Asia Pacific Academy of Science Pte. Ltd.

Advances in Analytic Science 2024, 5(2), 2830.
https://doi.org/10.54517/aas.v5i2.2830

Review

Development of novel nanostructured biosensors for rapid detection of pathogens in clinical diagnostics

Md Jasim Uddin[1,2,*], Shahrin Risa Sejuti[3], Sharmin Lucky[3], Mili Akter[3], Sumaya Binty Hussain[3]

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur 50603, Malaysia
[2] School of Pharmacy, Brac University, Dhaka 1212, Bangladesh
[3] Drug Delivery & Therapeutics Lab, Dhaka 1212, Bangladesh
*** Corresponding author:** Md Jasim Uddin, j.uddin@um.edu.my

CITATION

Uddin MJ, Sejuti SR, Lucky S, et al. Development of novel nanostructured biosensors for rapid detection of pathogens in clinical diagnostics. Advances in Analytic Science. 2024; 5(2): 2830. https://doi.org/10.54517/aas.v5i2.2830

ARTICLE INFO

Received: 15 July 2024
Accepted: 14 September 2024
Available online: 25 September 2024

Abstract: The prompt and precise identification of microorganisms is crucial for successful clinical diagnostics and the prevention of infectious disease outbreaks. Traditional diagnostic methods often suffer from limitations such as extended processing durations, elevated expenses, and the necessity for specialized laboratory equipment. In this research, we propose the development of novel nanostructured biosensors that utilize the distinct characteristics of nanomaterials to improve the accuracy, specificity, and efficiency of identifying pathogens. These biosensors are created with the intention of offering point-of-care testing functionality, thus rendering them appropriate for utilization in a range of clinical settings. The integration of advanced nanotechnology with bioanalytical methods aims to create a reliable system for the real-time identification of bacterial, viral, and fungal pathogens. This review encompasses the design, fabrication, and testing of the biosensors, along with a comprehensive analysis of their performance in comparison to conventional diagnostic techniques. The results demonstrate the potential of nanostructured biosensors to revolutionize pathogen detection, offering significant improvements in efficiency and accuracy, which are essential for timely medical intervention and public health management.

Keywords: nanostructured biosensor; pathogen; clinical diagnosis

1. Introduction

1.1. Background and significance

1.1.1. Importance of rapid detection of pathogens

Pathogens are disease-causing agents, which consist of microorganisms like fungi, protozoans, and bacteria, as well as molecular-sized infectious agents such as viruses and prions. These pathogens can invade the human body through various means of transmission, including contaminated food, water, or air, and are accountable for more than 15 million deaths every year across the globe [1]. Such disease-causing organisms differ in several aspects, like severity, ability to spread, method of transfer, and amount needed to cause infection. Some of the frequently encountered disease-causing agents are viruses like norovirus and the flu virus, bacteria like *E. coli* and *S. aureus*, and fungi like Aspergillosis and Candida auris [2]. Viruses, for example, are minuscule parasitic entities that invade cells and can cause a range of diseases such as influenza, varicella, herpes virus, Human papillomavirus (HPV), severe acute respiratory syndrome (SARS), Ebola, human immunodeficiency virus (HIV), and others [3]. Recent global outbreak of COVID-19 has highlighted the destructive nature of viruses on the human population. Bacterial and fungal assaults pose an equally

significant threat. Just like viral diseases, the detection and characterization of detrimental bacteria is vital in different sectors such as healthcare, food industry, and public health safety measures. Worldwide, bacterial infections are a leading reason of hospitalization and death due to the fact that these infections are either identified inaccurately or are diagnosed too slowly, despite the presence of antibiotics [4]. Furthermore, as a result of global warming and other climatic shifts, the Fungi Kingdom is always changing to adapt to all environments, including hotter ones. The first recorded case of a plant fungus infection in humans was documented in March 2023 [5]. Fungal infections such as Aspergillosis, Candida auris, Pneumocystis pneumonia, and Mucormycosis are additional harmful diseases caused by fungi [6]. These diseases are major causes of illness and death worldwide, leading to millions of deaths and hospitalizations every year. Therefore, early and quick identification of these diseases is essential as treatment options are limited.

Detecting pathogens typically includes using various molecular methods like RT-PCR, ELISA, immunofluorescence, immunoperoxidase, and gene sequencing. Traditional ways of identifying these pathogens usually involve isolating, growing, and conducting biochemical tests, making them time-consuming, labor-intensive, and costly [3,7]. On top of that, the outbreaks have created numerous issues in the areas of heath care, food production, and environment. Hence, to effectively handle and monitor the impacts of viral and bacterial outbreaks on human health, it is necessary to create new technologies that can quickly identify these pathogens. The properties of biosensors combined with nanotechnologies are now being regarded as a possible chance to accelerate the development of accurate, quick, sensitive, and targeted devices for the detection of pathogens [7,8].

1.1.2. Biosensor and nanomaterial-based biosensor

According to Chao et al., A biosensor is a tool that measures and detects substances by combining a living component with a device that measures physical and chemical properties [9]. In 1962, Clark and Lyons innovatively developed an oxidase enzyme electrode for the detection of glucose, marking the introduction of the concept of a biosensor for the very first time [10]. The biosensor's intended use and design determine how it will detect analytes. Biosensors can identify substances associated with illnesses, like proteins, genetic material such as nucleic acid, and cells. This is made feasible by its three main elements: The reading device (transducer), the detector element, and the biochemically sensitive element (Biorecognition element) (**Figure 1**) [8]. In essence, biosensors consist of a biologically sensitive component connected to a detector that transforms targeted substances attaching to biological receptors into a quantifiable or observable result (**Figure 1**) [4,11]. Biosensors can be categorized in several manners, for example based on the method of transmission of signals, including optical, mechanical, or electrical biosensors, or depending on the type of bioreceptor utilized, such as catalytic (enzyme-based) or affinity-based (which recognizes antibody, aptamer, lectin, bacteriophage, etc.). Due to their affinity-based nature, biosensors possess greater selectivity and specificity, making them more favorable than enzymatic biosensors for the detection of microbes as they do not necessitate extra reagents. The area of biosensors is growing quickly; over the last three decades, amperometric and optical methods have been commonly used, but

newer techniques such as impedance and fiber optics are becoming increasingly popular [4].

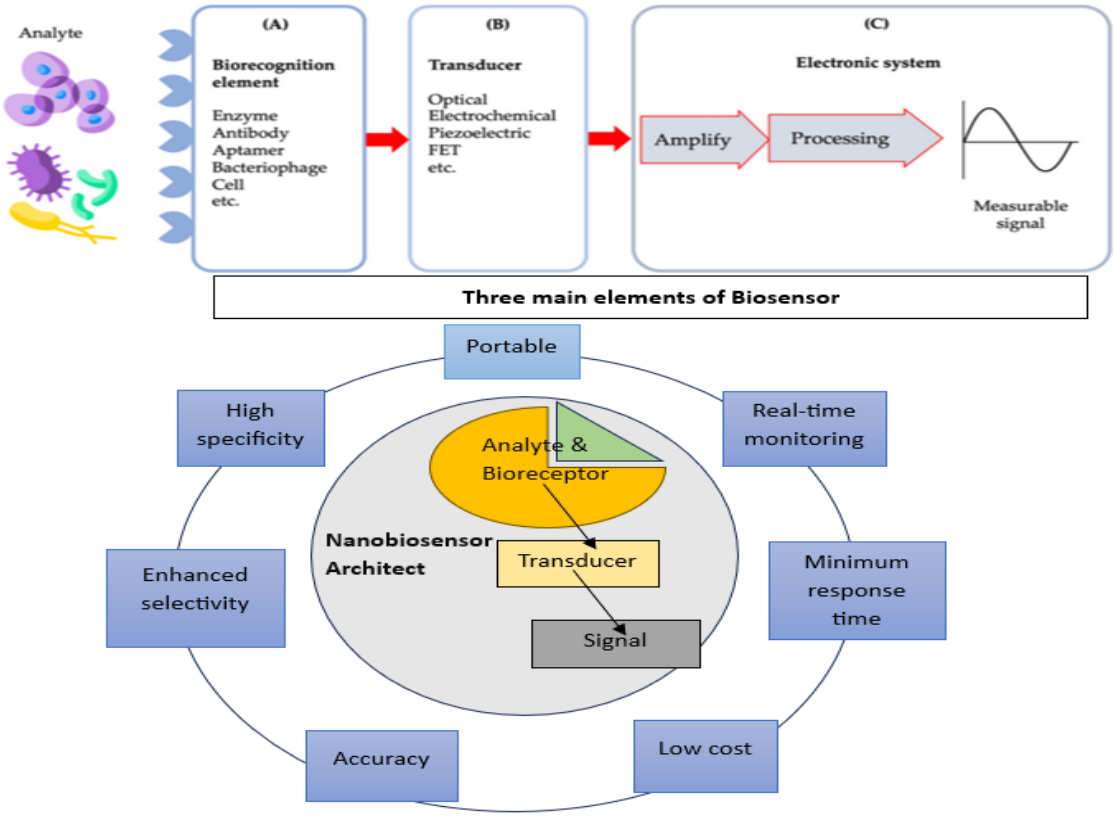

Figure 1. Schematic illustration of the three main elements a typical biosensor (Adopted with modification from [11]); **(A)** biorecognition elements- interact with the analytes such as cells, DNA, tissues, antigens etc.; **(B)** transducer- convert the analyte-bioreceptor interaction into a quantifiable signal; **(C)** electronic system-detector. And some advantages offered by biosensors.

Biosensors are crucial for quickly and precisely diagnosing illnesses and delivering substantial healthcare [12]. They are used at clinical level to identify biomolecules associated with various disease conditions, such as cholesterol, cardiovascular disease indicators, cancer/tumor biomarkers, allergic reactions, and bacterial, viral, and fungal infections [13]. Given the current focus on bacterial and viral infections, biosensors have become essential for detecting pathogens. In the last quarter-century, biosensors have been created to compete with PCR and ELISA in identifying and measuring pathogens. These devices integrate a sensitive transducer element and a selective biorecognition element, offering supplementary platforms to PCR and ELISA [2].

Furthermore, the ability to create electrodes on a very tiny size, known as nanoscale sensors, and recent developments in the field of nanoscience and nanotechnology have enabled the creation of a novel class of biosensors for diagnosis known as nano-biosensors [3]. The properties of biosensors combined with nanotechnologies are now being explored as a potential method to hasten the development of precise, rapid, responsive, and focused tools for identifying viruses and bacteria. Research on nanomaterials, including carbon nanotubes, quantum dots, metal oxide particles, small metal clusters, nanomaterials with optical properties,

composite materials with polymers, and gel nano particles, has significantly advanced the field of nanotechnology and biosensor development [8]. Nanomaterial-based biosensors are unique in that their material dimensions continuously drop from big to tiny in the 1–100 nm range. This process preserves the biosensor's characteristics while greatly enhancing its application. The incredibly high surface-to-volume ratios of nanoscale devices lead to highly efficient surface interactions between the sensors and the analyte [14]. According to Ahmed et al., researchers have referred to biosensors based on nanomaterials as "lab-in-the-chip" methods for clinical diagnostics [4]. Several types of nano-biosensors are grouped together with profound considerations of nanostructured materials and the biosensing process [15]. Many types of nanomaterials, including gold nanoparticles, carbon nanotubes (CNTs), magnetic nanoparticles, and quantum dots (QDs), are being combined with biosensors in consideration of unique qualities such as physical, chemical, mechanical, magnetic, etc. [3].

1.2. Objectives of the study

Infectious diseases are a primary factor in elevated rates of death and sickness on a global scale. Rapid detection allows for early diagnosis and prevention of complications and spread of diseases. Nanostructured biosensors offer higher sensitivity and specificity, making them particularly beneficial in point-of-care settings and resource-limited areas.

Hence, the main objective of this review is to discuss the creation of innovative nanostructured biosensors for the rapid and accurate detection of pathogens in clinical diagnostics. This involves the design, fabrication, and validation of biosensors with enhanced sensitivity and specificity, leveraging advanced nanomaterials and nanotechnology. We will also put a comparison traditional and advanced biosensor pathogen detection techniques, and provide case studies and real-world applications. The aim is to significantly improve the speed and accuracy of pathogen detection, which will help with timely diagnosis and treatment in clinical settings.

1.3. Scope/rationale of the study

This review article will provide an up-to-date and comprehensive understanding of the exploration and advancement, as well as publications, on biosensors utilizing nanomaterials and their uses in clinical diagnosis. Additionally, the aim of this review is to fill the informational void regarding biosensors and nanomaterials and explore how their integration can contribute to advancements of healthcare sector. It will also pave the way for researchers and clinicians to create innovative instruments and methods for real-time monitoring of pathogenic biomolecules in clinical samples, enhancing the upcoming research and development in clinical diagnosis. Additionally, this evaluation will be beneficial for researchers, teachers, healthcare professionals, laboratory specialists, biotech experts, medical professionals, and individuals working in biomedicine. It will also be of interest to students pursuing advanced degrees, as well as professionals in the field of medical device development.

2. Materials and methods

2.1. Selection and preparation of nanomaterials

2.1.1. Nanomaterials as components of biosensors

The intrigue surrounding nanomaterials has captured the attention of various fields, especially in the area of biological sciences, due to their remarkable properties such as excellent conductivity and suitability for interaction with biological cells. The capacity to enhance surface area and create innovative features not found in traditional materials are two highly esteemed benefits of nanomaterials. These characteristics have facilitated their application in a diverse range of methodologies for developing biosensors. In this section, we will introduce several nanomaterials that possess highly applicable properties for creating biosensors.

