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Biosensors Based Diagnosis Treatment of Infectious Diseases

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ABSTRACT:

The development of biosensors has been the focus of scientist's attention for current decades. Biosensors can centrally serve as low-cost and highly able devices for this aim in addition to being applied in other day to day applications. In the past five decades, biosensors have consolidated their impact in several fields, including clinical applications, due to advantages such as high selectivity and sensitivity, potential for miniaturization, portability, low cost and rapid response. Recent advances in biomarkers discovery and biotechnology are now clarifying the nuances of many biological processes in health and disease, highlighting new targets for diagnosis of therapeutics. This is especially important in the case of infectious disease, since the number of predicted deaths remains high with threats of epidemics and pandemics, emerging and re-emerging disease and pathogen resistance to antibiotics. Therefore, the availability of robust diagnosis method is crucial. Rapid diagnosis of infectious diseases and timely initiation of appropriate treatment are critical determinants that promote optimal clinical outcomes and general public health. Recent advances in biosensor technologies have potential to deliver point-of-care diagnostics that match or surpass conventional standards in regards to time, accuracy and cost.

I. INTRODUCTION:

The term biosensor refers to powerful and innovative analytical device involving biological sensing element with wide range of applications, such as drug discovery, diagnosis, biomedicine, food safety and processing, environmental monitoring, defense, and security. The history of biosensors began in the year 1962 with the establishment of enzyme electrodes by the scientist Leland C. Clark. Since then, experiment communities from different areas such as VLSI, Physics, Chemistry, and Material Science have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, Agriculture, biotechnology, as well as the military and bioterrorism detection and prevention.[1]

Biosensor is a device that consists of two main parts: A bioreceptor and a transducer. Bioreceptor is a biological component (tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.) that determines the target analyte. Difference fraction is transducer, a physicochemical detector component that changes the detection event into a measurable signal. [2,3] The function of a biosensor depends on the biochemical specificity of the biologically active material.

The choice of the biological material will depend on a number of factors via the specificity, storage, operational and environmental stability. [2,4] Biosensors can have a variety of biomedical, industry, and also military applications. The major application so far is in blood glucose sensing because of its abundant market potential. [1,5] Biomolecules such as enzymes, antibodies, receptors, organelles and microorganisms as well as animal and plant cells or tissues have been used as biological sensing factors. [2] Microorganisms have been composed with a variety of transducers such as amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescence and fluorescence to construct biosensor devices. [3, 6, 7] Infectious diseases are caused by pathogenic microorganisms, including bacteria, viruses, fungi and parasites. Some examples among those enumerated by the World Health Organization (WHO) include tuberculosis, meningococcal meningitis, malaria, AIDS, pneumonia, poliomyelitis, hepatitis, Ebola virus disease, dengue and Chikungunya, American trypanosomiasis (Chagas disease), leprosy, toxoplasmosis and leishmaniasis. [8] Biosensor fabrication is a multidisciplinary field as it involves chemistry, biology, physics, electronics, material science, engineering, etc. [9] Recently, various new elements, such as molecularly imprinted polymers, aptamers, nanomaterials, etc., have been developed in the field of biosensors. [10-14] The growth of nanotechnology has provided strong benefits to the

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biosensor field as the nanomaterials have a large surface-to-volume ratio that helps in achieving higher sensitivity and efficiency. [15]

II. WORKING PRINCIPLE OF BIOSENSOR:

Biosensors are operated based on the principle of signal transduction. These components include

- Bio-recognition element
- A bio transducer and
- An electronic system composed of a display, processor and amplifier.

The bio-recognition element, essentially a bioreceptor, is allowed to interact with a specific analyte. The transducer measures this interaction and outputs a signal. The intensity of the signal output is proportional to the concentration of the analyte. The signal is then amplified and processed by the electronic system. The biological element is immobilized on a surface. In the presence of a specific target analyte that binds to the immobilized biomolecule, the transducer converts the recognition event into a measurable signal, which can be then processed.

A biosensor is an analytical device with three major modules:

(i) A sensing bioreceptor;
(ii) A transducer; and
(iii) A detector with a digital output.

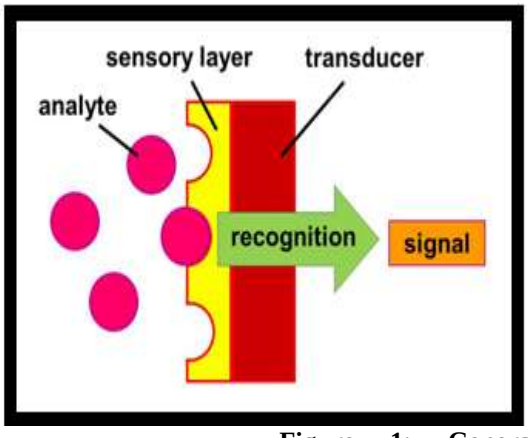

Figure 1: General Operation Principle of Biosensor

1. Principally, target analyte interacts with bioreceptor and the detecting component part specifically recognizes the analyte through a reaction, specific adsorption, or another process such as physical/chemical interaction.
2. Then, the transducer translates molecular changes to a quantifiable signal measured by the digital detector module. [17] The subject of the transduction principles can be separated as electrochemical, piezoelectric, optical, thermal, micromechanical, and magnetic.
3. Biosensors provide multiple capabilities, including exceptional performance, user-friendly operation, rapid response, high sensitivity and specificity, portability, relatively compact size, and real-time analysis. [18]

III. DIAGNOSIS STRATEGIES FOR INFECTIOUS DISAESES:

- The importance of simple, accurate, affordable and rapid diagnosis tests is justified by its impact in the clinical management, since early diagnosis affects therapy effectiveness and avoids long-term complications and pathogen transmission. [22]
- While the standard diagnosis techniques for infectious diseases include well-established methodologies, such as Enzyme- Linked Immunosorbent Assay (ELISA), nucleic acid-based assays, microscopy and microorganism culture. [23] The diagnosis tests for infectious diseases should present a set of desirable characteristics, such as sensitivity, specificity and reproducibility. [22]
- Historically, the identification of infectious agents was initially performed by culture and microscopy. Then, antigen detection and Polymerase Chain Reaction (PCR) became widely used. Currently, pathogen identification and host response (e.g., antibodies detection) are both used to diagnosis pathological states. [25]
- PCR, DNA microarrays and Matrix-Assisted Laser Desorption/Ionization Time- Of-Flight Mass Spectrometry (MALDI-TOF MS) are widely used nucleic acids technologies.

