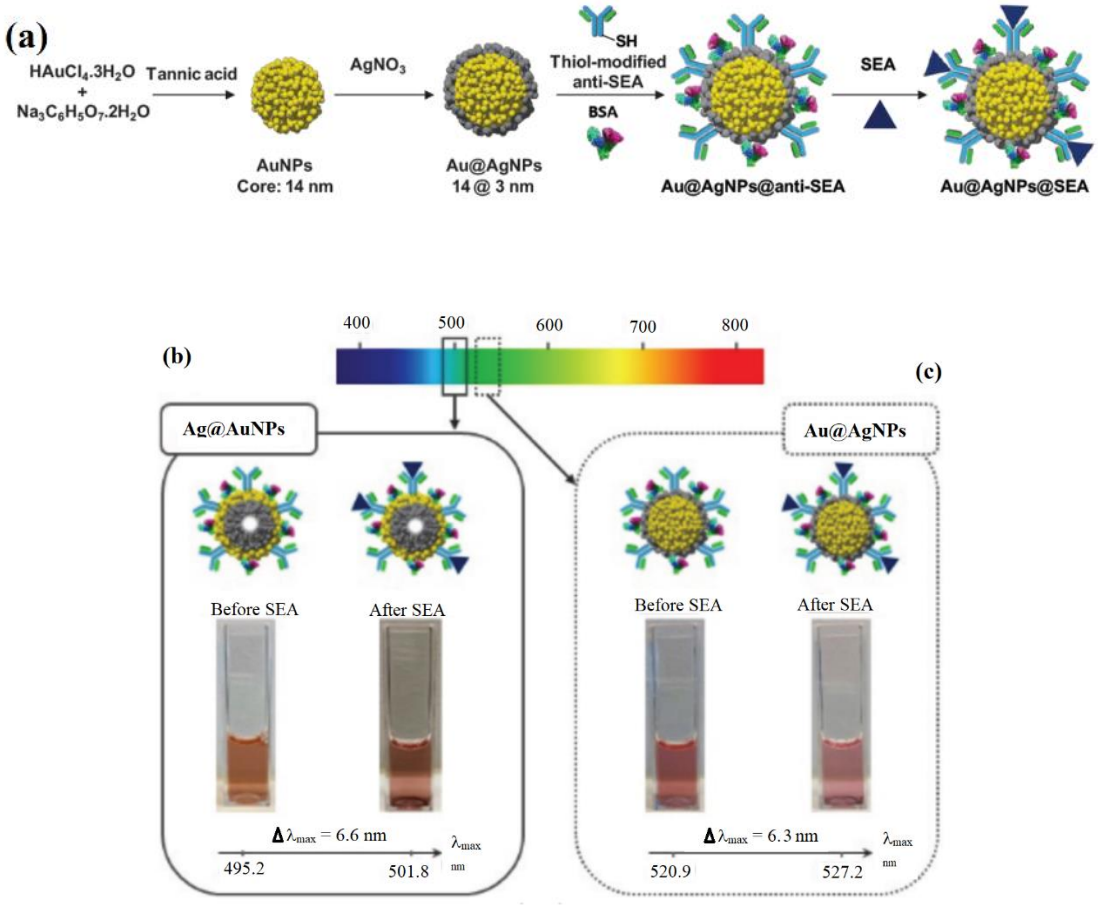

Figure 5:**(a)** Schematic of synthesis core-shell nanoparticles (Au@AgNPs), their functionalization with conjugated-antibody, and binding with SEA **(b)** Ag@AuNPs change in color before and after binding with SEA **(c)** Au@AgNPs, change in colour before and after binding (printed with the permission)[5].

4.1.2 Surface-enhanced Raman scattering based

The Surface-Enhanced Raman Scattering (SERS) is a phenomenon that enhances signal intensity by many orders of magnitude (~10^6-10^{14}) and overcomes the inherent weakness of a Raman signal. It is highly sensitive and selective due to unique fingerprint spectra. Therefore, even just a single molecule [7] can be detected using this technique

SERS can be utilized for developing different biosensors such as *in-vivo* glucose detection [8,9] nucleic acid and proteins [10], DNA detection [11], and rapid detection of bacteria [12,13]. The detection methodology with label-based and label-free technique utilizing nanoparticles is shown in Figure *6*.

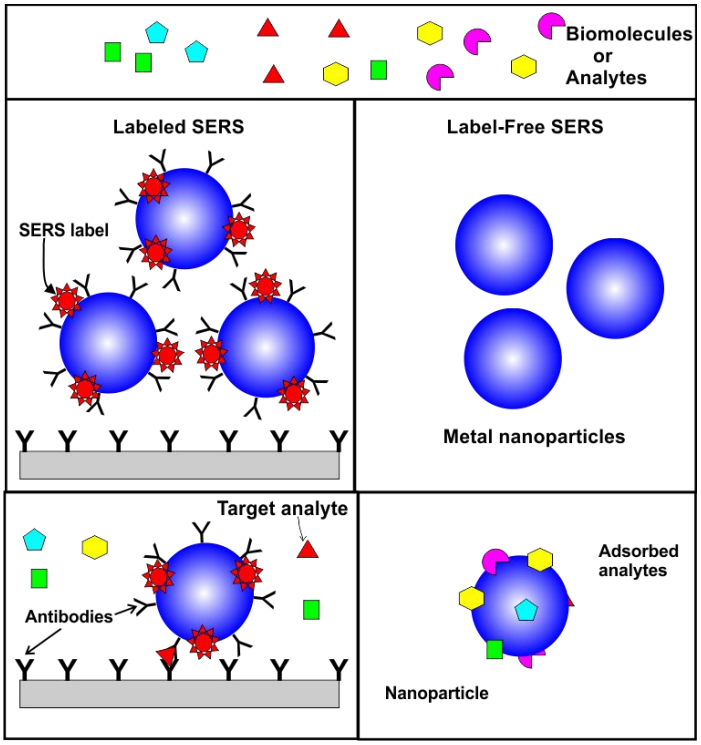

Figure 6: Schematics label-based and label-free surface-enhanced Raman scattering phenomena for designing biosensors