2.1.2. Characteristics and classification of biosensors

A biosensor prototype that is carefully crafted and expertly made needs to showcase specific fundamental features in order to guarantee its success in enhancing society's health and well-being.

- Selectivity: The primary factor to keep in mind when creating a biosensor is its selectivity, guaranteeing that it can precisely identify the desired substance within a mixture containing numerous akin or distinct substances or impurities. This emphasizes the importance of selectivity as the key element of a biosensor [16].
- Reproducibility: A biosensor must consistently produce the same results across repeated experiments, which is a critical requirement. High reproducibility, coupled with accuracy and precision, makes the biosensor highly reliable and in demand [16].
- Stability: The stability of a biosensor plays a crucial role in determining its profitability in the market. Biosensors can lose signal strength over time, and this degradation is often accelerated by increasing temperature. Therefore, maintaining stability is essential [16].
- Sensitivity and Linearity: Biosensors are highly valued for their sensitivity, especially in detecting pollutants in air, water, and soil at ppm levels, and in medical diagnostics at nanogram to femtogram levels per milliliter. The linearity of a biosensor indicates the accuracy of its response across varying analyte concentrations [16].

2.1.3. Types of nanomaterials used for biosensors

Metal Oxide-Based Biosensors: Metal-containing nanoparticles demonstrate great efficacy in biosensing because of their altered surface properties upon interaction with biological molecules and their unique quantum mechanical characteristics, including enhanced electron motion and intense electromagnetic forces [17]. Metal oxides such as nickel oxide (NiO), cobalt oxide (Co_3O_4), and manganese oxide (MnO_2) are widely favored for their fast and reversible Faradic redox reactions at the boundary between the electrode and electrolyte. An example of this is a bioelectrode made of NiO and other two-dimensional substances that has been used to detect the influenza virus. Likewise, Co_3O_4 has demonstrated potential in electrochemically detecting specific molecules because of its strong electrocatalytic capabilities, excellent

durability, and simple structural layout [17].

Zinc Oxide-Based Biosensors: Zinc oxide (ZnO) is a strong contender for creating biosensors because of its lofty isoelectric point (IEP), cost efficiency, environmentally friendly characteristics, and chemical durability. Its elevated IEP aids in attracting substances such as enzymes, DNA, and proteins due to electric interactions. Moreover, ZnO, being an n-type semiconductor that has a broad band gap of 3.37 eV, significant exciton binding energy of 6.0 meV, and solid electron movement, is ideal for constructing biosensors. The wide band gap of ZnO allows it to withstand high electric fields, ensuring a high breakdown voltage and stability within the visible spectrum [18].

Quantum Dot-Based Biosensors: Tiny particles known as quantum dots are small, crystalline materials that range in size from 2.0 nm to 10.0 nm, and display various colors depending on their dimensions. For instance, quantum dots measuring 5.0–6.0 nm emit an orange or red hue, while those between 2.0–3.0 nm appear blue or green. The characteristics of quantum dots are primarily influenced by their size, shape, and composition. One common method used to create quantum dots is the top-down approach, in which large carbon-based materials such as graphite, graphene oxide, carbon nanotubes, and carbon fibers are reduced to nanoscale quantum dots [19]. Quantum dots are widely utilized as imitation fluorescent molecules in optical sensors designed to detect both organic substances and large molecules [20].

Nanowire-Based Biosensors: Nanowires are slim, thread-like formations with extremely small sizes, crafted from semiconducting metal oxides, carbon, and metal nanotubes. Because of their minuscule dimensions, nanowires display outstanding characteristics in terms of mechanics, heat conduction, chemical reactivity, light interaction, and electrical conductivity that are hard to obtain in case of materials that are large in size. These minute constructions are frequently utilized in creating biosensors that are capable of recognizing and detecting with increased precision and exactness.

Nanorod-Based Biosensors: Microscopic rods, known as nanorods, are manufactured through chemical methods using materials like graphene, graphene oxide, different metal oxides, and semiconductors. These nanorods, which typically measure between 1 and 100 nanometers in size, exhibit promising capabilities in the realm of biosensors, specifically in detecting nucleic acids, carbohydrates, metallic ions, and other substances [21].

Carbon Nanotube-Based Biosensors: In 1991, Sumio Iijima first discovered carbon nanotubes, also called buckytubes. These hollow carbon formations have very small diameters and are constructed with carbon atoms joined through sp2 bonds, giving them exceptional durability and rigidity. CNTs are widely studied for creating biosensors utilized in healthcare diagnostics and various fields of study, acting as frameworks for fixing biological molecules on their exterior. In 2006, Tang and colleagues fashioned a DNA sensor using single-walled carbon nanotubes (SWNT) that displayed remarkable sensitivity and quick reaction times [22].

Dendrimer-Based Biosensors: Dendrimers have recently captured considerable interest as flexible microscopic structures in biosensors. These intricate, multi-branched large molecules provide precise configurations, adjustable surface features, and abundant branching concentrations. These qualities render dendrimers perfect for

designing biosensor systems with improved precision, specificity, and durability [23].

Table 1. An overview of various types of nanomaterials used for biosensors, along with their respective advantages and disadvantages.

Type of Nanomaterial	Advantages	Disadvantages	Reference
Metal Oxide-Based Biosensors	• Fast and reversible redox reactions • Strong electrocatalytic capabilities • Enhanced electron mobility • Durable and stable	• Limited by complex fabrication techniques • Potential toxicity of metal oxides • Sensitivity to environmental factors	[17]
Zinc Oxide (ZnO)-Based Biosensors	• High isoelectric point, attracting various biomolecules • Cost-effective and environmentally friendly • Chemical and thermal stability	• May suffer from photodegradation under UV exposure • Limited by the need for complex surface modifications • May exhibit poor selectivity in some applications	[18]
Quantum Dot-Based Biosensors	• High sensitivity and tunable optical properties based on size • High photostability and fluorescence • Capable of multiplexing and real-time monitoring	• Potential cytotoxicity due to heavy metal content • Complex and expensive synthesis processes • Possible degradation and quenching of fluorescence over time	[20]
Nanowire-Based Biosensors	• High surface area to volume ratio, enhancing sensitivity • Excellent electrical and mechanical properties • Rapid and accurate detection	• Difficult and costly manufacturing processes • Challenges in integrating with existing electronic systems • Potential issues with reproducibility and consistency in large-scale production	[21]
Nanorod-Based Biosensors	• High aspect ratio, enhancing surface interactions with analytes • Versatile and can be synthesized from various materials • Suitable for a wide range of sensing applications	• May be susceptible to aggregation, reducing efficiency • Stability and dispersion issues in aqueous environments • Potential toxicity and environmental concerns	[21]
Carbon Nanotube (CNT)-Based Biosensors	• High mechanical strength and electrical conductivity • High surface area for biomolecule attachment • Rapid and highly sensitive detection	• Difficulty in uniform dispersion and aggregation control • Expensive production methods and purification challenges • Potential toxicity and biocompatibility concerns	[22]
Dendrimer-Based Biosensors	• High degree of structural control and surface functionalization • Enhanced sensitivity and specificity due to multi-branched structure • Versatility in designing customized biosensors	• Complex and costly synthesis • Limited by potential instability and toxicity of dendrimers in biological systems • Challenges in large-scale production and consistency	[23]

Table 1 provides an overview of various nanomaterials used in biosensors, highlighting their unique advantages and limitations. Metal oxides and carbon nanotubes offer high sensitivity and stability, but they can be complex to fabricate and may pose toxicity concerns. Zinc oxide and quantum dots are noted for their strong chemical and optical properties, though they may require careful handling due to potential degradation and toxicity, whereas, dendrimers and nanorods offer versatility and precision but are often challenged by complex synthesis and stability issues.

2.1.4. Synthesis techniques of nanostructured biosensors

Nanostructured biosensors utilize the distinct characteristics of nanomaterials to enhance the precision of identifying pathogens. The synthesis of these biosensors typically involves several key steps:

- Selection of Nanomaterials: Select the right nanomaterials like gold nanospheres, graphite nanofibers, subatomic particles-Quantum dots, or carbon grids. Biosensor combines with nanomaterials, and nanostructure to enhance critical features, selectivity, and sensitivity. Based on classification (**Figure 2**) organic based nanomaterial includes nanofilms, nano gels, dendrimers, nanoMIPs etc. Inorganic-based nanomaterials include quantum dots, AuNPs, nano shells, silver NPs, and magnetic NPs. Also, there is carbon-allotrope based nanomaterials includes fullerene, carbon dot, nanotube, graphene, etc. [8]. These substances are chosen for their extensive surface area, electrical conductivity, and ability to interact well with living organisms [21].

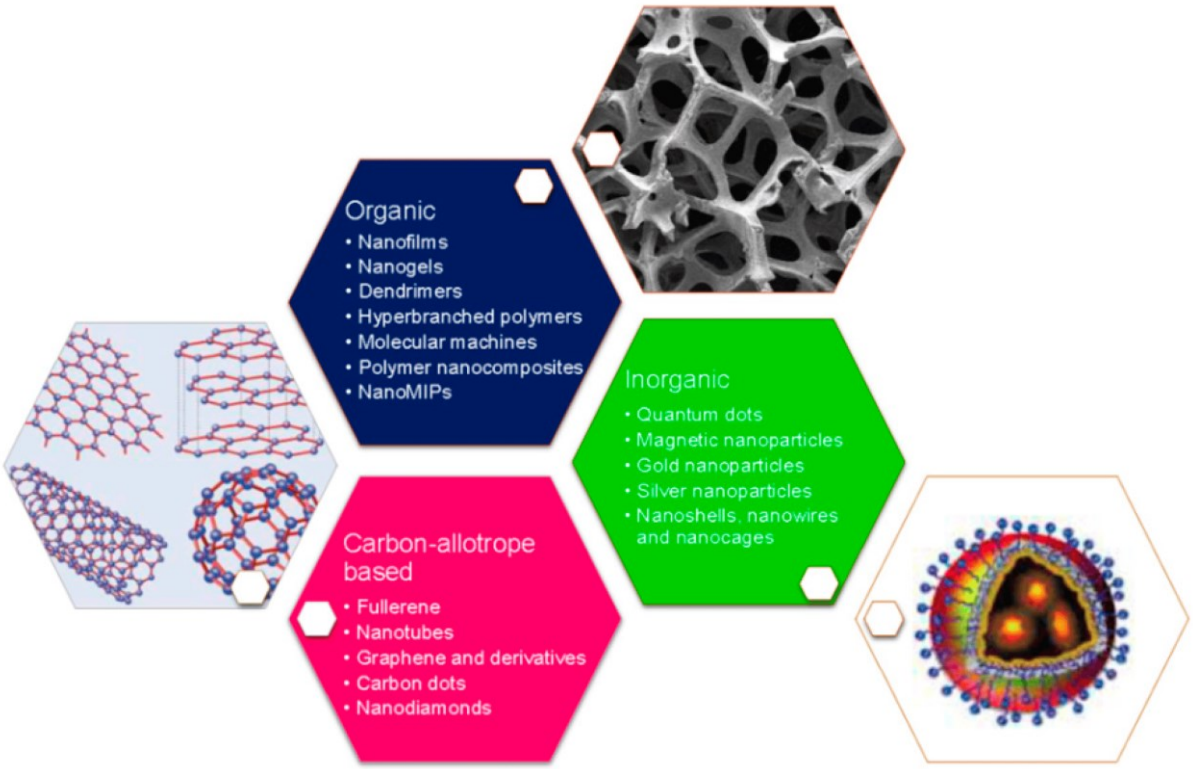

Figure 2. Different classifications of nanomaterial, and nanostructures [8].

- Functionalization of Nanomaterials: Modify the surface of the nanomaterials to attach biorecognition elements such as antibodies, DNA probes, or enzymes. This is often achieved through chemical conjugation techniques such as thiol-gold interactions, covalent bonding, or adsorption [24].
- Immobilization of Biorecognition Elements: Attach the functionalized nanomaterials to a suitable substrate, such as glass slides, silicon wafers, or electrode surfaces. This step ensures that the biorecognition elements are properly oriented and active [24].
- Assembly of the Biosensor: Integrate the immobilized nanomaterials into the biosensor device. This may involve the creation of microfluidic channels, the incorporation of electrodes, or the assembly of optical components [24].
- Calibration and Validation: Test the biosensor using known concentrations of the target pathogen to calibrate the device. This step ensures accuracy and

reproducibility in pathogen detection [24].

- Application in Clinical Diagnostics: Deploy the biosensor in a clinical setting to detect pathogens in patient samples such as blood, urine, or saliva. The rapid response and high sensitivity of the nanostructured biosensor enable timely and accurate diagnosis [24].

2.1.5. Functionalization processes

- Selection of Nanomaterials: Select small-scale materials like tiny particles of gold, slender tubes made of carbon, tiny crystals known as quantum dots, or a form of carbon called graphene because of their characteristics such as large surface area, ability to conduct electricity, and compatibility with living organisms [24].
- Surface Modification: Modify the surface of the nanomaterials to introduce functional groups that can bind to biorecognition elements. This can be done using chemical methods such as silanization, thiolation, or polymer coating. Example: For gold nanoparticles, thiol groups (-SH) can be used to create a stable gold-sulfur bond.
- Attachment of Biorecognition Elements: Attach biorecognition elements (antibodies, DNA probes, enzymes) to the functionalized nanomaterials. This step is crucial for the specific detection of pathogens.