1) **Polymerase Chain Reaction (PCR):** PCR employs oligonucleotide primers that are complementary to pathogen genetic material to amplify it, if present in the sample. The reaction product is detected during or after the process. The use of PCR in clinical settings can be broadly into three categories.

- To amplify human genes to check for mutation,
- To amplify a microbial gene in a sample.

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- To amplify human gene from a limited sample for creating a complete DNA profile of an individual.

2) **DNA Microarray:** DNA Microarray technology allows multiple target detection through hybridization with the probes immobilized on a surface. [26] Microarray was first used as a diagnostic tool in 1993 to identify Hantavirus. The DNA microarray technique includes several steps, such as manual or automatic extraction of nucleic acid from clinical sample, their amplification by PCR. Then hybridization of labeled nucleic acid on DNA microarray which is later scanned measure the specific probe /DNA interaction of the sample. These interactions are measured by fluorescence as numerical data.

3) **Matrix-Assisted Laser Desorption/Ionization Time- Of-Flight Mass Spectrometry (MALDI-TOF MS):** Recently this technique is being adopted in the comparison of protein fingerprint obtained in a sample with the available databases, for the identification of bacteria, fungi. [27] and viral pathogens. [28] There is also the potential of Next Generation Sequencing (NGS) methodologies to revolutionize infectious diseases diagnosis, since it does not rely on pre-established sequence targets, allowing the identification of emerging or mutating pathogens. [29,30] Other strategies are also being developed for clinical diagnosis, including microfluidic and nano technological devices. [31,32]

IV. TYPES OF BIOSENSORS:

4.1 RESONANT BIOSENSOR:

In this type of biosensor, an acoustic wave transducer is coupled with an antibody (bio-factor). The analyte molecule (or antigen) gets added to the membrane, the mass of the membrane diversities. Resulting diversification in the mass subsequently diversities the resonant frequency of the transducer. This frequency change is then measured. [33]

4.2 OPTICAL BIOSENSOR:

Principle: Optical biosensors are the devices that utilize the principle of optical measurements (absorbance, fluorescence, chemiluminescence etc.). They employ the use of fiber optics and optoelectronic transducers.The output transduced signal that is measured is light for this type of biosensor. Optical transducers are particularly attractive for application to direct (label-free) detection of bacteria. These sensors are accomplished to discover minute conversions in the refractive index or thickness which happens when cells fasten to receptors immobilized on the transducer surface. They correlate changes in concentration, mass or number of molecules to direct changes in characteristics of light. Several optical techniques have been reported for detection of bacterial pathogens including: monomode dielectric waveguides, surface plasmon resonance (SPR), ellipsometry, the resonant mirror and the interferometer etc. [34-36]

Working: It measures light absorbed or emitted during a biochemical reaction. In these biosensors, the optical fibers detect the analytes on the basis of light scattering fluorescence or absorption. [38] These biosensors measure both the affinity and catalytic reactions. The sensing element causes a change in absorbance or fluorescence which changes the refractive index between two media having different densities. They are superior to nonelectrical biosensors as they allow multiple analyte detection using various monitoring wavelengths. The adaptability of using optic probes is because of their ability to transmit signals that account on changes in polarity, time, wavelength, wave propagation, distribution of the spectrum, or intensity of the light. They are solely based on the principle of light scattering absorption, internal reflection, fluorescence, surface plasmon resonance, or luminescence spectroscopy. [39]

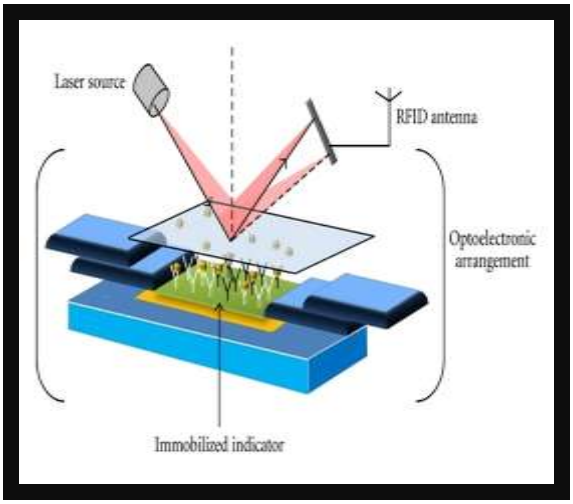

Figure 4: Optical Biosensor

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➢ **Advantages**:
- No reference electrode is needed
- No electric interference
- Multiple analyte response with different wavelengths
- Potential for a higher information content than electrical transducer

➢ **Disadvantages:**
- Limited dynamic range
- Response time may be slow
- Problems with long term stability
- Difficult to miniaturized
- Background ambient light interference

4.2.1 SURFACE PLASMON RESONANCE (SPR) BIOSENSOR:

Surface Plasmon resonance (SPR) has become an important optical biosensing technology due to its real-time, label-free, and noninvasive nature. These techniques allow for rapid and ultra-sensitive detection of biological analytes, with applications in medical diagnostics, environmental monitoring, and agriculture.

Principle: Surface Plasmon resonance occurs when a photon of incident light hits a metal surface. At a certain angle of incident, a portion of light energy couples with the electrons in the metal surface layer, which then move due to excitation.

Working: This is an evanescent area based optical sensors applying thin gold film for sensing approaches. The interaction between analyte flowing over immobilized interact ant on gold surface is probed through the detection of reflection minima on photo-detector array sensors. SPR has successfully been applied to the detection of pathogen bacteria by means of immunoreactions. [11-12]

In this study, we developed an SPR biosensor to provide a highly sensitive and specific approach to early-stage detection of viral and malignant diseases, such as cancer tumors, for which biomarker detection is very important. A cancer cell line (HeLa cells) with biomarker Rodamine 6G was experimentally analyzed in vitro with our constructed SPR biosensor. It was observed that the biosensor can offer a potentially powerful solution for tumor screening with dominant angular shift. The angular shift for both regents is dominant with a time curve at a wavelength of 632.8 nm of a He-Ne laser. We have successfully captured and detected a biomarker in vitro for cancer diagnostics using the developed instrument.

