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Review Article

Nanomaterials in designing biosensor for diagnosis of animal disease - A review

M. S. Murtuza[1*], S. Batabyal[2], A. Maity[2], S. Polley[2], S. S. Kesh[3], S. Biswas[2], M. Habib[2], A. Palli[1], L. Naveen[4], C. B. Reddy[5] and D. Singh[2]

[1]Department of Veterinary Physiology and Biochemistry, School of Veterinary and Animal Sciences, Centurion University of Technology and Managment, Paralakhemundi, Gajapati - 761 211, Odisha, India; [2]Department of Veterinary Biochemistry, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Science, 37 K. B. Sarani, Kolkata - 700 037, West Bengal, India; [3]Department of Veterinary Clinical Complex, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Science, 37 K. B. Sarani, Kolkata - 700 037, West Bengal, India; [4]Department of Veterinary Biochemistry, College of Veterinary Science (Mamnoor), P. V. Narshima Rao Telengana Veterinary University, Hyderabad - 506 166, Telengana, India; [5]Department of Veterinary Surgery and Radiology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Science, 37 K. B. Sarani, Kolkata - 700 037, West Bengal, India

Abstract

Disease diagnosis is an important factor for the well-being of an individual healthcare. Different disease diagnostic equipments are available for the recognition of the specific diseases. Technological advancement has led to the development of the point-of-care diagnostic tools such as biosensors. With the emergence of nanotechnology, the use of various nanomaterials in the biosensor designing provides a platform for a more sensitive as well as specific diagnostic approach. The nanomaterials mainly act as bridge between the biological recognition element and the transducer, magnifying the biological interaction generating a remarkable signal even at low concentration of analytes. As the global animal health is of critical concern, the addition of nanomaterial in the designing of biosensor will be helpful in rapid disease diagnosis. These nanomaterials-based biosensors can resolve the limitations of traditional diagnostic methods as they can be easily carried into the field, thus providing a point of care testing at an early stage of infectious diseases.

Keywords: Animal disease diagnosis, Bioreceptor, Biosensors, Nano sensors, Nanomaterials

Highlights

- Biosensors are sensitive diagnostic tools for disease monitoring.
- Bioreceptor captures the analyte through highly specific affinity binding.
- Nanomaterial-enhanced transducers magnify signals for disease diagnosis at low pathogen counts.

INTRODUCTION

Biosensors are vital analytical instruments that offer great sensitivity, portability, affordability, and user-friendliness in environmental monitoring, biological diagnostics and healthcare. Numerous biosensing technologies, including electro-chemical impedance sensors, amperometric sensors, luminescence sensors, and photoelectrochemical sensors, have become widely used at electrode interfaces in the detection of chemical and biological targets through precise measurements of dynamic changes (Shen et al., 2021).

The capabilities of biosensors have been transformed by the incorporation of nanotechnology. Nanostructure integration has overcome the limitations of conventional sensing platforms, particularly in response and sensitivity dynamics (Yang et al., 2010). Examples of nanoscale materials that significantly enhance sensor performance include nanoparticles, nanosheets, nanowires, nanotubes and nanoneedles. These materials increase signal transduction, boost sensitivity, improve selectivity, lower detection limits, and accelerate response dynamics (Ma et al., 2024). These advancements have made it possible to identify targets that were previously undetected, expanding applications beyond conventional biomedical research into clinical diagnostics and personalized healthcare (Yu et al., 2023).

Animals are essential to our existence because they produce the milk, meat, and other byproducts we use on a daily basis. At the same time, because humans and animals live in the same habitat, human health is either directly or indirectly impacted. Because the One Health idea will affect both our health and the economy, it is essential that animals maintain their health. From this perspective, it is crucial to diagnose

*Corresponding Author: E-mail: msmurtuza@gmail.com

diseases in animals, monitor their health, prevent them, and control them. In order to diagnose animal diseases early and implement control measures, tools for quick diagnosis are essential (Tewari et al., 2021). The use of nanoparticles in diagnostic technologies transformed the field by making it possible to identify pathogens, biomarkers, and pathological alterations at the molecular and cellular level with extreme sensitivity. This review emphasizes, the current usefulness and versatility of nanotechnology in biosensor based diagnostic technique.