  Method: Use covalent bonding, electrostatic interactions, or affinity binding (e.g., streptavidin-biotin).
- Immobilization on Substrate: Immobilize the functionalized nanomaterials on a solid substrate (glass, silicon wafer, electrode surface) to create a stable and usable biosensor platform.

  Method: Various methods like spin coating, dropping liquid, or building up layers can be utilized.
- Optimization and Calibration: Optimize the biosensor's performance by adjusting parameters such as pH, temperature, and incubation time. Calibrate the sensor using known concentrations of the target pathogen.
- Validation and Testing: Validate the biosensor's performance using clinical samples. Ensure that the sensor can accurately and rapidly detect pathogens in various sample matrices (blood, urine, saliva) [25].

2.2. Design and fabrication of biosensors

2.2.1. Sensor architecture

Biosensors, nano-based biosensors are highly used for viral, and bacterial detection. It can detect biological components via a physicochemical detector. These sensors can be used for pathogen detection in water and food. Biosensors have some sensing elements; these are also known as bioreceptor that simulate in vivo molecular identification affairs. Sensing elements are cells, cell receptors, enzymes, antibiotic, nucleic acids, microbes, etc. The process consists of three parts including analyte, transducer and signal processor [8]. There are mainly two components of biosensor including a bioreceptor and a transducer. This transducer can convert the recognition matter into electrical signal [26]. The classification of a transducer is based on optical, electrochemical, and mass-based (**Figure 3**). Optical transducer includes colorimetry,

fluorescence, SERS, and SPR. Electrochemical transducers include aerometric, potentiometric, impedance, and conductometric. And mass-based transducers can be piezoelectric and magnetoelastic. There can be many more [27]. A bioreceptor is mainly a molecular segment that can identify its target via catalyzed biochemical mechanism where receptors bind to target analytes in complex bio-fluid [28]. There are various bio receptors, for instance, antibody bio-receptors, enzyme bio-receptors, nucleic acid bio receptors, cellular optical biosensors, biomimetic bio-receptors, bacteriophage bio receptors etc. Antibody bio receptors appear with distinctive different classes of monoclonal, polyclonal antibodies or Recombinant antibodies [27]. Enzyme bio-receptor uses enzymes with a transducer to the same target analytes. To gain more advances in enzyme bio receptors, inorganic nanoparticles, and organic complexes having catalytic properties have developed which are called biomimetic enzyme bio-receptors [28]. Moreover, nucleic acid bio-receptors work by providing a recognition process and mainly based on a complementary base pairing system of purine and Pyramid [27]. They work through hybridization, aptamers, and DNAzymes methods [28]. Another one is using cell organelles including mitochondria, cell walls or tissue-cultured cells for identification of pathogens. Even, whole cell-based biosensor detects its target through metabolic transformation between whole cell and the target analytes [29]. Furthermore, bacteriophage receptors which are especially for bacterial pathogen diagnosis and the phage is attached to the surface of the biosensor where it determines phage-host characteristics or biological agents [28]. There are countless bio-receptors including peptides, proteins, peptide-nucleic acid, Ion sensing electrodes (ISE), Molecular imprinted polymers (MIPs), cell imprinted polymers (CIPs) etc. [29]. Biosensor generally operates based on analytical interaction with receptor, where one or both need immobilization over a solid support [29]. To increase the stability of biosensors, there are two types of methods to immobilize the receptor upon the surface area. One is covalent and another one is non-covalent immobilization which are much perfect for nucleic acid bio-receptors, peptide bio-receptors, protein bio-receptors etc. [29].

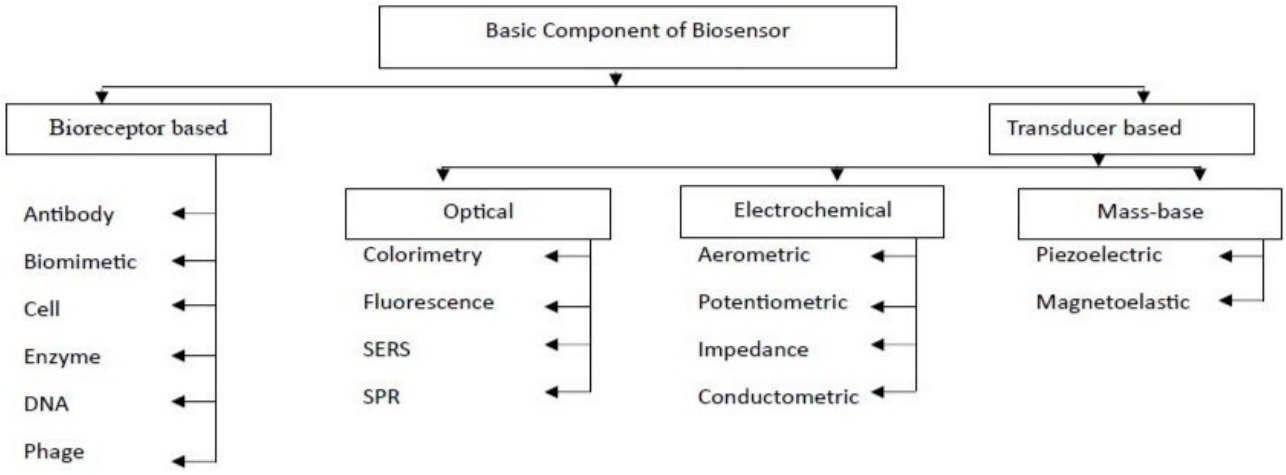

Figure 3. A basic classification of biosensor.

Conventional techniques work via metabolic reaction, or via growing reaction in a sufficient substratum after an enough incubation period. These systems include

Multiple tube Fermentation, microscopic techniques, and membrane filters. Moreover, there are many advanced techniques such as immunological methods, nucleic acid-based methods, Fluorescence In-Situ Hybridization method, Lamp-based, DNA micro-array, Next-Generation sequencing, enzymatic method etc. However, to enhance and improve the quality of performance, sensitivities and limitations of detection here are nano material-based systems available, such as quantum dots (QDS), Carbon based (Graphene nanomaterial), polymer etc. [30].

2.2.2. Quantum dots nanomaterial-based sensor

Quantum dots mainly nanocrystals with fluorescent semiconductor. It has the controllable photoluminescence [31]. This semiconductor crystals have very small size of about 2–10 nm diameter with unique electrical and unique optical properties [19]. Its unique feature is that, it can be illuminated perfectly at any wavelength and if the wavelength is shorter than the emission-peak, it will emit same symmetric narrow, properties spectrum [29]. Quantum dot shows unique features compare to organic fluorescent dyes in the area of symmetric size photoluminescence, wide resistance etc. Another advantage its light emission is very useful property for many medical labelling, sensing application to compare between normal cell and tumor cell [19].

2.2.3. Graphene nanomaterial-based sensor

Graphene oxide (GO) which is mainly carbon-based nanomaterial is made through graphite exfoliation. It includes epoxide, phenol hydroxyl, carboxylic group. Interested features are size controllability of nano sheets, changes in oxidation, antimicrobial properties. Moreover, employing QDs Fluorescence together with graphene can identify especially viruses [19].

- Detection of Influenza virus by Graphen based sensor: In a report it states that, Graphene oxide was immobilized with H5-polychonal antibody to make electrochemical immunosensor detector and thus amplify the signal. GO-PAb-BSA combination linkers were used on thiourea, AuNPs, Au electrode, H5-monoclonal antibody, H5 antigen. The detection limit was 2^{-15} HA unit per 50 micro litter and the linear range was 2^{-15} to 2^{-8} HA unit per 50 micro litter [19].
- Detecting of HIV virus by Graphen based sensor: The combination of Graphene oxide and carbon nanotube for electrochemical biosensor to detect HIV, silica-carbon nanotubes were used to encapsulation of the horseradish peroxide enzyme, later grafted to Graphene. The limit of detection was 0.15 pg mL^{-1} with 0.5 pg mL^{-1} to 8.5 ng mL^{-1} linear range [19].

Another research showed immobilization of GO and DNA strand with using DNA strand both as probe part and immobilization part to create nano-structured device. During the presence of HIV, the probe part was attached to DNA double helix. When negative charge was produced upon GO, it was identified through spectroscopy. The linear range was 10^{-12} to 10^{-6} M and the detection limit was 1.1×10^{-13} M [19].

Interested features of Graphene-based sensors are the size controllability of nanosheets, changes in oxidation, and antimicrobial properties. Moreover, employing QDs Fluorescence together with graphene can identify special viruses [19]. However, still ongoing research on this material is going on. Graphene-based sensors production is very limited at present and needs to test in a very controlled place [32]. Moreover, ensuring its homogeneity is difficult according to thickness, size and number of the

surface functional group. Inhomogeneity affects both efficiency of surface functionalization and reproducibility. Even, a pre-treatment process can be needed considering the risk of microbial and other contaminations of the complex environment [33].

2.2.4. Aptamer nanomaterial-based sensor

Another advanced nanostructured sensor is Aptamer-based nano sensing. This aptamer is a small sequence of peptides produced by ligands via exponential enrichment [34]. It refers a short single DNA/RNA and a promising, great alternative to antibodies as a molecular detection tool. They are selected in vivo which is from synthesized random libraries through exponential enrichment using a systemic evolution of the ligands [35]. They have greater affinity and specificity to many targets. Mostly carbon-based materials are used for sensor designing. Such as single wall carbon nanotubes, and multi-wall carbon nanotubes. Even metal NPS (Au), magnetic beads are also used as sensor [34].

2.2.5. Testing procedure using electrochemical aptamer-based sensor

A recent case study shows the developing novel ratiometric, dual signal aptasensor which can detect single-cell leveling bacteria. It was mainly based on a rolling circle amplification (RCA) or, G-quadruplex techniques which can interact with different signaling molecules including methylene blue **(Figure 4)**. The amplification was made from two probes including employ to target bacteria and the primer sequence with anchoring gold electrode using a sulfhydryl probe. Two probes created a complex that was disrupted due to bacterial (*S. aureus*) presence. And that is how this method detects pathogen [36].

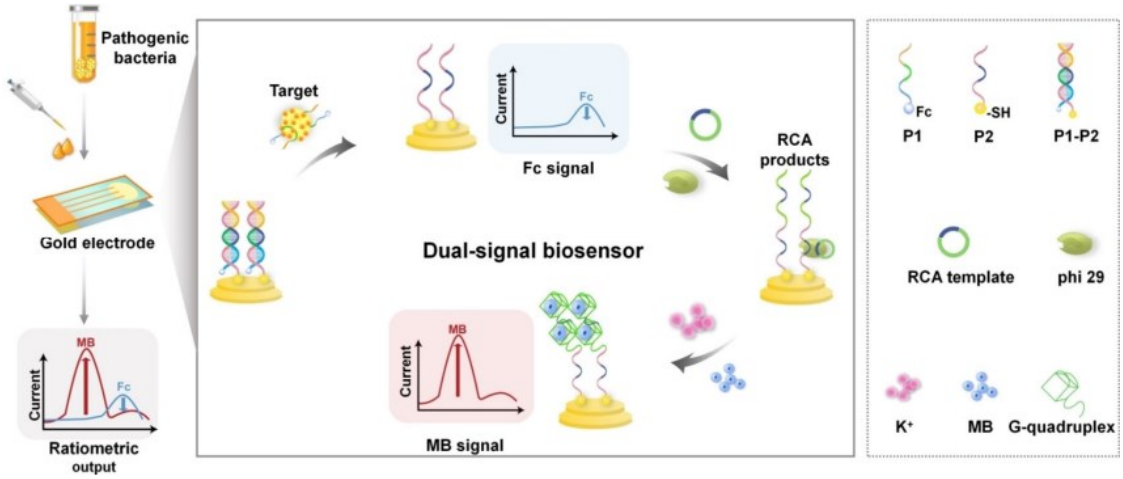

Figure 4. Presenting a novel ratiometric dual signal aptasensor to detect pathogenic bacteria based on RCA/G-quadruplex technique [36].

Compared to antibodies, aptamers are less batch-to-batch transform, highly flexible modifying strategies, and low cost. Targets like toxins, haptens can be used to produce aptamers with having high affinity where antibody cannot. However, more effective screening techniques are needed to detect pathogen-specific aptamers. Complicated pathogen detection requires the development of a group-specific aptamer to ensure higher reliability. Therefore, these nano-materials need further chemical

optimization before the aptasensor fabrication [35].

Electrochemical nucleic acid sensors: These types of sensors used for the identification of DNA to DNA, DNA to RNA, and DNA to aptamers in case of nucleic acid pathogen identification. The measurement is based on biochemical reactions, by which the sensor can recognize potential induce, and changes. It is used as sensitive and as simple method for DNA hybridization detection via bracing of single-stranded DNA for the complementary strands.

Bacterial pathogen detection by using AuNPs: There are many detection processes such as thin films of polymers like polyaniline, self-assembled monolayers of silanes, fullerene, and nanostructured metal oxides. Following the ELISA assay, there are similar principles are using for the biosensor detection systems. For that, the platforms are used based on AuNPs. AuNPs can be used perfectly to identify pathogenic cells, nucleic acids, proteins, etc. A wide range of application can be done through functionalization of gold nanoparticles with carbohydrates, aptamers, proteins, phages, antibodies, nucleic acids, and small molecules. AuNPs can be coated with cystine, polyethyleneimine, electrochemical peptides, and oligonucleotides on the surface area which will serve as proof to react with target analytes [37].