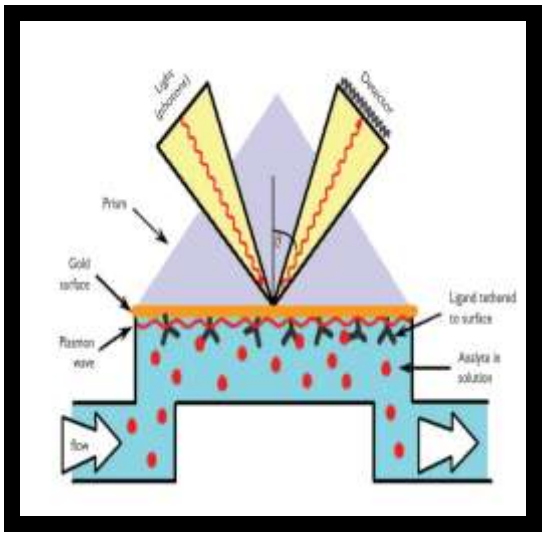

Figure 5: Surface Plasma Resonance

➢ **Advantages:**
- High sensitivity to small changes of blood glucose concentration
- No need for statistical calibration models due to its conventional electrical model number.

➢ **Disadvantages:**
- Sensitive to motion
- Long calibration prices
- Sensitive to sweat and temperature
- Bulky in size.

4.2.2 PIEOZOELECTRIC BIOSENSOR:

Principle: It involves measurement of mass change during bimolecular interaction. They are also considered as mass-based biosensors and are based on the principle of sound vibrations and also called as acoustic biosensors.[43]

Working: The sensing molecules and analyte and translate them to electrical signals. [41] They produce an electrical signal when mechanical force is applied. [44] Sensing modules are directly attached to a piezoelectric surface which is piezoelectric in nature. Hence, mechanical vibrations arise from the interaction between the sensing molecules and analyte and translate them to electrical signals. [41]

Piezoelectric (PZ) biosensor offers a real-time output, simplicity of use and cost effectiveness. The chief idea is based on coating the surface of the PZ sensor with a selectively binding material, for instance, antibodies to bacteria, and then locating it in a solution containing bacteria. The bacteria will bind to the antibodies and the mass of the crystal will increase

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while the resonance frequency of oscillation will decrease proportionally. [9-11]

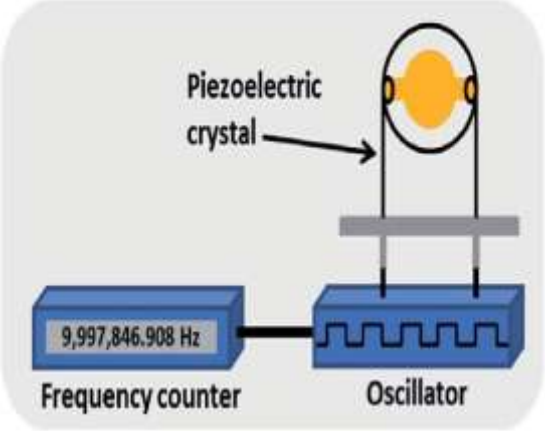

Figure 6: Piezoelectric Biosensors

➢ **Advantages:**
- Good frequency response
- Small in size, easy to handle
- Available in desired shape
- Rugged construction

➢ **Disadvantages:**
- High temperature sensitivity
- Poor mechanical characteristics
- Low output current

4.3 THERMAL BIOSENSOR

Principle: This type of biosensors works on the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place.

Working: This type of biosensor is exploiting one of the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. They are combined by combining immobilized enzyme molecules with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration. Common applications of this type of biosensor include the detection of pesticides and pathogenic bacteria. [11]

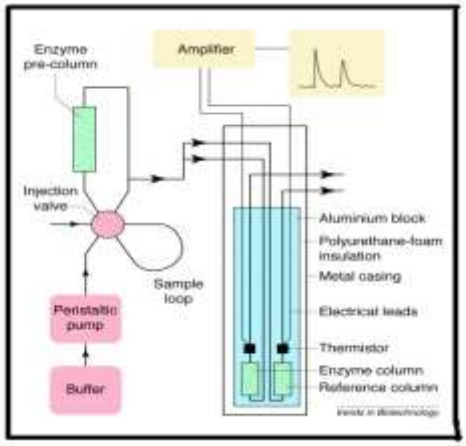

Figure 7: Thermal Biosensor

➢ **Advantages**
- Long term stability
- Less cost
- Accuracy

➢ **Disadvantages**
- Complexity
- Very week sensitivity
- Poor reputation

4.4 ELECTROCHEMICAL BIOSENSOR:

Principle: Electrochemical biosensors provide an attractive means to analyte the content of biological sample due to direct conversion of biological event to an electronic signal.

Working: An electrochemical biosensor should sense and convert the signal effectively and the same signal must trigger the therapeutic release. However, an additional therapeutic reservoir in the biosensor may make the system complicated. In these systems, an electrical signal should open or close the drug reservoir. An additional mechanism between the electrical signal and reservoir increases the complexity and challenges the maintenance of the system. Still, since electrochemical-based sensors are widely used and established, combining the existing ones with drug delivery systems is promising for the treatment of various diseases.

The electrochemical biosensor is a special type of biosensor where a biological entity is detected by converting the information into an electrical signal, i.e., voltage, current, impedance, etc. [45,46] The first electrochemical biosensor was developed by Clark to monitor the glucose level in human blood serum. The electrochemical biosensors have been developed to detect various biological entities such as enzymes, proteins, viruses, antibodies, etc. [47]

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The electrode is the most vital component of an electrochemical biosensor as it controls the flow of electrons and bioagents. Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration

- **Advantages:**
- Wide Linear range of sensor response
- High stability
- Inexpensive
- Fast response

- **Disadvantages:**
- Requirement of sample preparation
- Tedious measurement conditions
- Temperature parameter influence the performance of biosensors sensitivity

4.4.1 CONDUCTIM ETRIC BIOSENSOR:

Principle: Conductimetric biosensors are based on measurement of electrical conductivity in sample solution between two electrodes, as a consequence of the biochemical reaction.