Overview of biosensor components

A biosensor uses an immobilized sensing element called a bioreceptor (monoclonal antibody, DNA, RNA, enzyme, lectin, glycan, tissue, and whole-cell) to identify a target biomarker that is unique to a pathogen (Gattani et al., 2019). Because of its biochemical characteristics, which guarantee high selectivity and sensitivity of biomarker detection and enable the avoidance of interference from other microbes or chemicals present in the tested sample, the bioreceptor is a crucial component. The distinct biochemical interaction between the biomarker and the bioreceptor is converted into an observable by the transductor (Vidic et al., 2017). A typical biosensor comprises five key functional components, as detailed in Table 1 (Darsanaki et al., 2013).

Types of biosensor sensors

Biosensor development can be broadly categorized into five generations. The initial generation of biosensors measures two distinct things: the analyte's composition and the product of the bioreceptor interaction, which ultimately generates the signal as a response (Malik et al., 2023). The first person to work on this kind of biosensor was Leland Charles Clark Jr. (Heineman & Jensen, 2006). Clark also used an amperometric enzyme electrode to detect glucose in 1962 (Clark & Lyons, 1962). Updike and Hicks later improved Clark's research and created the first enzyme electrode in 1967 (Updike & Hicks, 1967). Guilbault and Montalvo also created a potentiometric electrode sensor for urea detection in 1969 (Guilbault & Montalvo, 1969). Similar to this, Guilbault and Lubrano used a lactate/glucose enzyme-based sensor in 1973 to identify hydrogen peroxide (Guilbault & Lubrano, 1973).

In order to detect temperature changes, the Klaus Mosbach group designed and developed thermistors in 1974 (Mosbach & Danielsson, 1974). Lubbers and Opitz developed an optical biosensor to detect alcohol in 1975 (Lübbers & Opitz, 1975). Auxiliary enzymes and co-reactants were added to the second-generation biosensors to increase their analytical efficiency (Singh et al., 2021). Mediator amperometric biosensors were the name given to these sensors. The bio-receptors develop into a crucial component of the sensing element in the third and fifth generations. Without the need for an intermediary, the transmission of electrons between the electrode and bioreceptor (enzymes) produced a direct interface. These generation sensors' primary benefits were their great sensitivity, low cost, and repeatability (Li et al., 2017). This thorough analysis emphasizes the major developments and discoveries that have influenced the development of biosensing technologies, showcasing the astounding progress made over time.

Nanomaterials in biosensor design

The integration of recent advances in nanotechnology and advanced electronics manufacturing technology has created a new class of biosensors known as nano biosensors, bringing a new era of bio nanotechnology for disease diagnosis. Biological agents like antibodies, nucleic acids, infections, and metabolites can be found using a nano biosensor. The basic idea is that bioanalytes of interest are bound to bioreceptors, as a result of these binding physiochemical signals are altered. A transducer then captures and transforms these physiochemical signals into an electrical signal. Signal variations are tracked, including current, electric potential, impedance, conductance, electromagnetic radiation intensity and phase, mass, temperature, and viscosity. The presence or absence of bioagents is quantified by analyzing the

Table 1. Biosensor components

Component	Description
Analyte	A material of interest that must be found, such as glucose in the case of diabetes
Bioreceptor	An analyte-recognizing molecule, such as an enzyme, antibody, or nucleic acid
Transducer	Converts the biorecognition event into a quantifiable signal through signalization
Electronics	Processes the transduced signal and prepares it for display
Display	Typically uses liquid crystal displays in conjunction with hardware and software to generate biosensor results in an approachable way

variation in one or more of these parameters. The transducer is paired with nanomaterials to create a biosensor, and the nanostructures in nano biosensors serve as an intermediary layer between biological agents and physicochemical detector components or biological agents (Fig. 1) (Adamson & Gast, 1967; Prasad, 2014).

