A Comprehensive Review of Recent Progress in Nanosensor Engineering for Biosensing Applications

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Abstract

Biosensors are advanced bioanalytical devices designed for the highly sensitive and quantitative detection of target molecules within complex biological matrices. Recent progress in nanotechnology, surface engineering, and advanced materials has enabled the development of a new generation of optical, mechanical, and electrochemical biosensors capable of producing stable, selective, and anti-fouling signals using minimal sample volumes. This review examines the role of nanomaterials in enhancing the effective surface area, improving charge transfer, amplifying signal output, and creating anti-fouling interfaces. Strategies for immobilizing biological recognition elements through covalent and non-covalent interactions, as well as the use of conductive polymers, carbon-based nanostructures, nanozymes, and two-dimensional materials to improve sensor sensitivity and stability, are also discussed. Furthermore, recent advancements in wearable biosensors, lab-on-a-chip systems, and portable devices for real-time monitoring of biological analytes are highlighted. Despite these advancements, challenges such as nanomaterial stability under physiological conditions, improving signal-to-noise ratios, and developing low-cost, anti-fouling surfaces remain unresolved. Overall, this review demonstrates that the integration of nanotechnology, surface engineering, and advanced material design can pave the way for highly accurate, stable, and user-friendly biosensors suitable for routine applications.

Keywords: ***Biosensing Techniques; Nanotechnology; Nanostructures; Biofouling***

Background and Objective

Sensitive and accurate detection of biological molecules and chemical compound—particularly in diseases such as cancer, where early diagnosis plays a decisive role in patient survival—is a fundamental pillar of clinical diagnostics. However, in the early stages of disease, the concentration of these molecules is extremely low.

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These compounds, known as biomarkers, not only confirm the presence of disease but also reflect its stage of progression. Conventional biomarker detection techniques, including microscopy, cell culture, proteomics, and molecular biology-based assays, are highly sensitive but require sophisticated equipment, trained personnel, dedicated laboratory environments, and long processing times. Moreover, the large size of the instruments and the need for controlled conditions limit their applicability in remote settings or emergency situations. Therefore, the development of rapid, portable, and highly sensitive diagnostic tools has become essential.[1]

Biosensors are analytical devices that convert biological or chemical interactions into measurable signals. They have gained significant attention in clinical diagnostics, pharmaceuticals, biomedicine, and healthcare due to their high sensitivity, ease of use, and portability. Beyond disease detection, biosensors are also employed in prevention, rehabilitation, patient monitoring, and personalized health management. These devices can track biochemical changes in real time without invasive sampling and transmit data directly to patients or physicians via mobile devices.[2]

A typical biosensor consists of the following key components: the target analyte, a biological recognition element, a transducer, an electronic signal-processing unit, and a display interface. The recognition element interacts specifically with the analyte, and the transducer converts this interaction into an optical or electrical signal. The processed signal is then presented to the user in numerical, graphical, or visual form.[3]

In addition to quantifying target molecules, biosensors are widely used to study cellular physiology, cell mechanics, and drug responses. Their analytical performance is evaluated based on parameters such as sensitivity, selectivity, limit of detection (LOD), linear range, and reusability. However, interfering molecules present in biological samples can compromise signal accuracy. The incorporation of nanomaterials, including metallic nanostructures, advanced polymers, carbon-based nanomaterials, and quantum dots, has significantly enhanced sensitivity, reduced interference, and improved overall biosensor performance.[4]

Integrating nanomaterials into biosensor design has enabled true point-of-care diagnostics, allowing portable kits to deliver rapid, accurate, and highly sensitive results. Nanomaterials, owing to their high surface-to-volume ratio, size-dependent optical and electronic properties, suitable electrical conductivity, strong catalytic activity, or desirable inertness (e.g., gold nanoparticles), play a crucial role in signal amplification and sensitivity enhancement. Surface modification of nanomaterials further facilitates efficient immobilization of biological recognition elements and increases detection capacity through covalent or non-covalent interactions.[5-6]

This review aims to examine the principles of biosensor design, the role of nanomaterials and surface engineering in improving biosensor performance, and emerging technologies in the field. The following sections discuss various biosensor types, operational principles, signal-enhancement strategies, and current trends in the development of advanced biosensing platforms.