Working: Conductimetric biosensors can measure the change of the electrical conductivity of cell solution. Most reactions involve a change in the composition of solution. Thus conductimetric biosensors can detect any reactive change occuring in a solution.The measured parameter is the electrical: conductance/resistance of the solution. When electrochemical reactions create ions or electrons, the overall conductivity or resistivity of the solution has been altering. This convert is ended and calibrated to an appropriate degree. Conductance measurements have relatively low sensitivity [9].

4.4.2 Amperometric Biosensors:

Principle: The principle of Amperometric sensor is based on measuring current generated by enzymatic or bioaffinity reaction at the electrode surface, at a constant working potential with respect to the reference electrode.

Working: Amperometric biosensors transducer the activity of the biological species on the surface into a signal in the form of current which quantifies the analyte of interest in the complex sample matrix. Amperometric biosensors have three types of electrodes: (1) a working electrode made of gold, carbon or platinum; (2) a reference electrode made of silver (Ag) or silver chloride (AgCl) having a fixed potential that controls the potential of the working electrode; and (3) a counter or auxiliary electrode used to measure current flow. The flow of electrons (to or from) the electrode depends on the redox reaction (oxidation or reduction) of the molecules. Due to the simplicity of the transducer, low-cost portable devices can be fabricated, which are essential for applications such as medical diagnostics and environmental monitoring. In amperometric biosensors, the output current of the sensor is analyzed and used for the sensing process. The sensitivity of the amperometric biosensor is determined by comparing the current obtained for the different analyte concentrations. Such biosensors utilized only two electrodes, one for applying the voltage and the other for measuring the current flowing through the device. The amperometric biosensors do not utilize optical or electrochemical devices, but rather depend only on the current measurements. The development of biosensors started from glucose sensing and most of the glucose-sensing involves the catalytic reaction of the enzyme with the glucose oxidase. [50,51] The sensitivity of such glucose sensors is affected by the variation in temperature and pH. Two Electrochemical Techniques are used in sensing process in Amperometric glucose sensors.

1. Cyclic Voltammetry (CV)
2. Electrochemical impedance spectroscopy (EIS)

1. Cyclic Voltammetry: Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment, the working electrode potential is changed linearly versus time. Cyclic voltammetry experiment ends when it reaches a set potential value. When cyclic voltammetry reaches the set potential, potential ramp of the working electrode is inverted back. This inversion can happen multiple times during a single experiment until a set cycle number is obtained. The plot of the current at the working electrode vs. the applied voltage gives the cyclic voltammogram of the reaction. Cyclic voltammeter is a general way to study the electrochemical properties of an analyte in a solution.

2. Electrochemical Impedance spectroscopy: Electrochemical Impedance Spectroscopy (EIS) is aelectrochemical technique to measure the impedance of a system in dependence of the AC potentials frequency. Electrochemical impedance is normally measured using a small excitation signal. This is done so that the cell's response is pseudo-linear. In a linear (or pseudo-linear) system, the current response to a sinusoidal potential will be a sinusoid at the same frequency but shifted in phase.

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4.4.3. POTENTIOMETRIC BIOSENSOR:

Principle: Potentiometric biosensors use the ion-selective electrodes to convert the biological reaction to electronic response. Most commonly used electrodes are pH meter glass electrodes (for cations glass pH electrodes coated with a gas selective membrane for CO2, NH or H 2 S.) or solid-state electrodes.

Working: Potentiometric biosensors use the ion-selective electrodes to convert the biological reaction to electronic response. Most commonly used electrodes are pH meter glass electrodes (for cations glass pH Electrodes coated with a gas selective membrane for CO2, NH or H_2S.) or solid-state electrodes Biosensors Detects and measure the ions or electrons generated in many reactions, very weak buffer solutions are used in this case. Gas sensing electrodes detect and measure the amount of gas produced.

The two main advantages of using potentiometric biosensors are: the signal produced is in the form of potential and the biochemical component used is the part of the receptor. [52] To monitor the perspiration in humans, potentiometric biosensors in the form of tattoo has been developed. [53]. The potentiometric biosensor fabricated by coating polypyrrole on a gold electrode and using mat horseradish speroxidase as a biochemical agent has been developed for detecting tumor, hepatitis B, digoxin, and troponin. [54]

Example:

A potentiometric biosensor with a gold electrode and extended FET transistor was used to detect interleukin with LOD 1 pg mL□1. [55] The acetylcholinesterase was used as an antibody that produced thiol which was adsorbed on the electrode. [56]

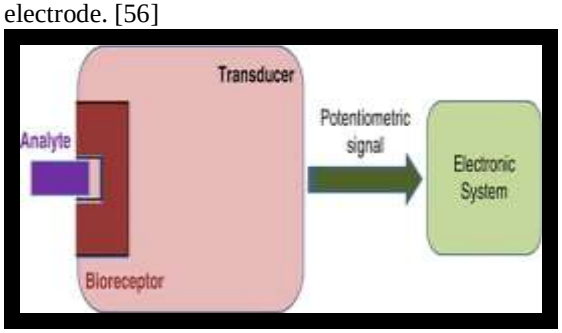

Figure 13: Potentiometric Biosensor

4.5. BIOLUMINESCENCE SENSOR:

Principle: Bioluminescent sensing involves detecting change in luminescence emitted by the bacteria in response to a target analyte in a dose dependent manner. The luciferase coded by the lux gene catalyses the oxidation of Flavin Mononucleotide (FMNH2) and a long chain fatty aldehyde to emit of blue-green light.