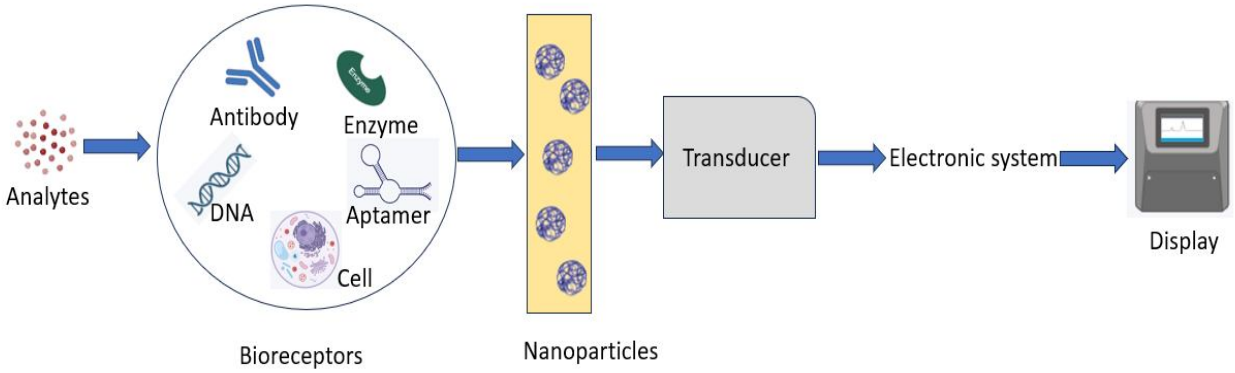


Fig. 1. Illustration of nanoparticles incorporated in biosensor design

The sensing component in a biosensor is biological in origin and reacts to the substance being measured. In order to record a signal that can be seen, it must be attached to some kind of transducer. Currently, nanoparticles can be used as transducers in sensors. The many desirable properties of nanomaterials are the driving force behind their widespread interest; in particular, the ability to customize the size, structure. These characteristics of nanoparticles present significant potential for creating innovative sensing systems and enhancing biosensor performance (Homola et al., 1999; Guedon et al., 2000)

The drawback of traditional (optical) sandwich bioaffinity assays is that they only record a limited number of labels per binding event. Because of their small size, nanoparticles can be tailored for certain bioassays, and the binding of target biomolecules has a significant impact on their characteristics (Van Den Berg et al., 2000). The signal from the assays is much increased when nanoparticles are used. The use of nanoparticle-quantifying tags in optical bioassays has been motivated by the need to overcome the drawbacks of organic fluorophores.

Numerous applications, such as biomaterials, catalysis, nano biosensors, and nano-bioelectronics, heavily rely on nanomaterials (Mustafa et al., 2017). The application of nanoparticles, nanotubes, nanowires, nanopores, or any other nanostructures in biosensors and the creation of diagnostic devices has been thoroughly investigated (Hassan et al., 2017). Solid surface functionalization and the production of electrochemical transducers are two applications for nanomaterials. Because of their nanoscale dimension, the designed nanostructured materials have a desirable biocompatibility, high mechanical strength, electrical conductivity, and catalytic activity, and an expandable surface-to-volume ratio (Yoon et al., 2022).

Furthermore, the use of certain nanomaterials facilitates direct charge/electron transfer (Holzinger et al., 2014). For instance, biomolecules (DNA, antibodies, enzymes, or even cells) have been conjugated with electrochemical transducers using carbon nanomaterials (Zhang & Wei, 2016). Nanomaterials are divided into four categories based on their sizes and dimensions: zero-dimension, one dimension, two dimensions, and three dimensions. Metallic nanoparticles (such as silver, gold, platinum, gold, and palladium) and quantum dots exhibit all three dimensions of nanoscale materials in zero dimensions (0D). Nanoparticles have an average diameter of 1–50 nm and can be spherical in shape. Only one dimension in one dimension (1D) falls between 1 and 100 nm, while the other two dimensions may be macroscale.

Nanotubes, nanorods, nanowires, and nanofibers are a few types of 1D nanomaterials. Two dimensions (2D) consist of one macroscale dimension and two nanoscale dimensions. 2D-like structures, such as nano-walls, nano-sheets, nano-thin-films, or nano-thin-multilayers, are produced by coating solid surfaces with nanomaterials (Gezgin et al., 2013). The entire area of nanomaterials' 2D-like structures might be within a few square micrometers if the thickness is kept constant in the nanoscale range. There are no dimensions in nanoscales for 3D nanostructures; all dimensions are found in macro-scales. Therefore, bulk materials with separate components (blocks) at the nanoscale (less than 100 nm) make up 3D nanostructures. The most popular detection modalities are optical and electrochemical nanosensors because of their mobility and ease of usage (Wang & Duncan, 2017).

Many nanostructured materials have been widely employed as sensing platforms with expanding surface areas to improve electrochemical signal outputs and receptor–analyte interaction. Because of their high electrical conductivity, vast surface area, ease of functionalization, and high biocompatibility, carbon nanomaterials – such as carbon nanotubes, carbon nanofibers, fullerene (C60) or graphene – are ideal materials for sensor development. Because of their strong electrocatalytic activity and propensity to directly promote electron transfer/charge in mediator-less biosensing systems, metal and metal oxide nanostructures are widely employed materials for sensor modification (Hassan, 2022).