2. Biosensors

1.2. Historical Background

The history of biosensors dates back to early 1906, when Kremer demonstrated that the concentration of an acid in solution correlates with the electrical potential generated across a glass membrane.[7]

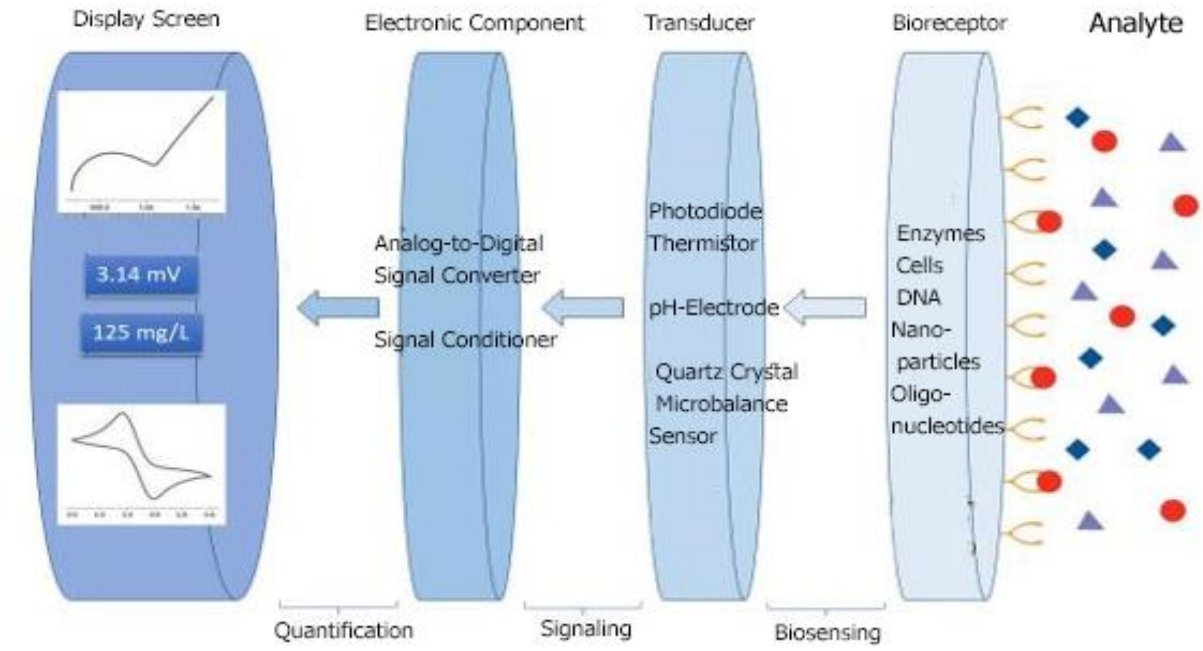


Figure 1. Schematic of a biosensor

A few years later, in 1909, Sørensen introduced the concept of hydrogen-ion concentration (pH) In 1922, Hughes developed the first electrode specifically designed for pH measurement.[8]

Between 1909 and 1922, Griffin and Nelson showed for the first time that the enzyme invertase could be immobilized on aluminum hydroxide and activated charcoal, a milestone in the development of enzyme, based biosensors.[9]

The first true biosensor was developed in 1956 by Leland C. Clark, who designed an oxygen-measuring device later known as the Clark electrode.[10] In 1962, Clark introduced the first amperometric enzyme biosensor for glucose detection. A few years later, in 1969, Guilbault and Montalvo developed the first potentiometric biosensor for urea.[11]

In 1975, Yellow Springs Instruments (YSI) introduced the first commercial biosensor. Since then, significant advancements have been made, including the development of portable sensors and rapid diagnostic tools. Today, biosensors represent a highly interdisciplinary field that integrates principles of physics, chemistry, and biology with micro- and nanotechnology, electronics, and applied medicine.

According to the Web of Science database, more than 84,000 research articles on biosensors were published between 2005 and 2015, highlighting the rapid growth and increasing importance of this field in diagnostics and health technologies.

2.2. Characteristics of Biosensors

Biosensors must exhibit specific static and dynamic characteristics, and optimizing these properties directly influences their performance.

Selectivity

Selectivity is perhaps the most important characteristic of a biosensor. It refers to the ability of a biorecognition element to identify a specific analyte within a sample containing additives or contaminants. A classic example is the antigen-antibody interaction: antibodies immobilized on the transducer surface selectively bind to their target antigens. Thus, choosing the appropriate bioreceptor is fundamental in biosensor design.

Repeatability

Repeatability refers to the ability of a biosensor to produce identical responses

across repeated measurements. It depends on the accuracy and precision of the transducer and electronic components.

- Accuracy indicates how close a measured value is to the true value.
- Precision indicates the consistency of repeated measurements.

High repeatability ensures the reliable interpretation of biosensor outputs.

Stability

Stability describes the sensitivity of the biosensor system to environmental disturbances. Such disturbances can cause signal drift or measurement errors, affecting both accuracy and precision. Stability is especially critical in applications requiring long incubation periods or continuous monitoring.