4.1.3 Magnetic nanoparticles based

Magnetic nanoparticles-based sensing is also being actively used for biosensing since the last decade. They are alternatives to fluorescent biosensors. Magnetic nanoparticles show super

magnetic behaviour at nanoscale dimensions due to a reduced number of domains, and magnetization can flip direction instantly. However, in the case of bulk, the external magnetic field is required to demagnetize and flip the direction, as shown in Figure *7* [14]. For nanoparticles, there is no thumb rule to determine magnetic behaviour (diamagnetic, paramagnetic, ferromagnetic) [15]. In the past few years, different types of magnetic nanoparticles are used for biosensing such as Co, Mn, Fe, Ni, Fe_2O_3, Fe_3O_4, FePt, FePt-Ag, CdS, etc. [16]. The magnetic behaviour of nanoparticles is now being integrated with transducers' materials, which impart the analytical merits such as low limit detection, high signal to noise ratio, enhanced sensitivity, etc. [17].

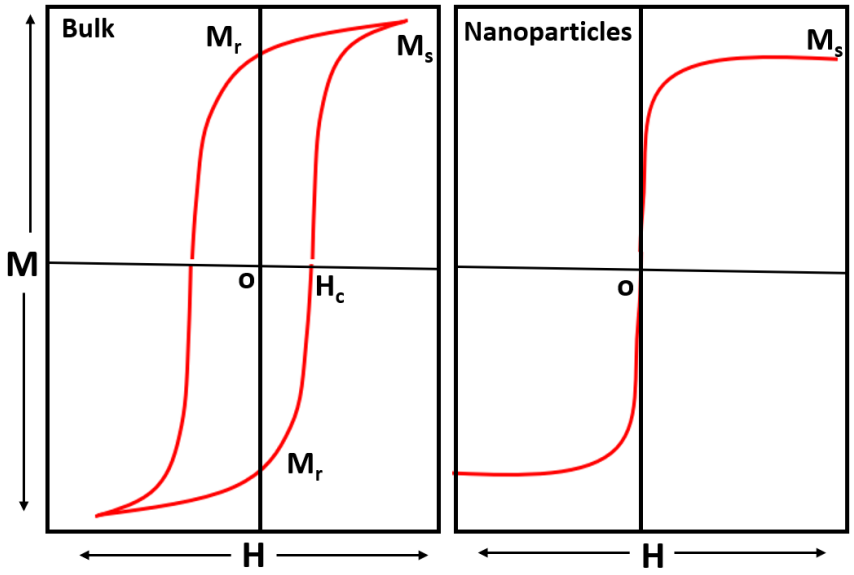

Figure 7: Schematics of the magnetic materials hysteresis loop of bulk magnetic vs nanoparticles (Ms: magnetic saturation, Mr: remnant magnetization, Hc: coercivity)

The separation of analytes from a large sample or other word concentration enhancement of analytes utilizing functionalized magnetic nanoparticles is a competent technique nowadays. In this technique, a nanoparticle functionalized with the receptor directly binds with the targeted analytes in a sample. Furthermore, analytes, along with magnetic nanoparticles, separates from the solution using an external magnetic field. In this way, centrifugation can be

avoided from sample preparation, which brings other impurities along with targeted analytes [15].

4.2 Nanowire (1 D materials)

A nanowire is a thin wire with a diameter below 100 nm. Nanowires made from various materials such as Ag, Au, Pt, carbon nanotubes, etc. have been developed. The frequency of vibration, stress response of piezoelectric nanowire, and an array of nanowires endows with photonic properties such as wire arrangement helps to trap the light in a broad range of wavelengths, and these properties of nanowires are utilized in biosensor development.

4.2.1 Nanomechanical based sensors:

The deformation and vibrations in a nanowire are sensitive properties, and the utilization of these unique properties is helpful in the development of biosensors. The nanowires functionalized with the receptors are purposely made to bind with biomolecules of interest. Whenever the analytes or biomolecules bind with the receptor on nanowires, it induces the stress and nanowire bend shown by a schematic in Figure *8*. The deflection of the nanowire can be measured through optical (laser deflection, optical interferometric, etc.) or electrical techniques (piezoelectric, etc.). Similarly, whenever biomolecules land onto a nanowire, it reduces their vibration frequency due to mass increase, which can be measured through optical techniques [18,19].

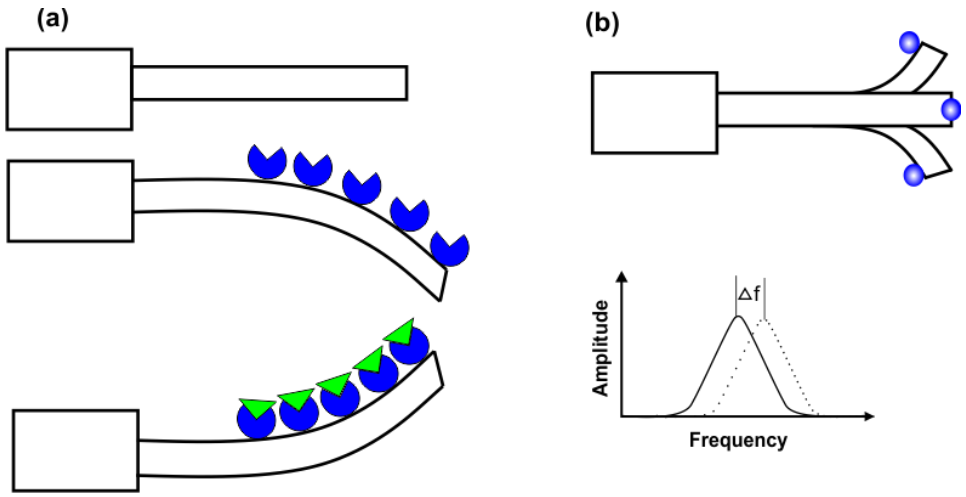

Figure 8:Nanomechanical biosensor principle **(a)** static; bending of nanowire **(b)** dynamic; changing the vibration of the nanowire