In order to facilitate quicker electron transfer kinetics between the electrode and the active sites of immobilized bio-receptors, ZnO, CuO, NiO, TiO2, and Fe3O4 have been extensively employed in electrochemical biosensing (Subbaiah et al., 2021). This has resulted in a synergistic improvement in sensing performance.

Because of their superior biocompatibility and inertness against oxidation reactions, precious metal nanostructures, such as Pt, Pd, Au and Ag, have been used for modification of the electrode (Kashish et al., 2015; Kuzin et al., 2015; Madianos et al., 2018). These nanostructures may be positioned on the electrode surface or combined with other elements in the electrode matrix, such as polymeric or sol-gel materials (Chen et al., 2016). In the end, nano-sensor devices enable the creation of nano-sensor chips and portable sensing devices for quick and precise multi-target in intricate biological and environmental matrices.

Nanosensors in animal disease diagnosis

Any biomarker, such as a monoclonal antibody, RNA, DNA, lectin, enzyme, tissue, or cells, can be the analyte from the sample. The transducer transforms the interaction between the bioreceptor and the analyte into a signal that may be used for either quantitative or qualitative pathogen detection. Even before the animal exhibits clinical symptoms, nanosensors can make a highly specific diagnosis. In essence, nanosensors identify pathogen-specific biomarker chemicals by binding to bioreceptors. With the aid of a transducer, the bioreceptor element interacts with the molecule of interest in a very sensitive and particular manner without interfering with other molecules in the sample. This interaction is then transformed into quantifiable signals (Yoo & Lee, 2016). Using the signals generated by the transducer, this technique can be used for both quantitative and qualitative investigations of analyte molecules. The detection of biomarkers from complicated media and suitable bioassays for the detection are two key concerns that, if properly addressed, can make nanosensors the most practical and efficient diagnostic devices. The rapid spread of pathogens within the animal body necessitates the development of extremely sensitive techniques to identify the pathogen before clinical signs manifest. According to Zhu et al. (2015), nanosensors can provide suitable methods for the real-time analysis of veterinary illnesses.

Nanosensors are extremely sensitive nanoscale devices that are equipped with mobile probe biomolecules, employed for diagnostic purposes and are composed of nanomaterials. They can be modified to diagnose hormonal, metabolic, and vaginal tract infections, as well as to identify estrus (Scott, 2007; Saragusty & Arav, 2011). In a similar vein, the nanotubes can be employed to identify estrus. When the cows enter estrus, the tubes are inserted beneath their skin and start to glow. The test is based on estradiol sensors that measure the amount of hormone in the blood and transmit the cows' real-time readings to a central computer for cow monitoring (El-Sayed & Kamel, 2020). Nanocapsules containing bull semen can be applied to the ovum in order to fertilize cows. Sperm and oocytes can be sorted using nanotechnology. Biochips are being developed to allow for fetus sexing, or selecting the fetus's sex (Patil et al., 2009).

Recent advances in biosensor technologies have demonstrated remarkable versatility in targeting a broad spectrum of veterinary pathogens with high sensitivity and specificity. For Avian influenza virus (AIV), a functionalized polycrystalline silicon nanowire field-effect transistor (poly-SiNW FET) has been developed, wherein complementary DNA capture probes immobilized on the nanowire surface enable the detection of target viral DNA, such as the *H5* subtype, through measurable changes in electrical signals upon hybridization (Li et al., 2011). Similarly, Bovine viral diarrhea virus (BVDV), the causative agent of one of the most economically significant livestock diseases globally, has been detected using a G-quadruplex-based aptasensor that combines a rationally designed aptamer (Apt31) with gold nanoparticles (AuNPs); in the presence of BVDV, the formation of an aptamer–virus complex triggers a salt-stimulated colorimetric shift in AuNPs from red to purple-blue, facilitating rapid visual detection and supporting the critical identification of persistently infected cattle central to BVD control programs (Rabiei et al., 2024). For the zoonotic nematode