Testing procedure by AuNPs with LSPR: Where AuNPs are modified with Antibodies and by measuring the color changes or, surface plasmon resonance (SPR) the identification of pathogen can be done. Due to target binding, aggregations or de-aggregations occur and thus the changing [34]. When a metal Nano particle is considerably smaller than the incident light wave length they show localized surface plasmon resonance (LSPR). As a result, responding to light the free electrons of the metallic nanoparticle terminal collectively and generate a localized electromagnetic field. This helps to detect substances of very lower concentration and UV-Vis Spectroscopy can measure absorption scattering of the light at resonance frequency which is shown in **Figure 5**. A new approach of using LSPR with gold nanoparticles (AuNPs) provides higher identifications to detect interactions of molecules and the changes at the Nanoscale. By editing morphology (shape and size) the color of the gold nanoparticle solution can be changed. In **Figure 6**, distributive AuNPs of 5–50 nm exhibit absorbance speak ranging from 515 to 545 nm. Au Nano-rods this place absorbance speaks with transverse banned and with longitudinal blend this absorbance can induce visible changes of colors and will sweat for on-side detection. As a result, the size and quantity of gold nanoparticles raise a clear red shift noticed in pick absorbance, giving a red stable AuNP solution [37]. In an experiment, it was seen that AuNP got a color transition from red to purple due to electrostatic aggregation and it can detect the pathogen *V. parahaemolyticus*. Moreover, AuNPs can capture *Salmonella enterica serovar* and *E. Coli* [38]. Even using fluorescent bacteria in printed polymer-level free AuNPs for 135 min can show significant detection [39].

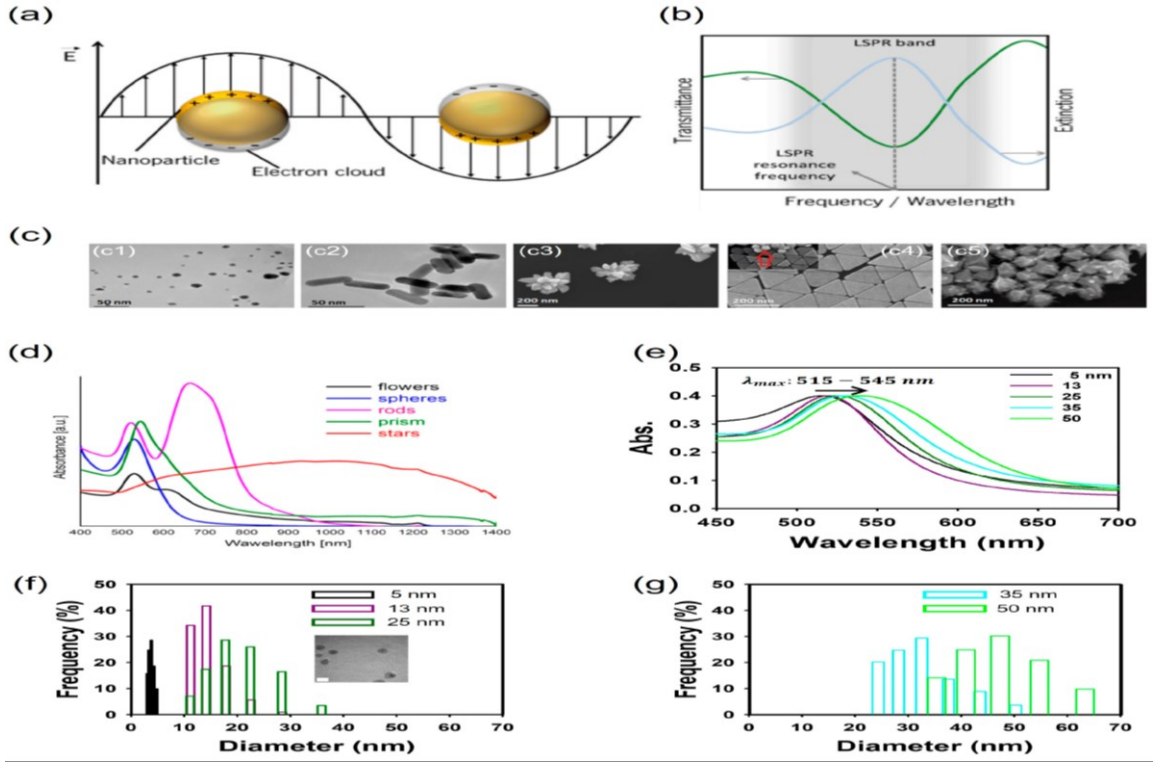

Figure 5. A typical LSPR of metal sphere. **(a)** Diagram of LSPR band, transmittance mode measurement, extinction spectrum; **(b)** Schematics of AuNPs; **(c)** Nano-Rods, Nano-Flowers, Nano-Prisms, Nano-Stars, UV-Vis absorption spectra; **(d)** Changes of five AuNPs in UV-Vis absorption spectra; **(e)** AuNPs distribution (5–25 nm); **(f)** 35, 50 nm; **(g)** Synthesizing via citrate reduction [37].

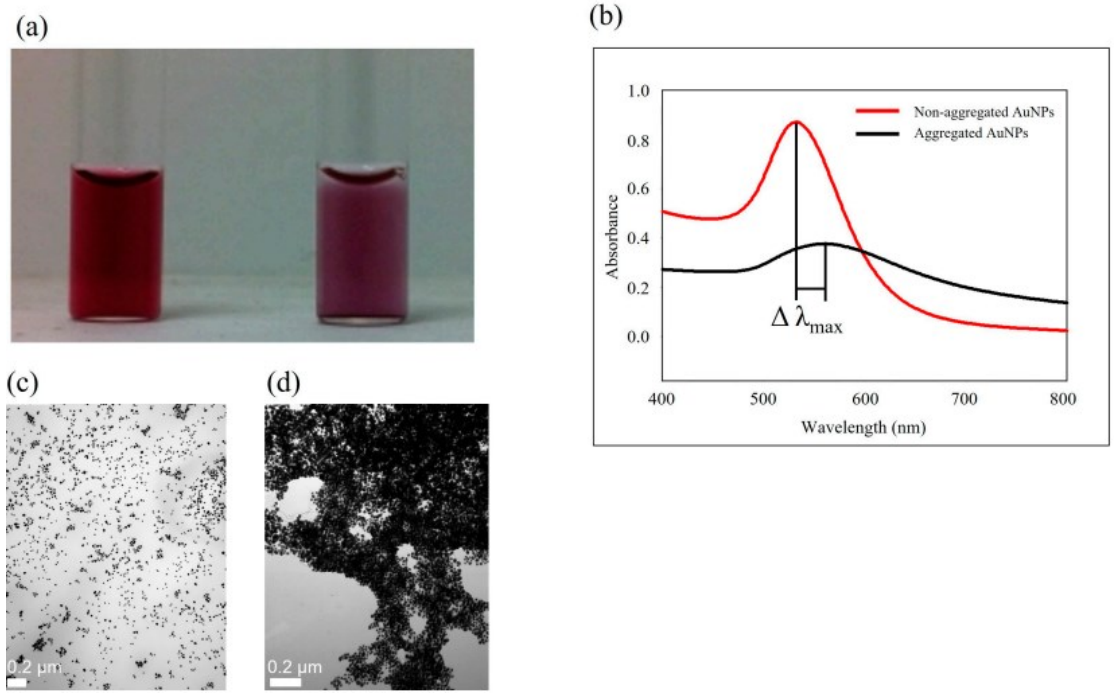

Figure 6. Color-aggregated AuNPs (right), Non-aggregated AuNPs (left). **(a)** AuNPs (aq) solutions after UV-Vis absorption spectra; Non-aggregated AuNPs (red), aggregated AuNPs (black); **(b)** TEM image (Non-aggregated AuNPs); **(c)** Aggregated AuNPs [37].

AuNPs decrease the limitation of detection location with high flexibility which

is a great advantage of this new method [37]. However, ensuring the reproducibility and sustainability of localized surface plasmon resonance (LSPR) with AuNPs poses great challenges. Another challenge is that to adjust sensor selectivity with a better understanding of colorimetric response by the influence of diverse matrix components. Moreover, before analysis preserving LSPR biosensor to apply an aggregation process is another concern. And the aggregation or unsustainability over time can have an effect over the reproducibility of the bio-sensor [37].

2.2.6. Integration with detection mechanism

A biosensor is comprised of two main elements: a bioreceptor that can identify the transducer target substance and a component that can transform the target substance into an electrical signal. The receptor can be aptamer, DNA, RNA, PNA etc. On the other hand, the transducer is optical, piezoelectric, electrochemical, etc. [22]. So, there is a basic concept of the full system of pathogen detection.

Immunosensors are an advanced type of biosensor that detect the pathogen via a stable complex formation between the pathogenic antigen and the capturing content such as antibody. As a result, in a rapid system, the attachment with the identifying agent and the identification can occur in a same time which was not possible in the conventional method. Immunosensors are of two types: Such as label-free and labeled sensors. Where the label-free sensors detect the chemical and physical changes of the identifying matters. Whereas labeled sensor generates a signal and resulting electron transfer. After that, the number of detected labels correlates with target analytes. The types of labeled sensors show a lower effect on non-specific signaling adsorption and higher sensitivity and versatility than label-free sensors (**Figure 7**) [26].

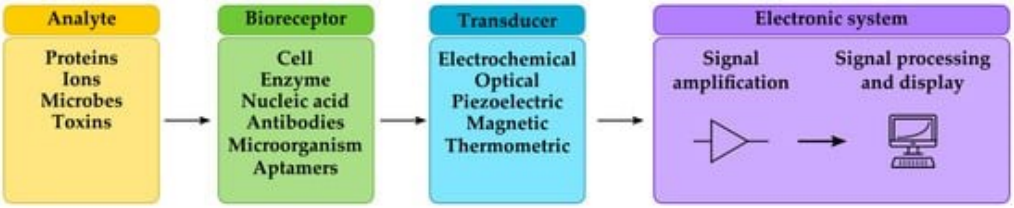

Figure 7. A basic structure of biosensor system [26].

Most of the electrochemical immunosensors follow aerometric measurement via adopting a constant potential. The working electrode is given a steady charge that is connected to a reference electrode, leading to the generation of electricity through electro reduction or electrochemical oxidation. There is a small amount of label-free immunosensors that are aerometric as most of the antigen, antibodies are inert to electricity and can't produce aerometric response. Some of the label-free immunosensors also belongs to potentiometric category immunosensors as they can form a complex between antigen and antibody. These potentiometric immunosensors allow simplicity of the operation [40].

2.3. Experimental procedures

In a recent application, a label-free electro-chemical sensor was used in the tumor detection study. The target was to detect necrosis factors using carbon nanotubes, ionic liquid which was Fullerene functionalized (**Figure 8**). The procedure was successfully

adopted for the serum samples.

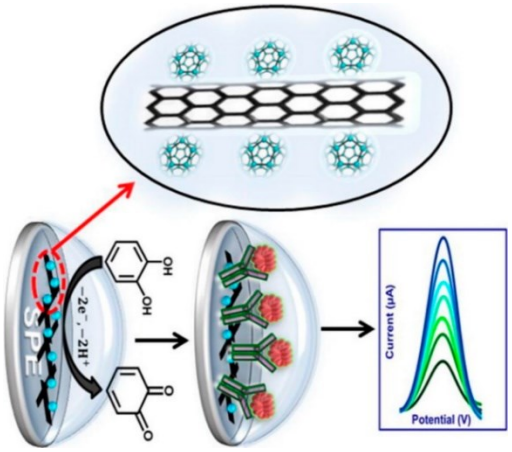

Figure 8. Fullerene-functionalized carbon nanotube [40].

In the application process cancer biomarker (AFO) was detected through a one-step electrochemical assay. Horseradish peroxidase-anti-AFP was operated over a nanogold functionalized graphene surface. Also, H2O2 was used in the solution which helps for antibody-antigen complexation. Using label-free sensors they successfully detected carcinoembryonic antigens based on using PtPd nanoparticles graphene quantum dots, Au nanoparticles [40].

3. Characterization and testing

3.1. Physical characterization and chemical characterization

In the development of innovative nanostructured biosensors for rapid pathogen detection in clinical diagnostics, thorough physical and chemical characterization of the nanomaterials is essential. This ensures that the biosensors possess the requisite properties for high sensitivity, selectivity, and stability. This section outlines the techniques employed for morphological, structural, and surface chemistry analysis of the nanostructured materials.

3.1.1. Morphological analysis (SEM, TEM) and structural analysis (XRD, FTIR)

1) Morphological analysis (SEM, TEM):

Scanning Electron Microscopy (SEM): SEM is utilized to examine the surface morphology of nanostructured biosensors at high magnifications. SEM images provide detailed insights into nanoparticle size, shape, and distribution on the biosensor surface, crucial for ensuring uniformity and reproducibility in pathogen detection [41]. Key observations using SEM include:

- Nanoparticle Size and Shape: SEM reveals whether nanoparticles are spherical, rod-like, or irregular.
- Distribution and Density: It indicates nanoparticle packing density and uniformity, impacting the biosensor's active surface area and, consequently, its sensitivity [42].

Transmission Electron Microscopy (TEM): TEM offers higher resolution

imaging than SEM, enabling detailed examination of nanoparticle internal structure, essential for understanding crystalline structure and identifying defects or irregularities [43]. Key observations using TEM include:

- Crystal Structure: TEM reveals lattice fringes, indicating nanoparticle crystallinity.
- Size Distribution: Precise measurements of nanoparticle size ensure uniformity, critical for consistent biosensor performance [44].