4.2.2 Opto-thermo-mechanical based

The working principle of Opto-thermo-mechanical based biosensor is slightly different from thermomechanical based biosensor. In this method, the array of the nanowire is vertically planted over the silicon-silicon dioxide membrane. In this way, the high surface area achieved makes it highly sensitive to respond to even a few analytes molecules. These nanowires are capable of trapping light of broadband due to nanostructures. Also, nanowires are functionalized with the single-stranded probe DNA. When analytes molecules come in contact with a target single-stranded DNA, the relevant molecules bind together, changing the mass detected by the device. Also, the vibration frequency of nanowire changes due to the addition of mass. 90% of the laser light exposed over the surface of array nanowire gets trapped or absorbed due to nanostructure array, which activates the efficient opto-thermo-mechanical excitation of the resonator. An optical signal of laser light (variation in frequency) is used to read for interpretation of the results. In this way, there is no requirement of wires to connect with the biorecognition platform [20].

4.2.3 Field-effect based (semiconductor) biosensing

A material having its conductivity between conductor and insulator known as a semiconductor, which is defined in terms of a bandgap. Therefore, a nanowire having a bandgap and its ability to bind different analytes over the surface makes it more popular to design a wide range of biosensors. Silicon is a popular semiconductor used in most electronics applications. Cui *et al.* [21], reported for the first time to use the silicon nanowire building block to develop different sensors, which can detect protein, viruses, nucleic acid, etc. Silicon nanowire acts as a resistor when the number of charge molecules binds over nanowire, and it leads to depletion or accumulation charge carrier, as shown in Figure *9*. As a result, the conductivity of the nanowire can be harnessed as a signal, and it is ultrasensitive up to a single virus/molecule detection [22,23]. The bio-molecules induce charges in an aqueous solution (proteins, nucleic acid) that can bind easily with appropriate receptor functionalized over the surface of the nanowire [24]. The different materials nanowire used for various bio analyte detection such as silicon nanowire for gastric cancer detection from exhaled volatolome [25], troponin detection [26], Follicle-Stimulating Hormone detection [27], etc.

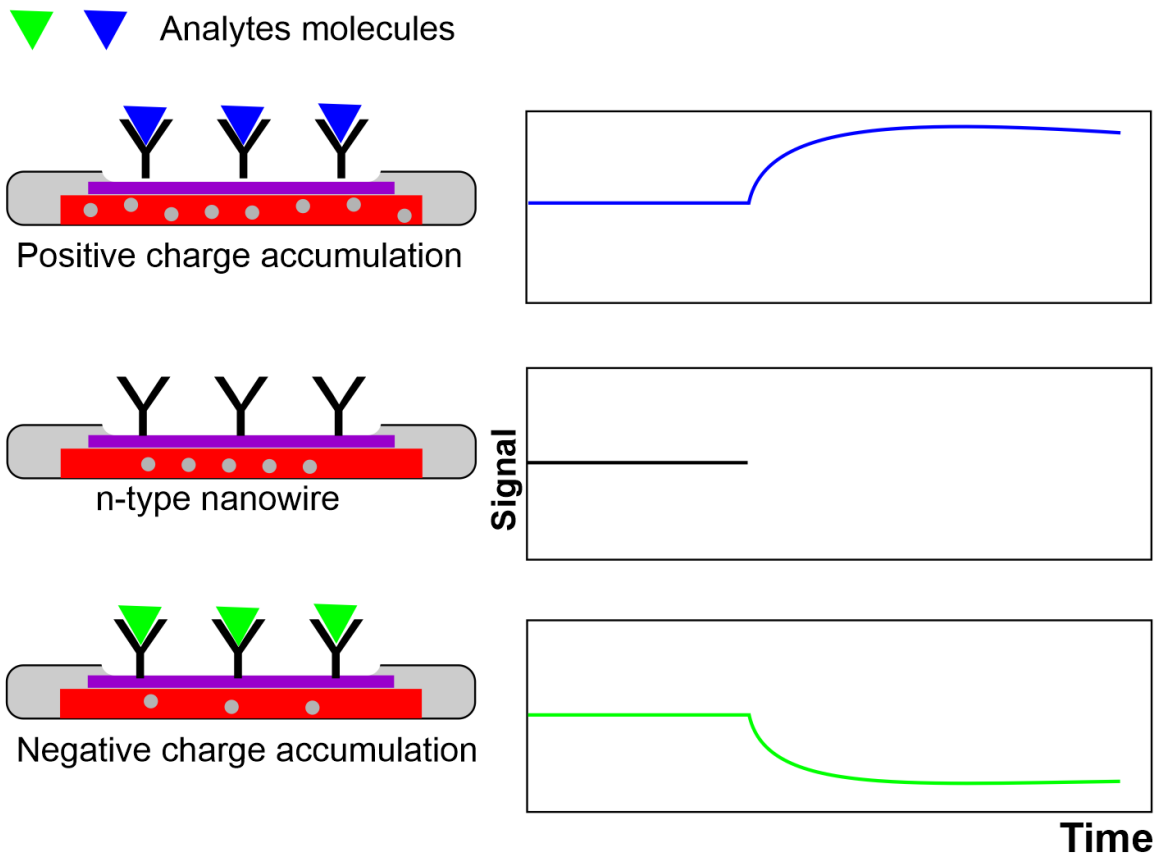

Figure 9: Semiconductor-based nanowire biosensor configured with receptor over nanowire surface

Similarly, the carbon nanotubes are very sensitive to response even at single-molecule interaction. There are carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs) being used for biosensor formulation. The SWCNTs having highly electrical transduction property and utilized paper-based chemiresistor sensor for the detection of human immunoglobulin G (HIgG) up to the picomolar level [28].