The bacterial luminescence lux gene has been broadly exercised as a reporter either in an inducible or constitutive mechanism. In the inducible manner, the reporter lux gene is fused to a promoter regulated by the concentration of a combine of interest. As an effect, the concentration of the compound can be quantitatively assayed by determining the bioluminescence intensity. Bioluminescence systems have been used for detection of a wide range of microorganisms. [2,11] Recent advances in bioanalytical sensors have led to the utilization of the ability of certain enzymes to emit photons as a byproduct of their reactions. This phenomenon is known as bioluminescence. The potential applications of bioluminescence for bacterial detection were begun by the development of luciferase reporter phages.

- **Advantages:**
- Low phototoxicity
- Low background
- High sensitivity
- Broad linear range
- **Disadvantages:**
- Low brightness
- Requirement of substrate
- Longer imaging time

4.6 NUCLEIC ACID-BASED BIOSENSOR:

Principle: The fundamental principle behind NABs depend on sequence complementarity as per Chargaff's rules of base pairing (for DNA, A=T, G=C) except in the case of aptamer Based detection s more akin to antigen –antibody or receptor-ligand interactions.

Working: A nucleic acid biosensor is an analytical instrument that integrates an oligonucleotide with a signal transducer. The nucleic acid probe is immobilized on the transducer and behaves as the bio-recognition molecule to detect DNA/RNA fragments. [11] The major component of DNA sensors is nucleic acids, mostly DNA. These sensing materials are the fragments commonly called DNA primers or DNA probes which reflect specificity of the whole DNA structure. These probes or primers are synthesized by amplification of DNA by PCR (polymerase chain reaction). [57] They are modified to increase the stability or to facilitate introduction of probes into biosensors. This type of biosensors helps in revealing non-macromolecular and protein compounds which

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interact with specific DNA fragments. [58] On the basis of the type of biorecognition unit used, they are classified as nucleic acid-based, enzymatic, whole cell-based, antibody-based, or aptamer-based biosensors. [59]

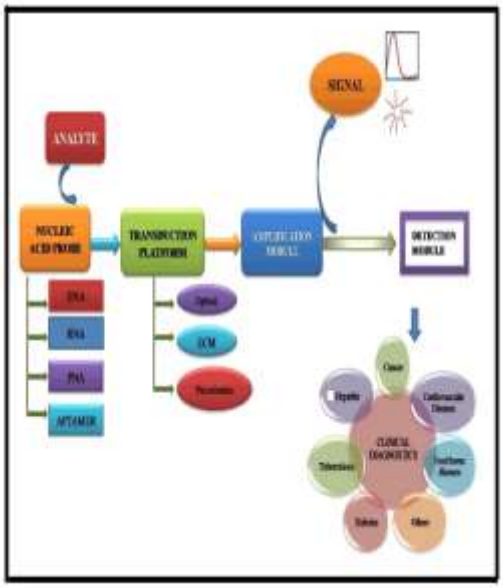

Figure 14: Nucleic Acid-based Biosensors

4.7 NANOBIOSENSOR:

- A nano biosensor is a means of detecting biological agents such as antibodies, nucleic acids, pathogens, and metabolites. The working principle consists of binding bioanalytes of interest onto bioreceptors, which in turn modulate the physiochemical signal associated with the binding.
- Nanotechnology is having a profound impact on the development of a new class of biosensors known as nano biosensors. Nanobiosensors commonly comprise a biological recognition molecule immobilized onto the surface of a signal transducer. The reaction between the biorecognition molecule and the analyte is a heterogeneous reaction and therefore the design of the biosensing interface is important in determining the performance of the nano biosensor.
- Nanobiosensors are being widely used for molecular detection of biomarkers associated with diagnosis of disease. The application of new nanomaterials in biosensing has influenced bio-sensing research. The use of high surface area nanomaterials has been important in producing nano biosensors with greater sensitivity and shorter response times. They have been used in disease diagnostics, primarily through the detection of molecular biomarkers, such as proteins and nucleic acids mediated by use of nano biosensors. Development of nano biosensor is one of the most current advancements in the area of Nanotechnology.
- The silver and certain other noble metal nanoparticles have many important applications in the field of biolabeling, drug delivery system, filters and also antimicrobial drugs, sensors.

➢ **Advantages:**

- Low cost
- Sensitivity and selectivity
- High efficiency
- Portability

➢ **Disadvantages:**

- Lack of statistical method to analyze data.

4.8 MICROBIAL BIOSENSOR:

- A microbial biosensor is an analytical device which integrates microorganism (s) with a physical transducer to generate a measurable signal proportional to the concentration of analytes. In recent years, a large number of microbial biosensors have been developed for environmental, food, and biomedical applications.
- Microbes have a number of advantages as biological sensing materials in the fabrication of biosensors. They are present ubiquitously and are able to metabolize a wide range of chemical compounds. Purified enzymes have been most commonly used in the construction of biosensors due to their high specific activities as well as high analytical specificity.
- Over 90% of the enzymes known to date are intracellular. In this respect, the utilization of whole cells as a source of intracellular enzymes has been shown to be a better alternative to purified enzymes in various industrial processes. Whole cells have been used either in a viable or non-viable class. Viable cells are advancing considerable import in the fabrication of biosensors. Viable microbes metabolize different organic compounds either anaerobically or aerobically resulting in various end products like ammonia, carbon dioxide, acids etc, which can be monitored using a variety of transducers. Viable cells are mainly used when the overall substrate assimilation capacity of microorganisms is acquired as an index of respiratory metabolic action, as for estimation of biological oxygen demand (BOD) or application of

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other growth or metabolically related nutrients like vitamins, sugars, organic acids and nitrogenous compounds.