2) Structural Analysis (XRD, FTIR):

X-Ray Diffraction (XRD): XRD determines nanomaterial crystalline structure and phase composition, providing insights into crystal lattice parameters essential for material property understanding [45]. Key observations using XRD include:

- Phase Identification: Matching XRD patterns with reference standards identifies phases present in the sample.
- Crystallinity: Sharpness and intensity of XRD peaks indicate crystallinity levels, influencing biosensor performance [46].

Fourier Transform Infrared Spectroscopy (FTIR): FTIR identifies functional groups on nanomaterial surfaces, crucial for understanding surface chemistry and interactions with target pathogens [47]. Key observations using FTIR include:

- Functional Groups: FTIR spectra identify hydroxyl, carboxyl, and amine groups affecting biosensor surface properties and binding efficiency.
- Chemical Bonding: Changes in peak positions and intensities indicate chemical interactions between nanomaterials and surface modifications [48].

3.1.2. Surface chemistry (XPS, EDS)

X-ray Photoelectron Spectroscopy (XPS): XPS analyzes elemental composition and chemical states on nanomaterial surfaces, providing quantitative data essential for assessing surface properties and biosensor reactivity [49]. Key observations using XPS include:

- Elemental Composition: Detection and quantification of elements like carbon, oxygen, nitrogen, and metals on nanomaterial surfaces.
- Chemical States: XPS spectra reveal oxidation states and specific functional group presence, influencing biosensor performance [50].

Energy Dispersive Spectroscopy (EDS): EDS, coupled with SEM, provides elemental analysis confirming nanomaterial composition and distribution across biosensor surfaces [51]. Key observations using EDS include:

- Elemental Mapping: Mapping specific element distribution on biosensor surfaces to ensure uniformity and identify potential contamination.
- Quantitative Analysis: EDS spectra offer semi-quantitative elemental composition data complementing XPS analyses [52].

Employing such physical and chemical characterization techniques provides comprehensive insights into nanostructured biosensors' morphology, structure, and surface chemistry. This detailed analysis is crucial for optimizing biosensor design and enhancing their efficacy in clinical diagnostics.

3.2. Performance characterization

3.2.1. Sensitivity tests and limit of detection (LOD)

The result of the sensitivity test is the limit of detection (LOD), which is the minimal amount of an infectious agent that a nanostructured biosensor is able to reliably detect [8]. It is critical to take into account the sensor's reaction time during the sensitivity test, which is the amount of time required for the pathogen to cause a detectable signal [53]. In clinical settings, where prompt diagnosis can have a substantial influence on patient outcomes, prompt reaction times are especially significant [54]. Another factor is the sensor's linear response, which guarantees a proportionate correlation between the pathogen concentration and the signal intensity, enabling precise measurement of pathogen levels [55]. Additionally, other chemicals including salts, proteins, and other microbes that may be present in the sample matrix and cause interference must be taken into consideration by the sensitivity test. The performance of the biosensor may be impacted by this interference, hence complex biological samples, such as blood or urine, are frequently included in the test to mimic actual clinical settings. The sensor's resilience and dependability in real-world applications are ensured by assessing its performance under certain circumstances [55,56]. To increase the biosensor's sensitivity, cutting-edge strategies are also investigated, including signal amplification techniques and the application of highly specific biorecognition components. Using special nanomaterials that enhance the surface area available to contact and enhance the translation of the biological detection event into a quantifiable signal is one of them [57].

3.2.2. Specificity tests

To ascertain if a nanostructured biosensor can reliably distinguish the target pathogen from other non-target chemicals and microorganisms, the sensor must undergo a selectivity test [58]. Reliable diagnostics and the prevention of false positives are ensured by high selectivity. In order to verify that there is little to no signal generated by these non-target entities, cross-reactivity tests are performed by subjecting the biosensor to a variety of non-target bacteria that are found in the sample matrix while monitoring the response [59,60]. Additionally, negative controls are used, in which the biosensor is examined without the target pathogen present to ensure that there are no false positives [60]. Competitive binding tests are an additional component that involves subjecting the biosensor to mixtures of the target pathogen and similar non-target chemicals in order to assess its binding selectivity [61]. In order to guarantee precise detection in actual clinical settings, the performance of the sensor is also evaluated in complicated biological specimens such as urine or blood, which include a variety of interfering compounds [62]. The selectivity test findings are analyzed statistically to determine the positive and negative predictive value of the sensor. These numbers guarantee the biosensor's efficacy in precisely detecting the target pathogen in clinical diagnostics by offering a thorough evaluation of its diagnostic capabilities and dependability [63].

4. Results and discussion

4.1. Comparison with conventional diagnostic techniques

To diagnose pathogens in clinical diagnostics, conventional or traditional techniques such as immunoassays, electrochemical analysis, polymerase chain

reaction (PCR), colorimetric methods, culture-based procedures, and fluorescence polarization, are commonly used [8]. Some of the disadvantages of these methods include the need for specialized lab equipment, high costs, and lengthy processing times [64]. Novel nanostructured biosensor approaches primarily address the above-listed problems, offering significant advantages in terms of pathogen detection rate, sensitivity, and specificity [65].

Because nanostructured biosensors are more sensitive, pathogens can be found at lower concentrations. Additionally, they provide increased specificity, which facilitates the precise identification of certain disorders [8]. Nanostructured biosensors are able to identify infections in a couple of hours or minutes, as opposed to standard approaches that may take several days. This short turnaround is highly helpful when compared to the lengthy turnover periods [64]. Additionally, the longer processing periods, sophisticated equipment, and pricey chemicals needed for conventional procedures result in greater costs [65]. Conversely, biosensor methods are more affordable and intuitive to utilize. They are easier to use and require less specialist equipment to operate, even for those with less expertise. Nanostructured biosensors solve many of the drawbacks of existing pathogen detection techniques and offer a quicker, more affordable, and easier-to-use substitute [8].

Table 2. Examples of different traditional techniques used for pathogen detection.

Pathogen	Detection Technique	Duration of Detection	Limit of detection (LOD)	Reference
E. coli O157:H7	Paper-based enzyme-linked immunosorbent assay (p-ELISA)	3 h	10^4 CFU/mL	[66]
Salmonella	Real-time PCR	4 h	0.1 CFU/g	[67]
Zika virus	Real-time RT-PCR, ELISA (Plasma or urine sample)	Hours to days	Varies	[68]
Norovirus	Nanomaterial-based sandwich immunoassay method	One to several days	10 and 53 pfu/mL	[69]
Corona virus	RT-PCR	3–6 h	Varies	[70]

Table 3. Examples of nanomaterial-based biosensors for detection of pathogens.

Pathogen	Detection Technique	Duration of Detection	Limit of detection (LOD)	Reference
E. coli O157:H7	Gold nanoparticle-based biosensor	50 min–1 h	10^1 CFU/mL	[71]
Salmonella	Aptamer based biosensor made in combination with quantum dots and carbon nanoparticles.	1 h 20 min	35 CFU/mL	[72]
Zika virus	Novel graphene-based nano biosensor	4–8 min	0.45 nM	[73]
Norovirus	photoelectrochemical biosensor coupled with customized monoclonal antibody	30 min	2×10^{-10} g mL^{-1} (4.9 pM)	[74]
Coronavirus	Gold nanoparticles (AuNPs) based calorimetric biosensor	5 min	150 ng/mL	[75]

A comparison between traditional pathogen detection methods and the use of nanomaterial-based biosensors is presented in **Tables 2** and **3**, including their respective detection times and limits of detection. Numerous studies have demonstrated the effectiveness of nanomaterial-based biosensors in detecting various pathogens. For instance, gold nanoparticles have been utilized for the electrochemical detection of Escherichia coli, significantly enhancing sensitivity. Similarly, carbon

nanotube-based sensors have shown promise in the rapid identification of viruses like influenza through specific binding assays (**Table 3**).

Here, traditional methods take a long time and limit detection into dynamic range of pathogens comparatively to nanomaterial-based biosensors. These methods (ELISA, PCR, Immunoassay including other conventional techniques) take a very long time, even days to weeks to give a result, and some fail to detect low concentrations of the pathogens [76]. On the other hand, nano-based sensor detecting methods can detect within hour to a maximum of one day according to mentioned table. These methods can detect multiple pathogens simultaneously and are effective with high specificity and sensitivity. Moreover, some conventional methods require laborious work, special equipment [76]. However, conventional methods like PCR can be very accurate in results which offers higher advantages of high reproducibility, and sensibility with the broadest dynamic range [27]. Again, paper-based ELISA adapts the same function as traditional but with low cost, low sample, portability, and analytical flexibility [77].

4.2. Case studies and real-world applications

4.2.1. Detection of bacterial pathogens

Numerous varieties of nanostructures have been utilized in the creation of biosensors for identifying antibiotics and bacteria, in response to the substantial global health risk presented by bacterial infections, specifically those arising from Gram-negative microbes [78]. Scientists developed a nanostructured biosensor that can quickly identify bacterial infections such as *Salmonella spp., Staphylococcus aureus*, and *Escherichia coli* [8].The detector employs gold nanoparticles functionalized with particular antibodies against *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA), which is highly sensitively detected by colorimetric changes and can be analyzed in less than an hour [79,80].

The use of gold nanoparticles in lateral flow assays (LFAs) is now widespread. These tests use conjugated gold nanoparticles with antibodies that target particular antigens on the outermost layer of the virus, bacterium, or fungus. By means of capillary action, a clinical sample such as urine, blood, or a swab, that is placed to the testing strip migrates along it. The gold nanoparticle-antibody complexes attach to the bacterial antigens if the target bacteria are present. The nanoparticles cluster at a particular line on the test strip as a result of this binding event, changing its apparent color—usually showing as a purple or red line **(Figure 9)** [81–83].

Rapid findings for therapeutic action are possible with carbon nanotube-based sensors, which have proven to be able to identify *S. aureus* through alterations in electrical impulses [84]. In an effort to identify exotoxins produced by bacteria, one study developed a potentiometric nano-biosensor, while another study designed a biosensor utilizing the proteolytic ability of pathogen proteases [85,86]. Methicillin-resistant Staphylococcus aureus (MRSA) has a unique nanostructured biosensor as a result of these innovations, which meet important demands in clinical diagnostics [84]. Similarly, the use of magnetic nanoparticles (MNP) has enabled the successful early detection of sepsis, a potentially lethal condition often caused by infections caused by bacteria such as *Escherichia coli*. These nanoparticles are functionalized to adhere to bacterial cells in blood samples (**Figure 10**). They may then be magnetically isolated

and recognized using a range of methods, such as electrochemical OR fluorescent signals. By reducing diagnosis timeframes from days to a few hours, pathogen identification expedites the initiation of life-saving medicines [87,88].

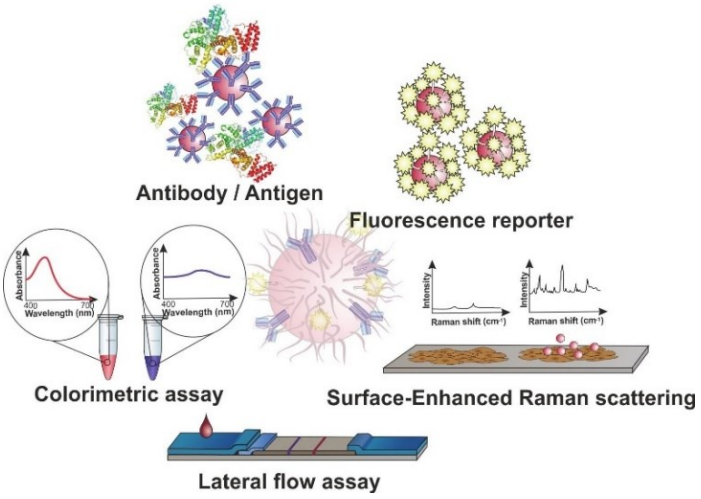

Figure 9. Basic principle of LFA [83].

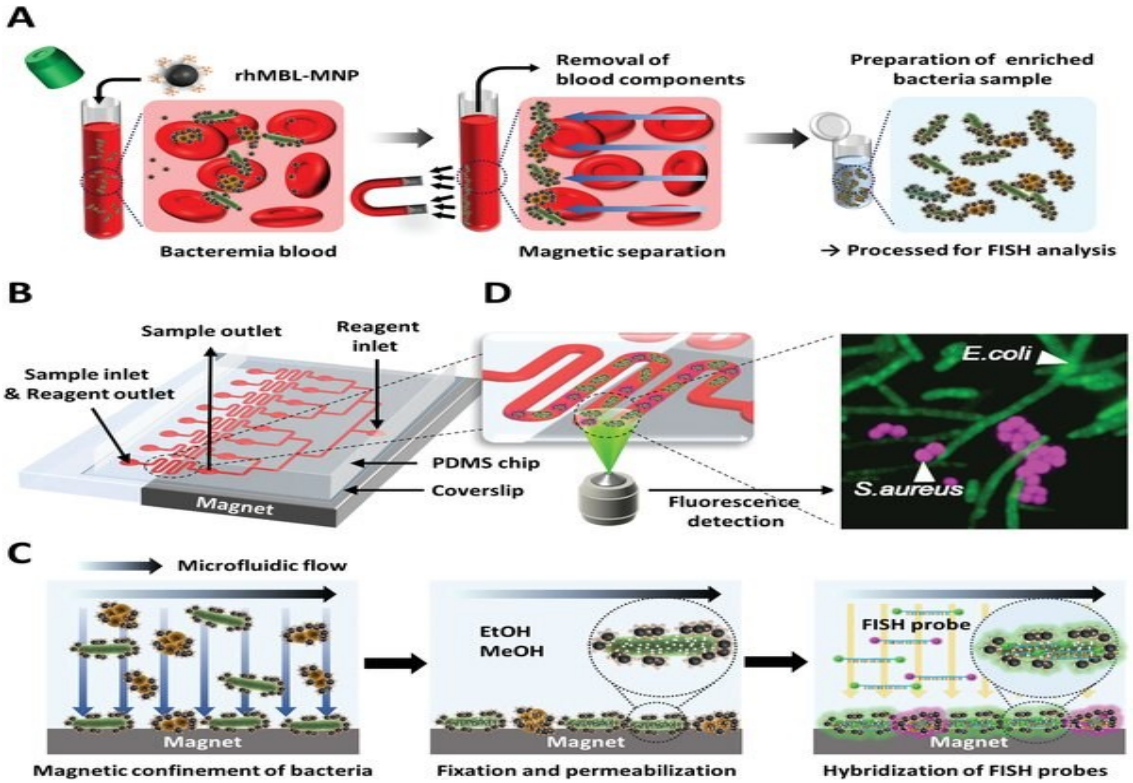

Figure 10. Quantitative detection of blood pathogens with μFISH and opsonin-coated MNP [89].