4.3 Nanosheet based (2D)

A 2-dimensional thinnest material, having a thickness of less than a few nanometers is referred to as a nanosheet or in short 2D nanostructure materials. These materials are having unique and excellent mechanical, thermal, optical, and electrical properties that have attracted researchers to harness their properties for many applications, one of them is to develop biosensors.

4.3.1 Fluorescence-based biosensing

Fluorescent molecules absorb light and in turn excites the electrons of the molecules, causing them to jump to a higher energy level. Upon returning from an excited state to a ground state, the energy is released in the form of radiation or heat. The light-emitted in this process is referred to as fluorescence, and molecules are known as a fluorophore. However, when the fluorophore interacts with the quencher (another molecule), the resultant complex may become non-fluorescent. A German scientist Theodor Förster proposed and explained the mechanism of two light sensitive (donor-acceptor) molecules interaction, which may emit light or can absorb [29]. It is known as Föster (Fluorescence) resonance energy transfer (FRET). Electronic excited state of a donor may transfer energy to an acceptor via the non-radiative dipole-dipole interaction. Energy transfer efficiency is inversely proportional to the sixth power of the distance between donor and acceptor making FRET extremely sensitive to small gaps [29].

The two-dimensional nanomaterials are extensively in use for biosensing because 2D nanosheets can act as a platform, where biomolecules can easily land or adsorb and alter the fluorescence efficiency (donor-acceptor). Now a day, many 2D materials are in practice to harness fluorescence property for use in biosensing, including the use of Vanadium disulfide (VS_2) nanosheet for detection of cytochrome c.

4.3.2 Field-effect based (semiconductor)

The field-effect transistor technology is a milestone in developing tiny and simplest biosensors. There are many-layered structure materials such as molybdenum disulfide (MoS_2), Graphite, Vanadium disulfide (VS_2), boron nitride (BN), tungsten disulfide (WS_2) that are easy to exfoliate in different solvents to prepare nanosheets. Additionally, graphene-based FET's are highly sensitive towards the detection of the analyte. Graphene has an extraordinary ability of the electron-transport property[30]. Therefore, graphene channels show conductivity change in response to the minor adsorption of analytes molecules on the sensor surface. Chen *et al*.[31]

described field-effect transistor-based detection of Ebola shown in Figure *10*. The reduced graphene oxide bridge between the source and the drain electrode, and layer protected with Al_2O_3 to protect underline rGO. The gold nanoparticles conjugated with antibody immobilized over the reduced graphene oxide layer, are highly prone to bind with Ebola virus glycoproteins. Therefore, the exposure of EGP (Ebola glycoprotein), makes the dynamic response of FET measured and the drain-voltage current to decrease with time as more protein binds with the platform (see Figure *10* (b)). The detection limit of such a biosensor is 1ng/ml EGP.

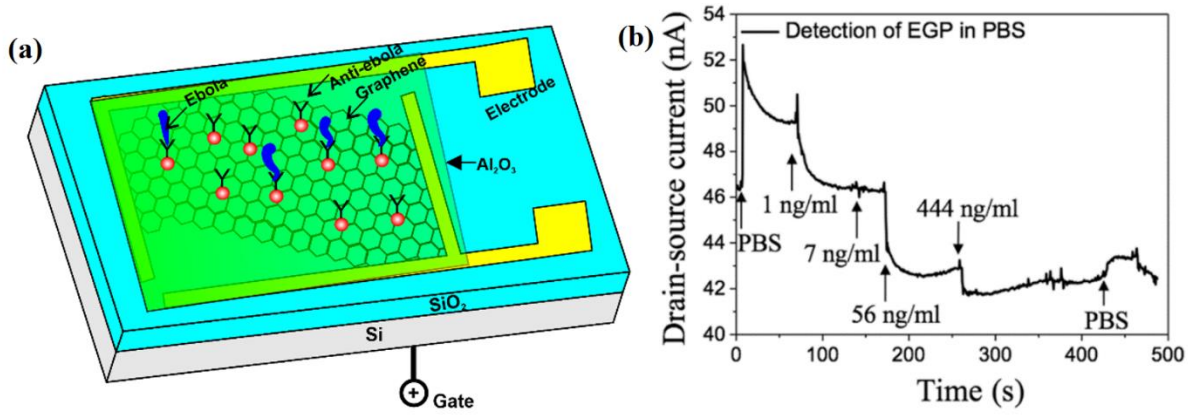

Figure 10:**(a)** Schematic of reduced graphene oxide-based Field-Effect Transistor (FET) biosensor. Ebola antibody conjugated with gold nanoparticles and Al_2O_3 coating for passivation, **(b)** dynamic response of FET sensor on the exposure of Ebola glycoprotein (EGP) (reprinted with permission) [31].

4.3.3 Optical biosensors

Graphene-based materials are mostly studied due to their extraordinary optical property such as surface plasmon and absorption polarization, which endowed the sensor to detect even single cells [32]. The distinction of graphene biosensors based on their electrical and optical properties are summarized in Table 1.

Table 1: Difference in electrical and optical principle-based graphene biosensor

Property	**Graphene electrical biosensor**	**Graphene optical biosensor**	**Ref**
Principle	Receptor functionalized graphene change conductivity on binding with analytes. Monitor the drain-source conductivity in Field effect transition mode.	Broad band absorption of graphene and their sensitivity with any change near to graphene surface or refractive index	[33-35]
Advantage	Fast response time High surface area Lower detection limit	Unlabelled sample Accurate detection High spatial resolution	
Disadvantage	Only measure current Sample damage	Light absorption of monolayer graphene too low. Aggregation of graphene affect the optical properties	

4.3.3.1 Surface plasmon based biosensing

The best property of optical sensors is that they respond ultra-fast and gives result in real-time. The electronic properties of graphene are excellent because Fermi surface of graphene lies in the overlapped/intersection of the empty conduction band and the filled valence band and also in the middle of the *p* band, where electrons are the valence electrons and have the mobility of about 1/300 the speed of light known as Dirac Fermion [36,37]. These fermions show a linear energy-momentum relationship near Dirac point, and this endowed graphene with optical resonance property at any frequency in the ultra-violet, infra-red region. Therefore, many different biosensors have been developed, in which the fiber optic sensor is simplest. The fiber optic integrated with graphene makes use of optical fiber used for transmission of light and to

receive the output. The change in intensity, wavelength, frequency, etc. after interaction with the graphene platform received through the optical fiber is analyzed [38,39]. There are many graphene SPR based biosensors used for a variety of purposes, such as specific protein detection [40], gas detection [41], and different chemical- biological species [42].