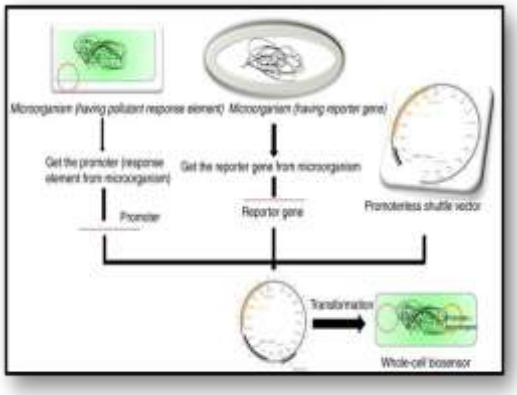

Figure 16: Microbial Biosensor

- Another mechanism used for the viable microbial biosensor involves the inhibition of microbial respiration by the analyte of interest, like environmental pollutants. Environmental applications of biosensors involve the detection of damaging bacteria or pesticides in air, water, or food. A microbial biosensor consisting of an oxygen microelectrode with microbial cells immobilized in polyvinyl alcohol has been fabricated for the measurement of bioavailable organic carbon in toxic sediments. Microbial biosensors have been developed for assaying BOD, a value related to total content of organic materials in wastewater. BOD sensors take advantage of the high reaction rates of microorganisms interfaced to electrodes to measure the oxygen depletion rates. [2,62,63]

➢ **Advantages:**

- Assess living microbes
- Able to recognize viable cells in sample
- High sensitivity with appropriate media

➢ **Disadvantages:**

- Risk of contamination
- Time and resources intensive
- High skill level is necessary for optimal results

V. BIOSENSOR AND PATHOGEN DETECTION

1. Bacteria, viruses and other microorganisms are found widely in nature and environment. Microbial diseases constitute the major cause of deaths in developing countries. [11]
2. Pathogen detection is of the utmost importance primarily for health and safety reasons.
3. Polymerase chain reaction (PCR), culture and colony counting methods as well as immunology-based procedures are the foremost frequent tools applied for pathogen identification.
4. They include DNA analysis, numbering of bacteria as well as antigen–antibody interactions, respectively. In spite of disadvantages such as the time required for the analysis or the complexity of their use, they still represent a field where progress is possible. Biosensors have recently been defined as analytical devices incorporating a biological material intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, magnetic or micromechanical. [9,64]
5. There are three central classes of biological identification elements which are applied in biosensor applications. These are enzymes, antibodies besides, nucleic acids. In the discovery of pathogenic bacteria, however, enzymes tend to function as labels rather than actual bacterial recognition elements. Enzymes can be used to label either antibodies or DNA probes much in the same fashion as in an ELISA assay.
6. In the case of amperometric biosensors enzymatic labels are critical. More advanced techniques may operate without labeling the recognition element, such as the case of surface plasmon resonance (SPR), piezoelectric biosensors.
7. The application of antibodies in biosensors is immediately more develop than that of DNA probes, the following sections deal mainly with antibody-based biosensors.

VI. 6. MACHINE LEARNING FOR BIOSENSOR:

- Firstly, for specimen or complicated matrices, large sensing data can be efficiently processed by machine learning.
- Secondly, the gain of ML in biosensors comprises the probability of getting sensible analytical results from disorderly and low-resolution sensing data which could closely overlie on one another.
- Furthermore, appropriate use of ML methods may find unseen relationships between signals of sensing and parameters of specimen via the

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visualization of data and interrelations between bioagents and signals.

- Particularly, raw sensing data can be analyzed by using ML from a biosensor in different ways: Categorization, anomaly detection, noise reduction, and pattern recognition. Based on the target analyte, the algorithms aids to categorize the sensing signals in different manners.
- It is also observed that the operating conditions inevitably affect the performance of a biosensor. On-site usage of biosensor generally interferes with contamination. In that case, ML plays a very important role in checking the quality of the signal. Because of interferences and bio fouling in real samples, the variations in sensor performance can be improved by using ML.
- It is also observed that sensing signals always contain noise. Hence, it is very important to train to develop the model of ML which extracts the good quality signal from the signal containing noise. Finally, the interpretation of sensing data occurs effectively and easily by developing the patterns and latent objects using ML algorithms. [65] For on-site diagnosis or detection, the ML can be significantly important to aid biosensors that can read out rapidly, accurately, automatically, and directly. Instead of predicting the model for electrochemical biosensor, the optical imaging method assisted by a convolutional neural network (CNN) was also developed to calculate the diagnostic consequences. [66] On the other hand,
- The pathology workforce takes thirty seconds to interpret the image. Additionally, for designing the desirable biosensors nowadays, ML has been preferred. Metamaterials with negative permittivity and permeability are used to enhance the ability to detect the signals of biosensors based on the surface plasmon resonance (SPR). [67]
- To ensure that the resonance is beneficial for SPR biosensors, the process of preparation of metamaterials with different reflectance characteristics is crucial. For predicting the reflectance characteristics of the metamaterial, SPR biosensors like multilayer perceptron (MLP) and Autoencoder (AE) are used. Afterwards, k-means clustering of the metamaterials was introduced for the dimensional reduction with the help of AE and t-Stochastic Neighbor embedding (t-SNE). Hence, without experimenting extensively, the designing of the optimized sensing devices can be boosted up with the clustering of the metamaterials.

VII. VARIOUS ALGORITHMS IN ML:

ML can be defined as a computer program that can gather information from raw data by extracting features. To deal with real-world difficulties, the newly gathered information can become beneficial to make decisions. [69] In the field of biosensors, especially electrochemical, ML is known as a method or tool that can be used for analyzing and processing data for instance concentration of the analytes, extracting features, and for the prediction of the species. It can be classified into unsupervised learning and supervised learning. [70] ML is very important to predict the sensing model for more than one analyte at a particular time. Until today, various algorithms used in ML were known. Those algorithms are preferred, which gives maximum accuracy of the results and give information related to hidden data. When ML algorithms are trained with their target outputs with a group of input data, then it is referred as supervised learning. During the training process, certain predictions can be made by the algorithms on the input data set and the predicted value can be improved by using the given real value, unless the algorithms get the acceptable accuracy. Particularly, in spectrometric biosensors, great progress has been achieved by these to perform regression and categorization. However, in the case of unsupervised learning, labelled training data sets along with their given outputs are not available. One of the most common unsupervised learning algorithms is k-Means clustering. [71]

VIII. POINT OF CARE BIOSENSOR FOR COVID-19:

- Currently, many viruses are being considered to have the capacity of causing future pandemics. Different factors such as fast dissemination, a high transmission rate of new variants, difficulties to develop efficient and sensible diagnostic techniques, as well as the lack of specific vaccines and safe drugs for treatment, make them one of the major threats for mankind. [72,73]
- The most recent case is the COVID-19 announced as a pandemic on March 13th, which is an infectious disease with rapid human-to-human transmission caused by