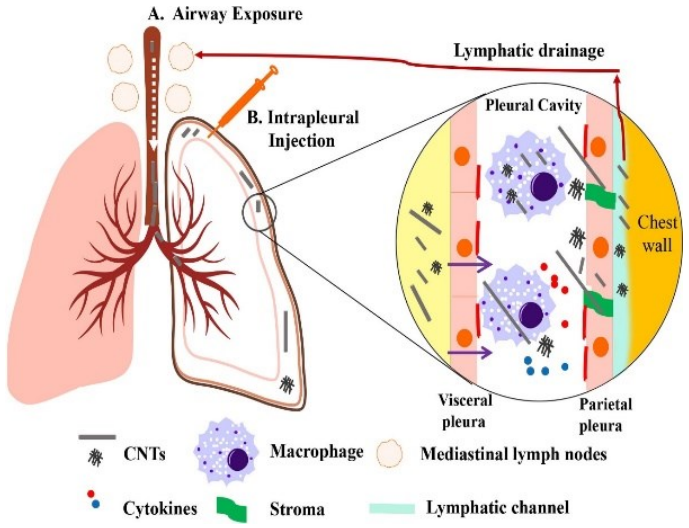

Figure 11. Diagrammatic representation of CNT outflow and pathological lesions caused by CNTs [90].

To concentrate bacteria identified in bacteremic blood, recombinant human mannose-binding lectin (rhMBL-MNP) combined with MNP exploits magnetic capture. The eight division channels, eight inlets, and eight outputs for samples and FISH reagents are features of the polydimethylsiloxane (PDMS) microfluidic system, which allows for high throughput. Next, before fluorescence-labeled FISH probes corresponding to the target microorganisms' sequences of rRNA hybridize, the MNP-captured bacteria are frozen, permeabilized, and magnetically trapped in the microfluidic channel. Lastly, fluorescence imaging of magnetically captured bacteria (*E. coli*: green, *S. aureus*: magenta) [89]. Carbon nanotubes (CNT) are being employed in identifying Pseudomonas aeruginosa, bacteria commonly found in the lungs of persons with cystic fibrosis (**Figure 11**), because of their exceptional electrical conductivity. These nanotubes are a part of biosensors, that provide a rapid and non-invasive means of identifying illnesses and setting up therapies by identifying alterations in their ability to conduct electricity in the event that a pathogen is identified [90,91].

In addition, innovative nanomaterials including quantum dots, zinc oxide nanostructures, and silicon nanowires have been created and used to biosensors to detect a range of bacterial illnesses [92]. Mycobacterium tuberculosis, the causal pathogen, is identified with high sensitivity and specificity utilizing quantum dots, which are known for their tunable fluorescence capabilities [93,94]. Zinc oxide nanostructures are used in biosensors which target Vibrio cholerae in order to enhance electrochemical detection methods [95]. However, Streptococcus pneumoniae sensors with silicon nanowires enable prompt detection of the pneumonia-causing germs [96].

4.2.2. Detection of viral pathogens

Many viruses are thought to have the potential to cause outbreaks in the future. The COVID-19 outbreak brought on by SARS-CoV-2 is the most recent example. Preventing an uncontrollably large-scale spread of the illness requires an early diagnosis.

So far, RT-PCR has been the most popular, appropriate, and efficient way to

detect SARS-CoV-2 infection. However, the process is expensive and time-consuming. A team of researchers has developed Lab-on-a-Chip biosensors for detecting SARS-CoV-2 [67]. Moreover, to identify influenza viruses, a unique biosensor evolved that employs gold nanoparticles infused with certain antibodies [97]. Additionally, a graphene-based biosensor was designed to identify the Zika virus in the blood specimens; it made use of graphene oxide nanosheets infused with Zika virus-specific DNA aptamers [98]. One additional major advancement is a silicon nanowire biosensor that can identify HIV quickly by using nanowires coated with the aptamers an which attach themselves particularly to HIV particles [64] and also by using gold nano particle the HIV can be detected [99,100]. When some antibodies or antigens interact with gold nanoparticles, their distinct optical characteristics enable a discernible color shift. These nanoparticles are coated by antibodies that bind to antigens associated with HIV found in patient blood samples for use in HIV diagnosis. As a result of this interaction, the gold nanoparticles cluster and produce a colorimetric signal which may be seen with the naked eye or identified using basic tools as shown in **Figure 9** [101]. Quantum dots (QD) are another novel way that nanomaterials are being used in viral diagnostics to identify viruses (**Figure 12**) such as COVID-19, HIV, and hepatitis. High-sensitivity biosensors can benefit greatly from the use of semiconductor nanocrystals known as quantum dots because of their strong and consistent fluorescence [102,103].

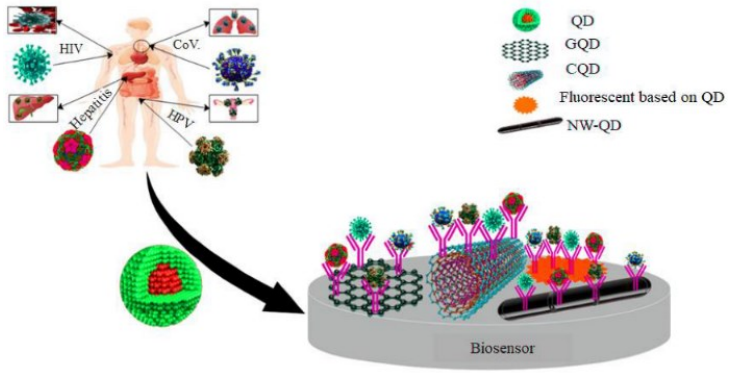

Figure 12. QD-based nanosensors to detect viruses [103].

4.2.3. Detection of fungal pathogens

Nanomaterials are transforming the diagnosis of fungal infections by offering state-of-the-art technologies that enhance the sensitivity, speed, and precision of clinical diagnostics. Numerous real-world examples have emerged, showcasing how these innovative materials are being integrated into diagnostic tools across multiple industries [6,104]. Gold nanoparticles are one well-known example of how to detect *Candida* infections, especially in immunocompromised individuals when a prompt diagnosis is essential [105]. Antibodies that bind to *Candida* antigens in samples from patients selectively are conjugated to gold nanoparticles. These nanoparticles link to the pathogen and cause a clearly visible color shift, making it possible to quickly and visually confirm the presence of the disease (**Figure 9**) [106]. Quantum dots are also employed in fluorescent biosensors for the purpose of identifying *Aspergillus* species, which cause serious respiratory infections. Because of their great sensitivity and

specificity, these biosensors enable earlier identification and better patient outcomes [107]. In order to identify *Cryptococcus* in human blood, nanoparticles with magnetic properties are also utilized. By facilitating the pathogen's separation and concentration, these particles greatly shorten the time needed for diagnosis [104,108].

Since these developments in nanostructured biosensors satisfy important demands in diagnostics for fungal infections, there is great potential for improving patient outcomes through timely and precise detection. They provide sensitive, fast, and targeted detection techniques [6].

5. Challenges and limitations

Notwithstanding their noteworthy benefits, innovative nanostructured biosensors encounter many obstacles and constraints in the field of clinical diagnostics. To guarantee accurateness and reliability in a variety of environments, one major obstacle is the requirement for rigorous standardization and validation processes [109]. A few elements that might impact the robustness and reproducibility of biosensor performance are sample matrix variability, interference from non-target molecules, and ambient conditions. Additionally, the cost-effectiveness and scalability of manufacturing processes provide two significant barriers to the widespread use of biosensor production in healthcare settings. Moreover, the challenge is ensuring user-friendliness and compatibility with existing diagnostic protocols [8]. Furthermore, nanostructured biosensors can detect things quickly, but there is still a technical challenge in maximizing sensitivity without sacrificing specificity. To fully realize the promise of nanostructured biosensors in transforming clinical diagnostics by offering quicker, more precise, and more affordable pathogen detection technologies, these obstacles must be overcome [109].

6. Future prospects

6.1. Potential improvements and innovations

The upcoming use of small-scale biosensors in medical testing shows great potential, especially for finding harmful organisms. These new biosensors use the special qualities of materials at a tiny scale, like their large surface area, ability to work well with living things, and improved ability to find harmful organisms even in very small amounts. Advancements in research will likely lead to the creation of more advanced and smaller biosensors that can quickly and accurately diagnose patients at the point of care. At this point, investing in the research, production, and marketing of intricate biomolecular sensors to meet the diverse needs of the healthcare industry becomes important.

Preventive treatments are preferred over treatments that are only administered after symptoms appear, as they require regular doctor visits that can only be maintained with a substantial investment of time and money spent at medical offices. Regular health monitoring through portable biosensors could potentially reduce or even eliminate the need for frequent visits to the doctor [110]. Creating biomolecular sensors to monitor specific biomolecules in real-time is a key step in reaching this future goal. These biological sensors and instant medical devices will enhance the

delivery of healthcare and the diagnosis of infectious diseases. Moreover, in the upcoming years, smart devices will have the capability to monitor various health indicators simultaneously and share the gathered information via a smartphone application. The progress of this technology will result in fast expansion, increased patient happiness, and improved customization in the healthcare field. If biosensors are coupled with IoT, AI, and 5G, it has the potential to make the sector more dependable, reactive, and personalized [15,110]. These innovations have the potential to result in timely and precise identification of communicable illnesses, enhancing the overall health outcomes of patients, and assisting in the efficient control of epidemics and pandemics. Furthermore, the fusion of nanostructured biosensors with digital health innovations will facilitate remote monitoring and data sharing, revolutionizing personalized medicine and public health surveillance.

6.2. Scalability and commercialization

The scalability of nanostructured biosensors for pathogen detection in clinical diagnosis holds significant promise for revolutionizing healthcare. Nanostructured biosensors, which utilize materials with nanoscale dimensions, offer enhanced sensitivity, specificity, and rapid response times due to their large surface area and unique physicochemical properties. Scalability concept, in this scenario, pertains to the capacity to manufacture a high volume of these biosensors without compromising their consistent functionality and dependability. Innovations in the field of nanofabrication methods, including lithography, self-assembly, and printing technologies, allow for the large-scale production of these sensors at an affordable rate [77,111]. Furthermore, the integration of nanostructured biosensors with microfluidic systems and portable electronic devices facilitates their deployment in point-of-care settings, making pathogen detection more accessible and timelier. As the technology continues to evolve, it is essential to overcome obstacles concerning standardization, reproducibility, and approval from regulatory bodies in order to fully maximize the capabilities of scalable biosensors with nanostructured materials in the field of clinical diagnostics. Biosensors made with nanostructures using methods that start from the smallest components may encounter challenges in terms of being able to consistently create nanoparticles, nanowires, or nanotubes with precise characteristics like shape, size, or electrical qualities on a larger scale [14]. Moving to commercialization, market acceptance is also crucial, necessitating collaborations with healthcare providers and clear demonstrations of the technology's advantages over existing diagnostic methods. Furthermore, investment in marketing, distribution channels, and customer education will be essential to drive adoption. With these considerations, the successful commercialization of nanostructured biosensors has the potential to enhance pathogen detection capabilities, improve patient outcomes, and reduce healthcare costs.

7. Conclusion

Detecting and diagnosing human diseases in their early stages is vital for successful treatment. Creating basic, highly responsive, and affordable diagnostic instruments like biosensors is essential for accurate detection of illnesses. Biosensors have a significant impact in a range of medical uses, such as monitoring diseases,

healthcare, preventative actions, patient data, and disease assessment. Recently, nanotechnology has been extensively employed in the development of sensors for biological applications. The fascination with nanostructured biosensors arises from their capability to detect a diverse array of compounds even at extremely minute concentrations. The use of nanomaterial-based biosensors has greatly improved upon conventional techniques, providing faster detection times and lower detection limits for various pathogens such as *E. coli*, *Salmonella*, *Zika virus*, *Norovirus*, and *Coronavirus*. Their reliability, portability, and potential for scalability and commercialization have made nano biosensors the preferred choice in recent times. However, it's important to note that traditional methods like PCR are still highly accurate, reproducible, and have a wide dynamic range. Additionally, paper-based ELISA offers similar functionality to traditional methods while also being cost-effective, portable, and flexible for analysis. The recent advancements in nano biosensors allow for unique features such as single molecule detection, multi-pathogen detection, and point-of-care testing. Moreover, the growing use of affordable and rapid nano biosensor technology has generated interest in the detection of fungal pathogens. Currently, limitations include technical challenges in maximizing sensitivity without sacrificing specificity, ensuring user-friendliness, and compatibility with existing diagnostic protocols. Efforts are being made to address these limitations. Therefore, as this area advances, it is probable that current methods of diagnosis will become obsolete, making room for a fresh wave of inexpensive, dependable, easy-to-use, and extremely sensitive diagnostic tools. This evolution could result in a rise in the accessibility of point-of-care diagnostics and diagnostic instruments for patient utilization. Although it is uncertain whether a solitary nano biosensor design will prevail or if several designs will move towards commercialization, these sensors will likely transform the identification of pathogens and illnesses.