4.3.3.2 Polarization absorption enhanced

Another interaction of light with graphene depends on the number of layers and prismatic total internal reflection (TIR) structure. In the graphene, the prismatic TIR structure shows the characteristic polarization absorption and broadband absorption enhancement [43]. When the refractive index of medium increases than the graphene, it starts to show a variation in the optical power of two polarization states [32,44]. Also, by utilization of change in polarization states, many sensitive biosensors have been designed [37].

4.3.4 Gravimetric based biosensors

The natural frequency of a piezo crystal or film is inversely proportional to its thickness, and also, we can make it oscillatory due to piezoelectricity by applying electricity using a simple circuit. Also, whenever any mass is bound to the piezoelectric film, its frequency gets reduced. This method can be utilized in different modes such as resonating crystal, in which well-known quartz crystal microbalance work, surface acoustic wave mode, etc [45]. This type of sensor sensitivity depends on the film thickness. Therefore, many piezoelectric polymer films have been developed, which can be drawn in very thin films to develop highly sensitive biosensors. The Polyvinylidene Fluoride (PVDF) polymer is one such well known piezoelectric polymer [45].

4.4 Electrochemical sensor

The electrochemical sensors work as electrochemical transduction, where electrochemical reactions (oxidation-reduction) results in the transfer of electrons, which can be detected

through amperometry or voltammetry. It can detect enzymes, cells, tissues, gas, ligand, etc. In principle, it is a conventional electrochemical cell containing three separate electrodes: the working electrode (WE), the counter electrode (CE), and the reference electrode (RE), and these three-electrodes can be printed on paper, polymer, etc. as shown in Figure *11*.

Nowadays, screen-printed carbon electrode (SPCE) biosensors are so famous due to easy, point-of-care, small fluid volume (analyte) [46]. The working electrode is functionalized with a biorecognition system (enzyme, etc.) utilizing nanoscale materials, and the analytes directly interact with the working electrode. As a result of this, the electrochemical cell becomes active and produces a signal.

Zelada-Guillén *et al*. [47] utilized carbon nanotubes as an ion-to-electron transducer, and covalently bonded aptamers for the detection of Staphylococcus aureus. The measurement of electromotive force (EMF) or potential, generated on the antigen-antibody interaction is known as a potentiometric biosensor. On the other hand, the measurement of the ionic strength of the solution, which affects the conductivity, is known as a conductometry biosensor [48]. Similarly, amperometric transducers measure the direct current from redox reaction under a constant potential applied to WE. The activity of the recognition element varies before and after the interaction with a target molecule. The product must be electroactive and undergoes a redox process [49]. However, if the biosensor measures the current-potential relationship, and the reference of potential at zero current applied is known as a voltammetric biosensor. Many analytes can be detected with their characteristic potentials. It has been divided into many such as cyclic voltammetry (CV), linear sweep voltammetry (LSV), and differential pulse voltammetry (DPV), etc. Currently, the most popular nanoscale materials being utilized for enhancing the sensitivity of biosensors are Au nanoparticles (NPs), Pt NPs, alloy nanoparticles, carbon nanotubes, graphene, MoS_2 nanosheet, VS_2 nanosheet. The utilization of different

nanoscale materials and various working principles used for analytes detection have been summarized in Table 2.

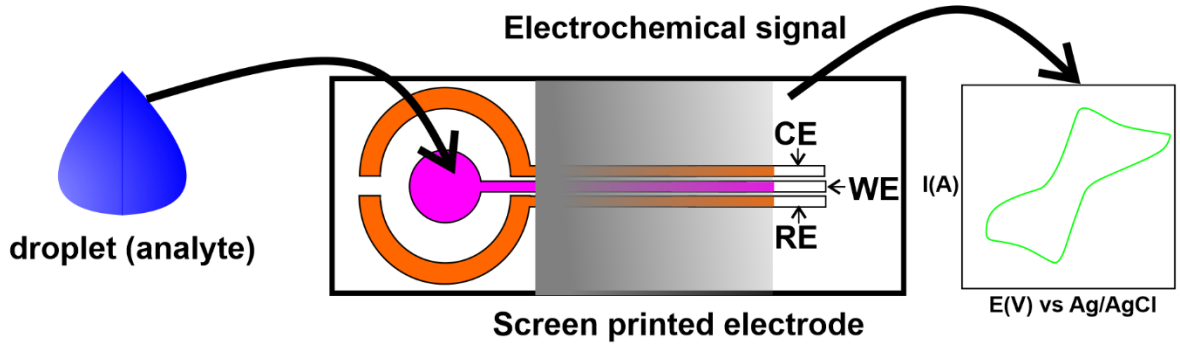

Figure 11:Screen printed electrode-based biosensor

Electrochemical Glucose biosensor: **How nanoscale materials appear to play a role?**

The glucose level detection in the human body is a very important task, which changes abruptly in a diabetic person, and needs careful monitoring. The electrochemical amperometric biosensors are so common in daily use. The working principle of this type of biosensor is based on a simple enzyme-catalyzed reaction of glucose as follows:

$$Glucose + O_2 \xrightarrow{Glucose\ oxidase\ (Enzyme)} Gluconic\ acid + H_2O_2 \quad (1)$$

$$O_2 + 4H^+ + 4e^- \xrightarrow{reduction} 2H_2O \quad (2)$$

$$H_2O_2 \xrightarrow{oxidation} O_2 + 2H^+ + 2e^- \quad (3)$$

Therefore, we can detect the consumption of oxygen or the generation of H_2O_2 electrochemically. The H_2O_2 oxidizes and produces electrons, and these electrons (anodic current) can be monitored electronically. These were classed as the first generation of glucose biosensors. In this process, the high potential required to apply at which other drugs may present in the blood sample becomes electroactive at such potential resulting in inaccuracy.