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SARS-CoV-2. This pathogen belongs to the positive-strand RNA viruses. [74,75] Like any other viral outbreak, an early diagnosis is fundamental for preventing an uncontrollable spread of the disease. However, this pandemic has the particularity that more than 30% of the confirmed cases are asymptomatic, thus making it harder to control .[75-77]

- RT-PCR is the most used suitable and reliable method for detecting SARS-CoV-2 infections until now. Nevertheless, the technique is time-consuming, labor-intensive, and unavailable in remote settings. [78,79]

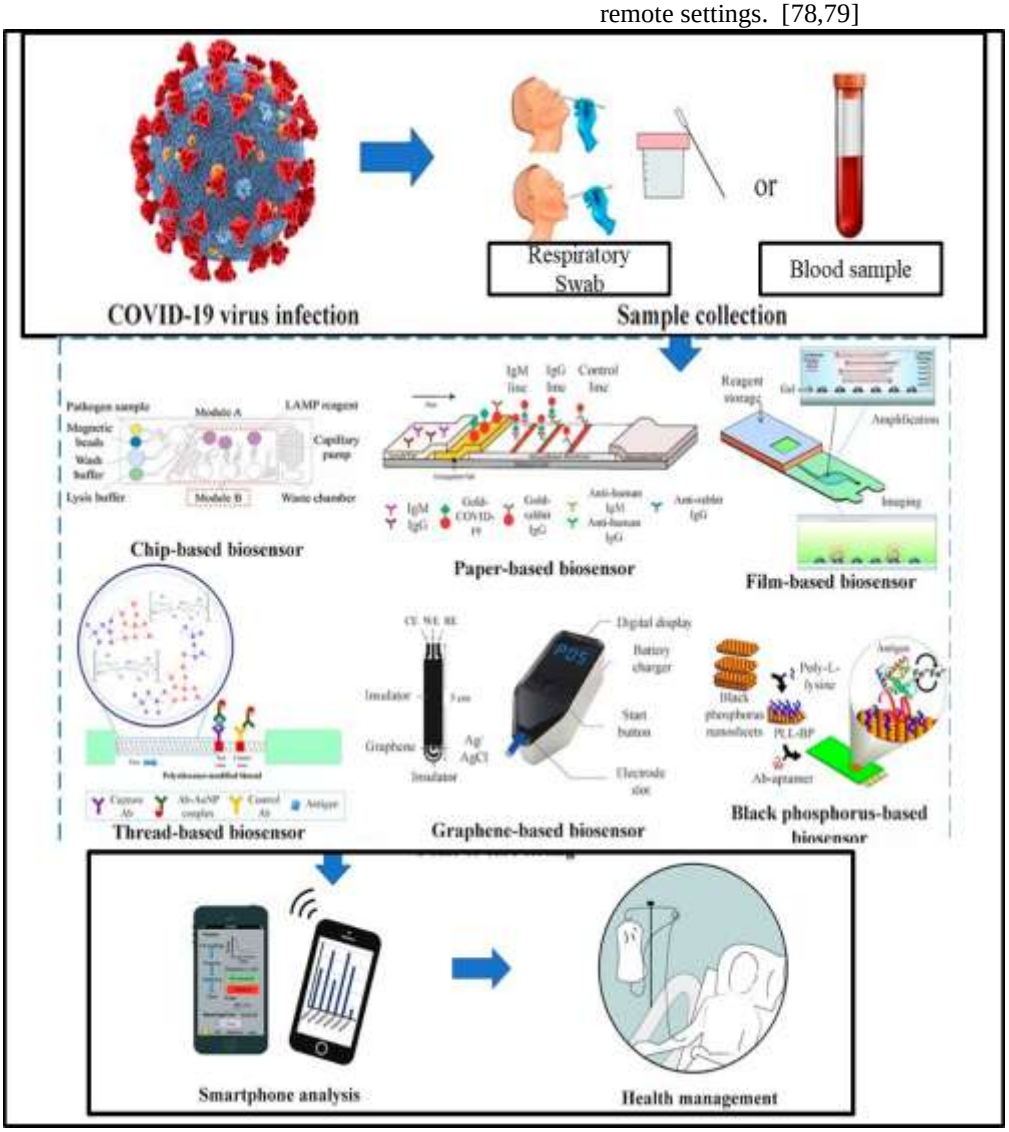

Figure 17: Development of Point of care for COVID-19

- Although several other methods can be employed for that purpose, such as immunological assays, thoracic imaging, portable X-rays, or amplification techniques, the pandemic spread of COVID-19 demands to develop POC devices for rapid detection. [80-83]

Table 1: Comparisons of Biosensors Platform for Virus Detection

Biosensor type	Recognition element	Dynamic range	Detection limit	Portability
HIV Virus				
Electrochemical	HIV aptamer	3.0fM-0.3Nm	0.3fM	No

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Piezoelectric	Glycoprotein 41	2-200 ng/Ml	2 ng/mL	No
Optical	Glycoprotein 120-antibody	10^4-10^8copies/Ml	10^5copies/mL	Yes
Ebola Virus				
Electrochemical	Biotinylated target strand DNA	0-5nM	4.7nM	No
Optical	Immobilized antiviral immunoglobulins	10^6-10^9 pfu/mL	10^5pfu/mL	No
Optical	Fluorescence single nucleic Acid	0.21-1.05*10^5pfu/mL	0.2pfu/mL	Yes
Zika Virus				
Electrochemical	Immobilized monoclonal antibody	500 ng/mL	0.45nM	Yes
Electrochemical	Specific envelop protein antibody	10 pM-1nM	<10pM	Yes
Optical	Envelope protein coding region	5-500pfu	5pfu	Yes
Noro Virus				
Optical	Anti-norovirus monoclonal antibody	0.01-100ng/mL	0.01ng/mL	Yes
Electrochemical	DNA	1pM-10nM	8.8pM	No
Optical	Norovirus-specific aptamer	20-1000viruses/mL	30viruses/mL	Yes
Dengue				
Electrochemical	Specific peptide nucleic acid	1-100fM	10fM	No
Electrochemical	Specific peptide	0.025-3.5ug/mL	0.025ug/mL	No
Electrochemical	Specific DNA probe	1*10^{-6}-1*10^{-12}M	2.7*10^{-12}M	No

Advantages

- Rapid and continuous measurement
- High specificity
- Very less usage of reagents required for calibration
- Fast response time
- Ability to measure non-polar molecules that cannot be estimated by other conventional device

Disadvantages:

- Requirement for sample preparation.
- Poor sensitivity
- Temperature parameter influences the performance of biosensor sensitivity
- Tedious measurement condition.