Conflict of interest: The authors declare no conflict of interest.

References

1. Dye C. After 2015: infectious diseases in a new era of health and development. Philosophical Transactions of the Royal Society B: Biological Sciences. 2014; 369(1645): 20130426. doi: 10.1098/rstb.2013.0426
2. Cesewski E, Johnson BN. Electrochemical biosensors for pathogen detection. Biosensors and Bioelectronics. 2020; 159: 112214. doi: 10.1016/j.bios.2020.112214
3. Mokhtarzadeh A, Eivazzadeh-Keihan R, Pashazadeh P, et al. Nanomaterial-based biosensors for detection of pathogenic virus. TrAC Trends in Analytical Chemistry. 2017; 97: 445-457. doi: 10.1016/j.trac.2017.10.005
4. Ahmed A, Rushworth JV, Hirst NA, et al. Biosensors for Whole-Cell Bacterial Detection. Clinical Microbiology Reviews. 2014; 27(3): 631-646. doi: 10.1128/cmr.00120-13
5. Dutta S, Ray U. Paratracheal abscess by plant fungus Chondrostereum purpureum- first case report of human infection. Medical Mycology Case Reports. 2023; 40: 30-32. doi: 10.1016/j.mmcr.2023.03.001
6. Stoia D, De Sio L, Petronella F, et al. Recent advances towards point-of-care devices for fungal detection: Emphasizing the role of plasmonic nanomaterials in current and future technologies. Biosensors and Bioelectronics. 2024; 255: 116243. doi: 10.1016/j.bios.2024.116243
7. Vidyadharani G, Vijaya Bhavadharani HK, Sathishnath P, et al. Present and pioneer methods of early detection of food borne pathogens. Journal of Food Science and Technology. 2021; 59(6): 2087-2107. doi: 10.1007/s13197-021-05130-4
8. Castillo-Henríquez L, Brenes-Acuña M, Castro-Rojas A, et al. Biosensors for the Detection of Bacterial and Viral Clinical Pathogens. Sensors. 2020; 20(23): 6926. doi: 10.3390/s20236926

9. Chao J, Zhu D, Zhang Y, et al. DNA nanotechnology-enabled biosensors. Biosensors and Bioelectronics. 2016; 76: 68-79. DOI: 10.1016/j.bios.2015.07.00
10. Clark LC, Lyons C. Electrode systems for continuous monitoring in cardiovascular surgery. Annals of the New York Academy of Sciences. 1962; 102(1): 29-45. doi: 10.1111/j.1749-6632.1962.tb13623.x
11. Awang MS, Bustami Y, Hamzah HH, et al. Advancement in Salmonella Detection Methods: From Conventional to Electrochemical-Based Sensing Detection. Biosensors. 2021; 11(9): 346. doi: 10.3390/bios11090346
12. Yunus G, Singh R, Raveendran S, et al. Electrochemical biosensors in healthcare services: bibliometric analysis and recent developments. PeerJ. 2023; 11: e15566. doi: 10.7717/peerj.15566
13. Choi C. Integrated nanobiosensor technology for biomedical application. Nanobiosensors in Disease Diagnosis. 2012: 1. doi: 10.2147/ndd.s26422
14. Bellan LM, Wu D, Langer RS. Current trends in nanobiosensor technology. WIREs Nanomedicine and Nanobiotechnology. 2011; 3(3): 229-246. doi: 10.1002/wnan.136
15. Kulkarni MB, Ayachit NH, Aminabhavi TM. Recent Advancements in Nanobiosensors: Current Trends, Challenges, Applications, and Future Scope. Biosensors. 2022; 12(10): 892. doi: 10.3390/bios12100892
16. Malik S, Singh J, Goyat R, et al. Nanomaterials-based biosensor and their applications: A review. Heliyon. 2023; 9(9): e19929. doi: 10.1016/j.heliyon.2023.e19929
17. Choi HK, Yoon J. Nanotechnology-Assisted Biosensors for the Detection of Viral Nucleic Acids: An Overview. Biosensors. 2023; 13(2): 208. doi: 10.3390/bios13020208
18. Naresh V, Lee N. A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. Sensors. 2021; 21(4): 1109. doi: 10.3390/s21041109
19. Ding R, Chen Y, Wang Q, et al. Recent advances in quantum dots-based biosensors for antibiotics detection. J Pharm Anal 2022; 12: 355–364. doi: 10.1016/j.jpha.2021.08.002.
20. Norouzi M, Ghobadi MZ, Golmimi M, et al. Quantum Dot-Based Biosensor for the Detection of Human T-Lymphotropic Virus-1. Analytical Letters. 2017; 50(15): 2402-2411. doi: 10.1080/00032719.2017.1287714
21. Karim SSA, Dee CF, Majlis BY, et al. Recent Progress on Fabrication of Zinc Oxide Nanorod-based Field Effect Transistor Biosensors. Sains Malaysiana. 2019; 48(6): 1301-1310. doi: 10.17576/jsm-2019-4806-19
22. Singh R, Mukherjee MD, Sumana G, et al. Biosensors for pathogen detection: A smart approach towards clinical diagnosis. Sensors and Actuators B: Chemical. 2014; 197: 385-404. doi: 10.1016/j.snb.2014.03.005
23. Thakare S, Shaikh A, Bodas D, et al. Application of dendrimer-based nanosensors in immunodiagnosis. Colloids and Surfaces B: Biointerfaces. 2022; 209: 112174. doi: 10.1016/j.colsurfb.2021.112174
24. Achi F, Attar AM, Ait Lahcen A. Electrochemical nanobiosensors for the detection of cancer biomarkers in real samples: Trends and challenges. TrAC Trends in Analytical Chemistry. 2024; 170: 117423. doi: 10.1016/j.trac.2023.117423
25. Zhu C, Yang G, Li H, et al. Electrochemical Sensors and Biosensors Based on Nanomaterials and Nanostructures. Analytical Chemistry. 2014; 87(1): 230-249. doi: 10.1021/ac5039863
26. Janik-Karpinska E, Ceremuga M, Niemcewicz M, et al. Immunosensors—The Future of Pathogen Real-Time Detection. Sensors. 2022; 22(24): 9757. doi: 10.3390/s22249757
27. Ahangari A, Mahmoodi P, Mohammadzadeh A. Advanced nano biosensors for rapid detection of zoonotic bacteria. Biotechnology and Bioengineering. 2022; 120(1): 41-56. doi: 10.1002/bit.28266
28. Ahovan ZA, Hashemi A, De Plano LM, et al. Bacteriophage Based Biosensors: Trends, Outcomes and Challenges. Nanomaterials. 2020; 10(3): 501. doi: 10.3390/nano10030501
29. Wang X, Zhou J, Wang H. Bioreceptors as the key components for electrochemical biosensing in medicine. Cell Reports Physical Science. 2024; 5(2): 101801. doi: 10.1016/j.xcrp.2024.101801
30. Ngashangva L, Hemdan B, El-Liethy M, et al. Emerging Bioanalytical Devices and Platforms for Rapid Detection of Pathogens in Environmental Samples. Micromachines. 2022; 13(7): 1083. doi: 10.3390/mi13071083
31. Sun F, Zhang J, Yang Q, et al. Quantum dot biosensor combined with antibody and aptamer for tracing food-borne pathogens. Food Quality and Safety. 2021; 5. doi: 10.1093/fqsafe/fyab019
32. Gao J, Chakraborthy A, He S, et al. Graphene-Based Sensors for the Detection of Microorganisms in Food: A Review. Biosensors. 2023; 13(6): 579. doi: 10.3390/bios13060579
33. Jiang Z, Feng B, Xu J, et al. Graphene biosensors for bacterial and viral pathogens. Biosensors and Bioelectronics. 2020; 166: 112471. doi: 10.1016/j.bios.2020.112471

34. Mustafa F, Hassan R, Andreescu S. Multifunctional Nanotechnology-Enabled Sensors for Rapid Capture and Detection of Pathogens. Sensors. 2017; 17(9): 2121. doi: 10.3390/s17092121
35. Zheng X, Gao S, Wu J, et al. Recent Advances in Aptamer-Based Biosensors for Detection of Pseudomonas aeruginosa. Frontiers in Microbiology. 2020; 11. doi: 10.3389/fmicb.2020.605229
36. Léguillier V, Heddi B, Vidic J. Recent Advances in Aptamer-Based Biosensors for Bacterial Detection. Biosensors. 2024; 14(5): 210. doi: 10.3390/bios14050210
37. Park SH, You Y. Gold Nanoparticle-Based Colorimetric Biosensing for Foodborne Pathogen Detection. Foods. 2023; 13(1): 95. doi: 10.3390/foods13010095
38. Wang P, Yu G, Wei J, et al. A single thiolated-phage displayed nanobody-based biosensor for label-free detection of foodborne pathogen. Journal of Hazardous Materials. 2023; 443: 130157. doi: 10.1016/j.jhazmat.2022.130157
39. Jiang T, Liu R, Huang X, et al. Colorimetric screening of bacterial enzyme activity and inhibition based on the aggregation of gold nanoparticles. Chemical Communications. 2009; (15): 1972. doi: 10.1039/b818853j
40. Mollarasouli F, Kurbanoglu S, Ozkan SA. The Role of Electrochemical Immunosensors in Clinical Analysis. Biosensors. 2019; 9(3): 86. doi: 10.3390/bios9030086
41. Goldstein JI, Newbury DE, Echlin P, et al. Scanning Electron Microscopy and X-Ray Microanalysis. Springer US; 2003. doi: 10.1007/978-1-4615-0215-9
42. Zhou W, Wang ZL. Scanning Microscopy for Nanotechnology. Springer New York; 2007. doi: 10.1007/978-0-387-39620-0
43. Pennycook SJ. Transmission Electron Microscopy: A Textbook for Materials Science, Second Edition by David B. Williams and C. Barry Carter. Microscopy and Microanalysis. 2010; 16: 111.
44. Egerton RF. Physical principles of electron microscopy: An introduction to TEM, SEM, and AEM. Springer; 2005.
45. Cullity BD. Elements of X-ray Diffraction - Bernard Dennis Cullity. Google Books. 1956.
46. Jenkins R, Snyder RL. Introduction to X-ray Powder Diffractometry. Wiley-Interscience; 1996. doi: 10.1002/9781118520994
47. Griffiths PR. The Early Days of Commercial FT-IR Spectrometry: A Personal Perspective. Applied Spectroscopy. 2017; 71(3): 329-340. doi: 10.1177/0003702816683529
48. Stuart B. Infrared Spectroscopy. Analytical Techniques in Forensic Science. John Wiley & Sons, Inc. 2020: 145-160. doi: 10.1002/9781119373421.ch7
49. Bubert H, Rivière JC, Werner WSM. X-Ray Photoelectron Spectroscopy (XPS). In: Surface and Thin Film Analysis. Wiley; 2011. pp. 7-41. doi: 10.1002/9783527636921.ch2
50. Skoog DA, Holler FJ, Crouch SR, et al. Principal of Instrumental Analysis, 7th ed. Sunder College Publisher, New York; 2017.
51. Watts JF, Wolstenholme J. An Introduction to Surface Analysis by XPS and AES. Wiley; 2003. doi: 10.1002/0470867930
52. Karakaş İ, Sağır L. B, Hacıoğlu Doğru N. Biological activities of green synthesis silver nanoparticles by Plantago lanceolata L. leaves. GSC Biological and Pharmaceutical Sciences. 2023; 22(2): 290-296. doi: 10.30574/gscbps.2023.22.2.0079
53. Yadav S, Parihar A, Sadique MA, et al. Emerging Point-of-Care Optical Biosensing Technologies for Diagnostics of Microbial Infections. ACS Applied Optical Materials. 2023; 1(7): 1245-1262. doi: 10.1021/acsaom.3c00129
54. Deng J, Zhao S, Liu Y, et al. Nanosensors for Diagnosis of Infectious Diseases. ACS Applied Bio Materials. 2020; 4(5): 3863-3879. doi: 10.1021/acsabm.0c01247
55. Noah NM, Ndangili PM. Current Trends of Nanobiosensors for Point-of-Care Diagnostics. Journal of Analytical Methods in Chemistry. 2019; 2019: 1-16. doi: 10.1155/2019/2179718
56. Ya N, Zhang D, Wang Y, et al. Recent advances of biocompatible optical nanobiosensors in liquid biopsy: towards early non-invasive diagnosis. Nanoscale. 2024; 16(29): 13784-13801. doi: 10.1039/d4nr01719f
57. Fu Y, Liu T, Wang H, et al. Applications of nanomaterial technology in biosensing. Journal of Science: Advanced Materials and Devices. 2024; 9(2): 100694. doi: 10.1016/j.jsamd.2024.100694
58. Welch EC, Powell JM, Clevinger TB, et al. Advances in Biosensors and Diagnostic Technologies Using Nanostructures and Nanomaterials. Advanced Functional Materials. 2021; 31(44). doi: 10.1002/adfm.202104126
59. Antiochia R. Nanobiosensors as new diagnostic tools for SARS, MERS and COVID-19: from past to perspectives. Microchimica Acta. 2020; 187(12). doi: 10.1007/s00604-020-04615-x
60. Khan A, Rao TS. Nanobiosensors for virus detection in the environment. In: Nanomaterials for Air Remediation. ResearchGate; 2020. pp. 61-87. doi: 10.1016/b978-0-12-818821-7.00004-x