Therefore, the influence of coexisting electroactive compounds reduced by a coating over the electrode surface with polymer layer (poly-(phenylendiamine), polyphenol, Nafion, cellulose acetate, etc.), and tuning the operational electrode potential of targeted analytes oxidation range preciously. However, the whole process depends on oxygen. Consequently, slight tension in oxygen and stoichiometry alter the sensor response [50]. Therefore, to counter this problem in a glucose biosensor, a mediator (non-physiological electron acceptor) is inserted between glucose oxidase enzyme and electrode, which carry forward electron from the reaction center to the electrode surface.

These are classed as the second-generation glucose biosensors and are based on the following equations as illustrated further in figure 12.

$$Glucose + Glucose\ oxidase(oxi) \rightarrow Gluconic\ acid + Glucose\ oxidase\ (red) \quad (4)$$

$$Glucose\ oxidase\ (red) + 2M(oxi) \rightarrow Glucoseoxidase\ (oxi) + 2M(red) + 2H^{+} \quad (5)$$

$$2M(red) \rightarrow 2M(oxi) + 2e^{-} \quad (6)$$

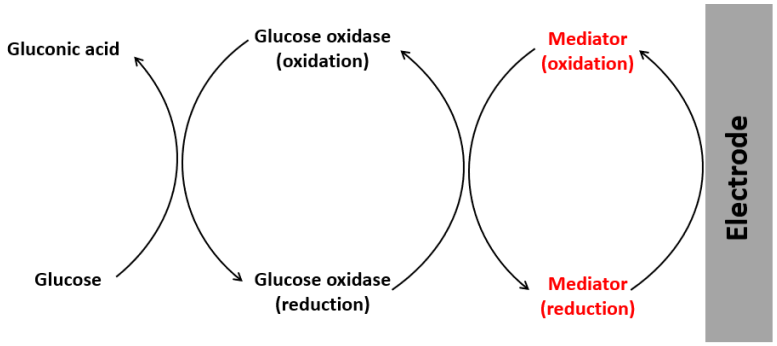

Figure 12: Sequence of reaction occur in second-generation glucose biosensor [50]

Many mediators are being used, such as ferricyanide, conducting organic salts, quinone compounds, transition-metal complexes, phenothiazine and phenoxazine compounds, etc. which are insoluble, non-toxic and chemically stable. The mediator is fast enough to react with

enzymes and transfer electrons, but still, oxygen remains a problem due to self-diffusing. It limits the accuracy of low glucose concentration.

Further improvements in the biosensor have been made by placing wiring as nanomaterials to fast electron transfer from the center of the redox enzyme to the electrode. It is fast communication between the redox enzyme and the electrode. Patolsky *et al*. [51,52] have constituted the glucose oxidase enzyme over the carbon nanotubes, which vertically connects to the electrode, and acts as a wire to fast transfer charge, as shown in Figure 13.

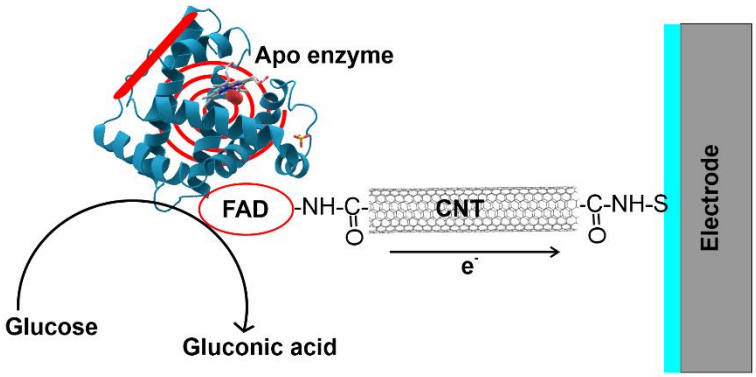

Figure 13: Carbon nanotube (CNT) as a connector in for electrical communication in glucose biosensor; (FAD: flavinadenine dinucleotide) [51];

It is known as the third generation of glucose biosensors based on amperometry. Modern technology is revolutionizing very fastly. These biosensors can now be screen printed with multilayers (electrode, enzyme, mediator, binder etc.) strips for one-time use.

Table 2: Some biosensors researching nanoscale materials, method and analytes

Materials	**Method Or Principle**	**Analytes To be measured**	**Dimension of nanomaterials**	**Ref.**
Ag@Au core shell nanoparticles	Surface plasmon resonance	Biotoxin staphylococcal enterotoxin A (SEA)	0 D	[6]
FeCo nanoparticles	Giant Magnetoresistive immunosensor	Endoglin detection from urine	0D	[53]
Carboxyl functionalized iron oxide nanoparticles	Superconducting quantum interference	breast cancer cells	0D	[54]
Manganese-doped ferrite (MnFe2O4)	Hall Sensor	Rare cells: MDA-MB-468 cancer cells (whole blood)	0D	[55]
Carbon nanotube	Chemiresistor	Proteins detection	1D	[28]
Polyaniline nanofibres (PANI-NF) functionalized with gold NPs	Electrochemical (Cyclic voltametry)	DNA detection	1D	[56]
Nickel oxide (NiO) nanofibres	Field effect	Alcohol detection	1D	[57]
Gold NanoWire arrays	Optical and electrocheical	Bacteria in kidney infection	1D	[58]
Platinum Nanowires	Electrochemical (Amperometer)	electrocatalytic reduction of hydrogen peroxide (H_2O_2)	1D	[59]

Graphene	Field Effect Transistor	Ebola virus detection	2D	[31]
Graphene	Field Effect Transistor	Acetylcholine	2D	[60]
Layered titanate sheets	Electrochemical based	Catalytic reduction of H_2O_2	2D	[61]
Flower-shaped copper oxide thin sheet	Electrochemicacal (amperometric)	Glucose	2D	[62]

5 Biological Recognition element

The biological recognition elements are different types of receptors or bio-probes, which are highly specific towards their stimuli. The bio probes are immobilized over the surface of nanoscale materials, which have the ability to form covalent bonds with analytes or adsorbed and bind with Van der-Waal forces, etc., such as antibodies, enzymes and cells. The most straightforward process is the physical adsorption of bio-probes. However, the salts, pH, and other contaminants appear as a nuisance, which creates noise in the output signals and losses in the precision quantification or detection. Contrarily, the chemical immobilization of bio-probes has attractive features, such as strong covalent bonding with nanoscale materials and is highly specific towards the target analytes; which increases precision and the probability of detection.