IX. APPLICATIONS:

1. Monitoring glucose level in diabetes patients:

Glucose testing tools — like glucose meter test strips and wearable sensors — are glucose biosensors. These compact devices are comprised of several crucial components for the detection and measurement of glucose.

The National center for biotechnology information (NCBI) features an exhaustive explanation of the parts of a biosensor. For a glucose biosensor, the following components are used:

- **Analyte:** A substance with chemical constituents that are being identified and measured. In this instance, glucose is the analyte that the biosensor is designed to detect.

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- **Bioreceptor:** This is a molecule that specifically recognizes the analyte. For the detection of glucose, specific enzymes are used, which are proteins that facilitate a chemical reaction. For example, the test strip for a blood glucose test contains the enzyme that interacts with the analyte in the drop of blood.
- **Transducer:** This part of the biosensor converts one form of energy into another. Specifically, it converts the recognition of the bioreceptor into a measurable signal. Most modern-day glucose meters and continuous glucose monitors measure electrical signals, although earlier generations of glucose meters used a colorimetric process (color change) that was measured optically.
- **Electronics and display:** These components process the transduced signal and prepare it for display. The processed signals are then quantified and shown on either the glucose meter's display or the receiver for a continuous glucose monitor (or compatible app).

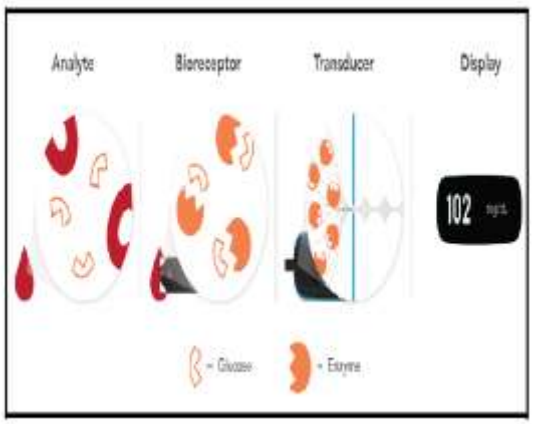

Figure18: Parts of Glucose Testing Tool

2. **Food analysis:** The food industry is a very sensitive one with high quality expectations. However, food products integrity has been compromised severally. This has led to many adverse effects. Numerous traditional testing methods may fall to identify hazardous contaminants in food at certain low levels. Bacterial luminescent sensors have been applied to detect toxicants in food at very negligible level. This has been applied successfully to monitor bacteria contamination and count in food. The food industry presents with a high need for quality assurance now than ever. Biosensors offer specific and affordable methods to meet this quality assurance needs.

3. **Environmental applications:** Waste monitoring, Water monitoring and Air monitoring
4. **Protein engineering and drug discovery applications**: Protein engineering is the process of controlling the development of useful or valuable proteins. Proteins were used for specific biosensor design. Affinity between protein and analyte is the basic principle of this study area. Scientists firstly determine the three-dimensional crystal structures of the proteins and build a protein data bank.. The interaction between protein and its ligand is determined with different types of transducers. If the presence of very low amounts of biomolecules is determined, various diseases and cancer types can be identified at early stages. Protein engineered biosensors can specifically identify chemical substrates with protein-based sensors. There are three main strategies employed in the engineering of more suitable biological components used in biosensors. These techniques do not exclusive to each other; also, they can be applied together. Rational protein design, directed evolution and de novo protein design are the main methods. Each design strategy has limitations, advantages and disadvantages respect to each other to be used in a biosensor format. The three design techniques are used to modify aspects of stability, sensitivity, selectivity, surface tethering, and signal +transduction within the biological environment [50].

5. **Waste water treatment.**

Many techniques are available for monitoring known water contaminants, but they are typically expensive and cumbersome requiring trained scientists or technicians to use them correctly. Biosensors provide the opportunity for simple to use, disposable or continuous tests, for monitoring many of the common contaminants and emerging contaminants that water-quality personnel are facing today. The trends in biosensor technology over the past 30 years have taken this equipment from simple and cheap components to the integration of several sensor systems into one unit including multiple analytes, making these systems smaller and tailored for mass production. The vision for the biosensor industry is to create

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microscale technology that will be suitable for performing sample preparation, analysis and diagnosis all with one chip.

X. CONCLUSION

Since the first ideas five decades ago, biosensors have shown their potential to revolutionize the diagnosis of a variety of health conditions. Today, their impact in clinical management is well established, since rapidity, specificity and sensibility are crucial characteristics for early diagnosis and therapy initiation. The development of new technologies, such as nanotechnology and microfluidics, together with biomarker discovery should improve their effectiveness. In the case of infectious diseases, which have the potential of transmission and outbreaks occurrence, with possible sequels development and lethality, the availability of robust diagnosis methods is crucial. Biosensors are also important in the democratization of diagnosis. Many methods currently available are inaccessible for a significant part of the world population, since they are expensive, centralized and require specialized technicians for operation. Therefore, the potential of cost reduction, portability and simplicity is largely appreciable, especially in the case of neglected diseases. Advances in fields such as genetics and epigenetics, chemistry and biochemistry, physiology and bioinformatics have the potential of clarifying the nuances of biological processes in the health and disease. New targets of study are emerging and other are being better understood, especially in diagnosis and therapeutics, or even both (i.e., theranostics). Therefore, the coupling of these findings with promising technologies such as biosensors may change the current landscape of clinical diagnosis.

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