61. Islam MA, Karim A, Ethiraj B, et al. Antimicrobial peptides: Promising alternatives over conventional capture ligands for biosensor-based detection of pathogenic bacteria. Biotechnology Advances. 2022; 55: 107901. doi: 10.1016/j.biotechadv.2021.107901
62. Kumari M, Gupta V, Kumar N, et al. Microfluidics-Based Nanobiosensors for Healthcare Monitoring. Molecular Biotechnology. 2023; 66(3): 378-401. doi: 10.1007/s12033-023-00760-9
63. Vakili S, Samare-Najaf M, Dehghanian A, et al. Gold Nanobiosensor Based on the Localized Surface Plasmon Resonance is Able to Diagnose Human Brucellosis, Introducing a Rapid and Affordable Method. Nanoscale Research Letters. 2021; 16(1). doi: 10.1186/s11671-021-03600-4
64. Lifson MA, Ozen MO, Inci F, et al. Advances in biosensing strategies for HIV-1 detection, diagnosis, and therapeutic monitoring. Advanced Drug Delivery Reviews. 2016; 103: 90-104. doi: 10.1016/j.addr.2016.05.018
65. Valenzuela-Amaro HM, Aguayo-Acosta A, Meléndez-Sánchez ER, et al. Emerging applications of nanobiosensors in pathogen detection in water and food. Biosensors 2023;13:922. doi: 10.3390/bios13100922.
66. Pang B, Zhao C, Li L, et al. Development of a low-cost paper-based ELISA method for rapid Escherichia coli O157: H7 detection. Analytical Biochemistry. 2018; 542: 58-62. doi: 10.1016/j.ab.2017.11.010
67. Hyeon JY, Deng X. Rapid detection of Salmonella in raw chicken breast using real-time PCR combined with immunomagnetic separation and whole genome amplification. Food Microbiology. 2017; 63: 111-116. doi: 10.1016/j.fm.2016.11.007
68. Herrada CA, Kabir MdA, Altamirano R, et al. Advances in Diagnostic Methods for Zika Virus Infection. Journal of Medical Devices. 2018; 12(4). doi: 10.1115/1.4041086
69. Alhadrami HA, Al-Amer S, Aloraij Y, et al. Development of a Simple, Fast, and Cost-Effective Nanobased Immunoassay Method for Detecting Norovirus in Food Samples. ACS Omega. 2020; 5(21): 12162-12165. doi: 10.1021/acsomega.0c00502
70. Carter LJ, Garner LV, Smoot JW, et al. Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Central Science. 2020; 6(5): 591-605. doi: 10.1021/acscentsci.0c00501
71. Wang Y, Alocilja EC. Gold nanoparticle-labeled biosensor for rapid and sensitive detection of bacterial pathogens. Journal of Biological Engineering. 2015; 9(1). doi: 10.1186/s13036-015-0014-z
72. Duan N, Wu S, Dai S, et al. Simultaneous detection of pathogenic bacteria using an Aptamer based biosensor and dual fluorescence resonance energy transfer from quantum dots to carbon nanoparticles. Microchimica Acta. 2014; 182(5-6): 917-923. doi: 10.1007/s00604-014-1406-3
73. Afsahi S, Lerner MB, Goldstein JM, et al. Novel graphene-based biosensor for early detection of Zika virus infection. Biosensors and Bioelectronics. 2018; 100: 85-88. doi: 10.1016/j.bios.2017.08.051
74. Guo J, Liu D, Yang Z, et al. A photoelectrochemical biosensor for rapid and ultrasensitive norovirus detection. Bioelectrochemistry. 2020; 136: 107591. doi: 10.1016/j.bioelechem.2020.107591
75. Behrouzi K, Lin L. Gold nanoparticle based plasmonic sensing for the detection of SARS-CoV-2 nucleocapsid proteins. Biosensors and Bioelectronics. 2022; 195: 113669. doi: 10.1016/j.bios.2021.113669
76. Mazzu-Nascimento T, Morbioli GG, Milan LA, et al. Improved assessment of accuracy and performance indicators in paper-based ELISA. Analytical Methods. 2017; 9(18): 2644-2653. doi: 10.1039/c7ay00505a
77. Fruncillo S, Su X, Liu H, et al. Lithographic Processes for the Scalable Fabrication of Micro- and Nanostructures for Biochips and Biosensors. ACS Sensors. 2021; 6(6): 2002-2024. doi: 10.1021/acssensors.0c02704
78. Ferreira D, Seca AML, Silva AMS. Targeting human pathogenic bacteria by siderophores: A proteomics review. Journal of Proteomics. 2016; 145: 153-166. doi: 10.1016/j.jprot.2016.04.006
79. Zhao VXT, Wong TI, Zheng XT, et al. Colorimetric biosensors for point-of-care virus detections. Materials Science for Energy Technologies. 2020; 3: 237-249. doi: 10.1016/j.mset.2019.10.002
80. Chan WS, Tang BSF, Boost MV, et al. Detection of methicillin-resistant Staphylococcus aureus using a gold nanoparticle-based colourimetric polymerase chain reaction assay. Biosensors and Bioelectronics. 2014; 53: 105-111. doi: 10.1016/j.bios.2013.09.027
81. Andrade S, Ramalho MJ, Santos SB, et al. Fighting Methicillin-Resistant Staphylococcus aureus with Targeted Nanoparticles. International Journal of Molecular Sciences. 2023; 24(10): 9030. doi: 10.3390/ijms24109030
82. Fong WK, Modrusan Z, Mcnevin JP, et al. Rapid Solid-Phase Immunoassay for Detection of Methicillin-Resistant Staphylococcus aureus Using Cycling Probe Technology. Journal of Clinical Microbiology. 2000; 38(7): 2525-2529. doi: 10.1128/jcm.38.7.2525-2529.2000

83. Fernandes AR, Baptista PV. Gold Nanoparticles for Diagnostics. Material MattersTM Publications. Available online: https://www.sigmaaldrich.com/BD/en/technical-documents/technical-article/materials-science-and-engineering/biosensors-and-imaging/gold-nanoparticles-for-biomolecular-diagnostics (accessed on 23 August 2024).
84. Hernández R, Vallés C, Benito AM, et al. Graphene-based potentiometric biosensor for the immediate detection of living bacteria. Biosensors and Bioelectronics. 2014; 54: 553-557. doi: 10.1016/j.bios.2013.11.053
85. Ahari H, Hedayati M, Akbari-adergani B, et al. Staphylococcus aureus exotoxin detection using potentiometric nanobiosensor for microbial electrode approach with the effects of pH and temperature. International Journal of Food Properties. 2017: 1-10. doi: 10.1080/10942912.2017.1347944
86. Suaifan GARY, Alhogail S, Zourob M. Rapid and low-cost biosensor for the detection of Staphylococcus aureus. Biosensors and Bioelectronics. 2017; 90: 230-237. doi: 10.1016/j.bios.2016.11.047
87. Allafchian A, Hosseini SS. Antibacterial magnetic nanoparticles for therapeutics: a review. IET Nanobiotechnology. 2019; 13(8): 786-799. doi: 10.1049/iet-nbt.2019.0146
88. Vasconcelos I, Santos T. Nanotechnology Applications in Sepsis: Essential Knowledge for Clinicians. Pharmaceutics. 2023; 15(6): 1682. doi: 10.3390/pharmaceutics15061682
89. Lee MS, Hyun H, Park I, et al. Quantitative Fluorescence in Situ Hybridization (FISH) of Magnetically Confined Bacteria Enables Early Detection of Human Bacteremia. Small Methods. 2022; 6(3). doi: 10.1002/smtd.202101239
90. Zhang C, Wu L, de Perrot M, et al. Carbon Nanotubes: A Summary of Beneficial and Dangerous Aspects of an Increasingly Popular Group of Nanomaterials. Frontiers in Oncology. 2021; 11. doi: 10.3389/fonc.2021.693814
91. Walling BE, Kuang Z, Hao Y, et al. Helical Carbon Nanotubes Enhance the Early Immune Response and Inhibit Macrophage-Mediated Phagocytosis of Pseudomonas aeruginosa. PLoS ONE. 2013; 8(11): e80283. doi: 10.1371/journal.pone.0080283
92. Ambhorkar P, Wang Z, Ko H, Lee S, Koo K-I, Kim K, et al. Nanowire-Based biosensors: From growth to applications. Micromachines 2018;9:679. doi: 10.3390/mi9120679.
93. Bhattacharyya D, Sarswat PK, Free ML. Quantum dots and carbon dots based fluorescent sensors for TB biomarkers detection. Vacuum. 2017; 146: 606-613. doi: 10.1016/j.vacuum.2017.02.003
94. Abdel-Salam M, Omran B, Whitehead K, et al. Superior Properties and Biomedical Applications of Microorganism-Derived Fluorescent Quantum Dots. Molecules. 2020; 25(19): 4486. doi: 10.3390/molecules25194486
95. Napi MLM, Sultan SM, Ismail R, et al. Electrochemical-Based Biosensors on Different Zinc Oxide Nanostructures: A Review. Materials. 2019; 12(18): 2985. doi: 10.3390/ma12182985
96. Wang F, Wang Y, Liu X, et al. Rapid, Simple, and Highly Specific Detection of Streptococcus pneumoniae With Visualized Recombinase Polymerase Amplification. Frontiers in Cellular and Infection Microbiology. 2022; 12. doi: 10.3389/fcimb.2022.878881
97. Nidzworski D, Pranszke P, Grudniewska M, et al. Universal biosensor for detection of influenza virus. Biosensors and Bioelectronics. 2014; 59: 239-242. doi: 10.1016/j.bios.2014.03.050
98. Chaudhari A, Dandekar P. Graphene-based biosensors for the detection of Zika virus. In: Zika Virus Impact, Diagnosis, Control, and Models. Academic Press. 2021; pp. 263–272. doi: 10.1016/b978-0-12-820267-8.00025-x
99. Thongkum W, Hadpech S, Tawon Y, et al. Semi-quantification of HIV-1 protease inhibitor concentrations in clinical samples of HIV-infected patients using a gold nanoparticle-based immunochromatographic assay. Analytica Chimica Acta. 2019; 1071: 86-97. doi: 10.1016/j.aca.2019.04.060
100. Kumar A, Mazinder Boruah B, Liang XJ. Gold Nanoparticles: Promising Nanomaterials for the Diagnosis of Cancer and HIV/AIDS. Journal of Nanomaterials. 2011; 2011: 1-17. doi: 10.1155/2011/202187
101. Gulati S, Singh P, Diwan A, et al. Functionalized gold nanoparticles: promising and efficient diagnostic and therapeutic tools for HIV/AIDS. RSC Medicinal Chemistry. 2020; 11(11): 1252-1266. doi: 10.1039/d0md00298d
102. Shi W, Li K, Zhang Y. The Advancement of Nanomaterials for the Detection of Hepatitis B Virus and Hepatitis C Virus. Molecules. 2023; 28(20): 7201. doi: 10.3390/molecules28207201
103. Mousavi SM, Hashemi SA, Yari Kalashgrani M, et al. The Pivotal Role of Quantum Dots-Based Biomarkers Integrated with Ultra-Sensitive Probes for Multiplex Detection of Human Viral Infections. Pharmaceuticals. 2022; 15(7): 880. doi: 10.3390/ph15070880
104. Hussain KK, Malavia D, Johnson E M, et al. Biosensors and Diagnostics for Fungal Detection. Journal of Fungi. 2020; 6(4): 349. doi: 10.3390/jof6040349

105. Seong M, Lee DG. Reactive oxygen species-independent apoptotic pathway by gold nanoparticles in Candida albicans. Microbiological Research. 2018; 207: 33-40. doi: 10.1016/j.micres.2017.11.003
106. Clack K, Sallam M, Matheson C, et al. Towards a Wearable Feminine Hygiene Platform for Detection of Invasive Fungal Pathogens via Gold Nanoparticle Aggregation. Micromachines. 2024; 15(7): 899. doi: 10.3390/mi15070899
107. Kattke MD, Gao EJ, Sapsford KE, et al. FRET-Based Quantum Dot Immunoassay for Rapid and Sensitive Detection of Aspergillus amstelodami. Sensors. 2011; 11(6): 6396-6410. doi: 10.3390/s110606396
108. Niemirowicz K, Durnaś B, Tokajuk G, et al. Formulation and candidacidal activity of magnetic nanoparticles coated with cathelicidin LL-37 and ceragenin CSA-13. Scientific Reports. 2017; 7(1). doi: 10.1038/s41598-017-04653-1
109. Prabowo BA, Cabral PD, Freitas P, et al. The Challenges of Developing Biosensors for Clinical Assessment: A Review. Chemosensors. 2021; 9(11): 299. doi: 10.3390/chemosensors9110299
110. Haleem A, Javaid M, Singh RP, et al. Biosensors applications in medical field: A brief review. Sensors International. 2021; 2: 100100. doi: 10.1016/j.sintl.2021.100100
111. Kang H, Lee D, Yang Y, et al. Emerging low-cost, large-scale photonic platforms with soft lithography and self-assembly. Photonics Insights. 2023; 2(2): R04. doi: 10.3788/pi.2023.r04