Each biosensor needs a unique combination of biorecognition element and transducer, which depends on the target analyte. A schematic map is shown in Figure *14* for selecting a suitable biorecognition element.

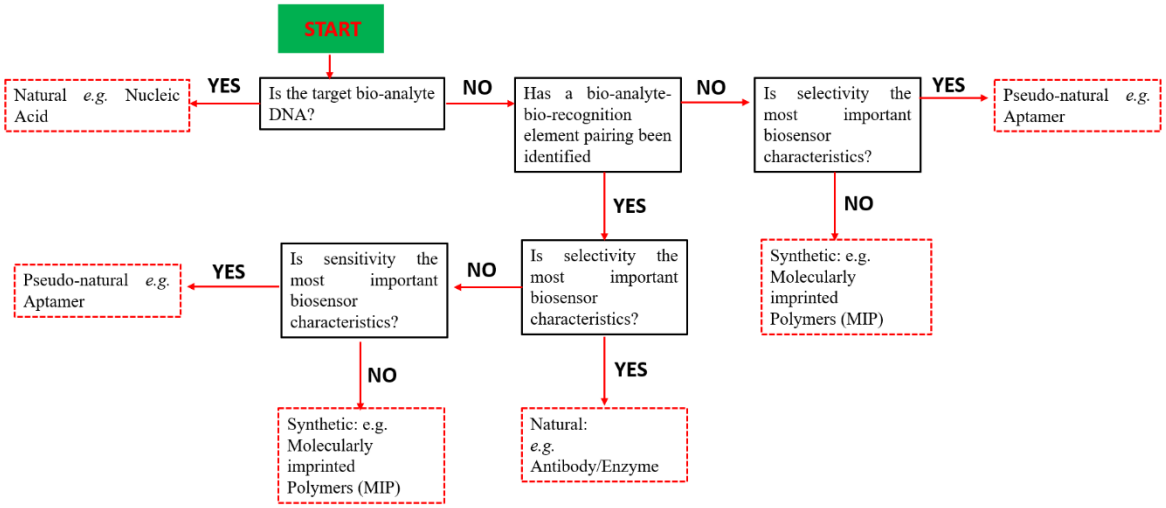

Figure 14: Map of selecting biorecognition element for developing biosensor (reprinted with permission) [63]

Antibodies

Antibodies are different types of proteins that are largely used as biorecognition elements. They are more specific and selective towards analytes that are being used for developing various types of biosensors. The antibodies may be categorized into two kinds monoclonal and polyclonal. Monoclonal antibodies can recognize a single epitope of a target molecule, whereas polyclonal antibodies can recognize different epitopes of the same target. The antibodies are more susceptible to the temperature and required to store at low temperatures. Otherwise, it gets denatured; as a result, it can lose the binding ability.

Deoxyribonucleic acid (DNA):

In modern biosensors, the DNA is being used as a probe in the biorecognition platform. It is a short DNA sequence, immobilized over the transducer surface, that can interact with the specific target DNA sequence, or it can bind to a complementary DNA sequence. In the case of metal ions detection, the phosphate backbone of DNA molecules have a negative charge and easily binds with the positively charged metal ions [64].

Cells:

The living cells are being used as biorecognition (bio-receptor), which is an economical alternative to enzymes and antibodies. Designing a biosensor recognition platform becomes easier by using cells in comparison to the other bio-receptors. The different types of cells with distinct functional strategies are in use for developing biosensors, as shown in Figure 15. The cells are immobilized over the transducer in such a way; the viability of the cell does not affect, such as direct immobilization on the electrode, hydrogel entrapment, and biofilm formation etc. [65].

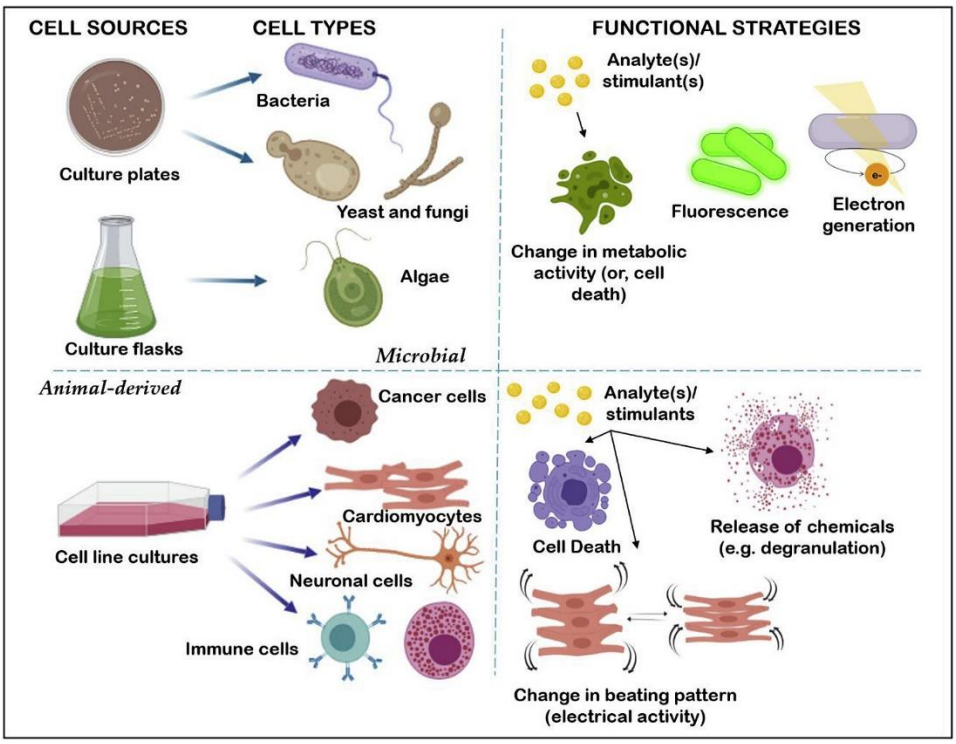

Figure 15: Schematic showing different cell types obtained (left), and functional strategies of cell-based biosensors (right) (reprinted with permission) [65].