Trichinella spiralis, transmitted through the consumption of undercooked meat, quantum dot-based fluorescent biosensors conjugated with specific antibodies have been employed to detect parasite antigens with high sensitivity and specificity, offering a rapid and accurate diagnostic alternative (Xu et al., 2021). Furthermore, for *Trypanosoma* spp., the causative agents of trypanosomiasis in both livestock and humans in endemic regions, a laboratory prototype biosensor utilizing fiber optic spectrometry has been developed to measure ultraviolet-visible-near-infrared (UV-Vis-NIR) absorbance of murine blood, detecting infection-related alterations in blood chemistry in accordance with the Beer-Lambert Law (Theint et al., 2019). African Swine Fever (ASF) is a critical infectious disease with a fatality rate reaching 100% upon infection, endangering the global swine industry. Although ASF may not directly induce obstetric abnormalities, its impact on the immune system, pregnancy management, and overall health may indirectly lead to reproductive issues. Zhang et al. (2024) developed a NanoBiT biosensor-based immunoassay for ASFV IgG detection by employing p30-fused LgBiT/SmBiT probes to reconstitute nanoluciferase upon antibody binding. This assay exhibited 98.19% concordance with clinical samples and no cross-reactivity, demonstrating a 16-fold increase in sensitivity compared to ELISA, so offering a rapid, accurate, and efficient method for ASFV antibody detection in clinical diagnostics (Zhang et al., 2024).

The intracellular parasite *Toxoplasma gondii* mostly infects cats as its definitive host. It spreads to humans through a variety of routes after infecting other species through cat feces (Doering et al., 2004). Controlling its spread is essential due to its widespread occurrence. In order to overcome this problem, Sanli et al. (2023) showed how to create an electrochemical biosensor based on antibodies for the direct detection of *T. gondii*, which may have uses in disease treatment and monitoring. The biosensor makes use of a screen-printed electrode that has been altered with chitosan and gold nanoparticles. Glutaraldehyde is used as a cross-linker to immobilize anti-*T. gondii* antibodies on the electrode surface.

E. coli-induced mastitis continues to pose a serious risk to dairy cattle in veterinary clinical settings, negatively impacting animal welfare and causing large financial losses (Guo et al., 2021). A field-effect transistor (FET)-based biosensor based on carbon nanotubes has been created to directly detect traces of bacterial genomic DNA. This biosensor detects genomic DNA from *E. coli* O157:H7 by immobilizing particular oligonucleotide probes on the FET array, which causes notable threshold voltage (Vth) variations.

The copper-doped nickel and zirconium oxide nanoparticles were effectively created and assessed by scanning electron microscopy (SEM), X-ray diffraction (XRD), UV-visible, and Fourier transform infrared (FTIR) spectroscopy. These were subsequently utilized as an effective matrix to create electrochemical immunosensors for the detection of *Brucella abortus* in phosphate buffer (pH=7.2, 0.1 M) between 1× 103 CFU/mL and 2×106 CFU/mL with and without antibody (Khan et al., 2018).

Using a magneto-immunoassay and gold nanoparticles (AuNPs) as a label for electrochemical detection, a disposable immunosensor for the detection of *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* LT2 (S) is created. The screen-printed carbon electrode (SPCE) with a permanent magnet below is the foundation of the immunosensor. Anti-Salmonella magnetic beads (MBs-pSAb) were used as the capture phase in the testing of Salmonella-containing samples (such as skim milk), and AuNPs-modified antibodies (sSAb-AuNPs) were then sandwiched and detected using differential pulse voltammetry (DPV). Salmonella was found to have a linear range of 10^3 to 10^6 cells mL^{-1} and a detection limit of 143 cells mL^{-1}, with a coefficient of variation of roughly 2.4% (Afonso et al., 2013; Zhao et al., 2025).

Conclusion

Biosensors, as an emerging diagnostic tool, already have application prospects in veterinary medicine and public health. Nanoparticles have shown great promise in the development of biosensors for the diagnosis of a variety of animal ailments. Pathogens and biomarkers linked to animal diseases have been effectively detected using these novel sensing technologies. Nanosensors provide promising solutions for accurate disease diagnosis by using particular recognition elements and transduction mechanisms to enable very sensitive and selective detection. Despite its many uses, nano technology has certain limitations in food safety, bioimaging, and diagnostics because it requires fewer hazardous nanoparticles and highly sensitive, biodegradable, and affordable equipment. While technical challenges remain, continuous innovation and optimization will ensure that biosensors become a key technology for advancing global animal health and public health in the future.

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Author's contribution: MSM: Writing of main draft; SB: Conceptualization, supervision, writing and

reviewing of original draft; AM: Correction, reviewing and editing; SP: Supervision, writing of main draft and editing; SSK: Reviewing and editing; SB: Reviewing of manuscript; MH, LN, CBR, DS: Editing of manuscript; AP: Final editing of manuscript.

Data availability statement: The corresponding author is willing to provide the raw data upon reasonable request.

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