Enzymes:

The enzymes are biocatalytic biomolecules, which can enhance the biological reaction and also liberate the measurable product. During the biocatalytic reaction, the intermediate product to

the final product formation process easily monitored through the transducer, electrochemical transducers (amperemeter, voltammeter, etc.).

6 Challenges

There are still many challenges to develop special type of biosensors, such as particular virus-detection biosensors. The virus is a tiny biological entity capable of destroying human body cells. Its detection at an early stage of infection has not been very much possible as yet, primarily because the affected person does not realize the asymptotic symptoms. However, early diagnosis of virus infection/biochemical disbalance/bacterial infection, etc. can be detected through *in-vivo* biosensor, and it will be a milestone in the clinical sector. However, the materials currently being utilised in making biosensors are foreign materials for the human body and their acceptance into the human body is still a challenge. Therefore, the utilisation of materials in biosensing needs to be compatible with the human body or whole biosensor must be biological. In addition, many biosensors are complicated and required to be stored at low temperatures. Therefore, the new technology needs to develop sensors that can be stored and operated at room temperature.

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Questions and answers to check your reading:

Objective types:

1) Surface plasmon resonance belongs to which type of biosensor?

a) Transducer (b) Recognition system

b) Signal Amplification (d) none

Ans: Transducer

2) What is an aptamer in a biosensor?

(a) Transducer (b) Recognition system

(b) Signal Amplification (d) none

Ans: Recognition system

(3) Please fill in the blank. An analyte can be detected through _____working principles.

(a) one (b) two

(c) three (d) many

Ans: Many

(4) A nanoscale material is defined to have its _____ ?

(a) one dimension <100 nm (b) two dimensions <100 nm

(b) three dimensions <100 nm (d) All dimensions <100 nm

Ans: one dimension <100 nm

Subjective types:

(5) What is a biosensor?

A biosensor is an analytical device, which is successfully used for the qualitative and quantitative estimation of several biologically important substances.

(6) What are the various types of biosensors?

Biosensors can be classified by their transduction mechanisms such as Electrochemical, Piezoelectrical, Thermometric, Optical, and Physical; one can also classify them by the mode of biorecognition, e.g., enzyme-based, antibody-based, nucleic acid-based, aptamer-based and whole cell-based. Also, the transducer-based classification can be further sub-divided. For example, electrochemical biosensors can be amperometric, potentiometric, conductometric, etc. In addition, biosensors can be defined by both the recognition mode and transducer, e.g., enzyme-based electrochemical biosensors or, more specifically, enzyme-based potentiometric biosensors.

(7) Describe the main components of biosensors and explain their function.

The biosensor consists of a biorecognition element, transducer, amplification, and display. The biorecognition system detects the analytes; the transducer converts action of recognition system into the signal; the amplification enhances the signal strength to read easily; display shows the result.

(8) Define precision and selectivity for a sensor.

Precision: The ability to have high reproducibility upon the repetition of measurements of the same sample. The sensor has good reproducibility of its response (low standard deviation).

Selectivity: The sensor performance towards targeted analytes. A sample has many chemical/biomolecules, but sensor detects particular analytes/biomolecules, for what it is tuned.

(9) What are nanomechanical biosensors (cantilever biosensors)? How have these been used for biosensor development? How can the biorecognition event be transduced?

These are microfabricated finger-like transducers that can bend/oscillate (depending on the material) as a result of a physical/electrical stimulation. The bending/oscillation of these devices is proportional to their mass. Cantilever based biosensors have been used for hybridization assay, for the screening of the effect of drugs on the growth of bacteria, for affinity (antibodies) based assay by immobilizing on the surface of the cantilever DNA probes, drugs or antibodies. Transduction can be optical or piezoelectrical. In optical, the reflection of a laser beam is used to evaluate the bending of the cantilever. In the case of piezoelectrical changes in the oscillation frequency of cantilever (made with piezoelectric material) are used to detect changes in the mass onto them.

(10) What is an enzyme? What is the function of an enzyme, when used as a biorecognition element?

The enzymes are protein and act as a biological catalyst. In the biorecognition system, it is attached to the transducer and catalyzes analyte conversion faster and efficiently. Correlate the analyte to a measurable event (loss in a substrate or generation of a product).

(11) What are the two main problems associated with the use of the Oxygen electrode (Clark electrode) as a transducer in the glucose electrochemical biosensor?

This is dependent on the starting concentration of oxygen in the starting solution. Use high potential: sensitive to interference from other molecules. The electrochemical reaction consumes oxygen (this can produce false positive).

(12) What are the main advantages and disadvantages of the transducer's functionalization via the adsorption process instead of covalent bonding?

Physical adsorption is a simple and easy process. However, pH variation and other analytes in the sample create interference in the signal, which appears as noise and reduces the probability of accurate detection.

Some further unsolved problems:

1) Explain a label-based and a label-free biosensor.
2) What is meant by spectral interrogation schemes in LSP sensors?
3) Describe Glucose biosensors. First, second and third generation of amperometric sensors.
4) Achieving selectivity and sensitivity in SPR biosensors. Immobilization strategies in SPR. Improving signal for small analytes.
5) Describe applications of carbon nanotubes and nanowires for biosensing.

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