Factors influencing stability include the following:

- Temperature-dependent behavior of transducers and electronics
- Binding affinity between the analyte and the bioreceptor
- Long-term degradation of the biorecognition element.

Bioreceptors with high affinity, achieved through electrostatic or covalent interactions, enhance the stability of biosensors.

Sensitivity

Sensitivity refers to the minimum detectable amount of the target analyte, commonly expressed as the LOD. In medical and environmental applications, biosensors may need to detect concentrations in the nanogram or even femtogram per milliliter range.

For example, when the concentration of prostate-specific antigen (PSA) in the blood reaches approximately 4 ng/mL, the likelihood of prostate cancer increases, prompting physicians to recommend a biopsy.

Linearity

Linearity indicates how proportionally the biosensor’s output changes with analyte concentration. A linear response is essential for accurate quantification over a defined range.

Related concepts include:

- Resolution: the smallest detectable change in analyte concentration.
- Linear range: the concentration interval over which the sensor response remains directly proportional to the analyte levels.[12]

3.2. Sensor Matrix/Platform

Biosensors are constructed on a supporting substrate, often referred to as the sensor matrix, which provides an appropriate surface for immobilizing biorecognition elements and creating the necessary conditions for accurate detection of the target molecule. The material, fabrication method, and structural design of this matrix directly influence the biosensor's overall performance and must be selected based on the target analyte and the signal transduction mechanism.

Common substrates used in biosensor fabrication include paper, graphite, carbon paste, glassy carbon electrodes, screen-printed electrodes, and thin films of indium tin oxide (ITO). Additionally, electrodes made from noble metals such as platinum, gold, and silver are widely used due to their high electron transfer rates and favorable stability. Among these metals, gold is the most frequently employed, offering stable performance over a relatively broad potential window.

Among ceramic electrodes, thin-film ITO has gained particular importance due to its excellent electrical conductivity, wide operational potential range, strong substrate adhesion, low capacitive current, and stable physical and electrochemical properties. ITO has been increasingly incorporated into biosensors through methods such as electrodeposition, polymerization, and adsorption-based surface modification for biomolecule immobilization. However, ITO is unstable in acidic environments and exhibits slower electron-transfer kinetics compared to glassy carbon and noble metal electrodes.

Glassy carbon electrodes are considered suitable substrates for biosensing due to their chemical stability, low oxidation rate, and wide operational potential window. Although generally regarded as chemically inert, they can catalyze certain electrochemical reactions even without

additional nanostructures, particularly at low analyte concentrations.

Screen-printed electrodes, produced by printing conductive inks—such as carbon, silver, or gold inks—onto ceramic or plastic substrates, are widely used as disposable electrodes. Their low cost, portability, ease of use, and ability to integrate reference, working, and auxiliary electrodes into a single platform make them highly suitable for portable biosensing systems.

Furthermore, disposable sensors based on secondary ion mass spectrometry (SIMS) have been developed due to their low cost and effective biosensing capabilities.

Overall, the selection of a sensor matrix must be made carefully, as each substrate possesses unique electrochemical, physical, and surface properties, and its behavior during sensing can differ significantly from others.[13-17]

4.2. Bioreceptors

Bioreceptors are immobilized on the transducer surface and are responsible for creating specific interactions with the target molecule. They can be broadly categorized into two groups:

- Catalytic biorecognition elements, such as enzymes,
- Molecular biorecognition elements, such as antibodies, nucleic acid sequences, and aptamers.

In addition, certain modified nanomaterials can enhance analyte detection through their catalytic properties, although they do not possess the selectivity characteristic of true bioreceptors. Selecting an appropriate bioreceptor requires balancing sensitivity, selectivity, repeatability, reusability, physical properties, and ease of fabrication. A systematic decision-making framework proposed by researchers can assist in identifying the optimal bioreceptor for biosensor design.[18]

1. Enzymes

Enzymes, known as biological catalysts, are among the most important bioreceptors due to their high substrate specificity. This specificity is often explained using the "lock-and-key" or "induced-fit" models. Enzyme-based biosensors function catalytically by converting the target molecule into a product, whose changes—such as color, charge, or reactivity—can be measured.

Enzymes are typically positioned near the transducer surface or embedded within conductive nanomaterials to shorten and accelerate electron transfer pathways. Glucose oxidase is the most widely used enzyme in glucose biosensors. Horseradish peroxidase is another commonly used enzyme, often conjugated to secondary antibodies to amplify the signal.[19]

2. Antibodies

Antibodies are key protein-based bioreceptors. Their three-dimensional recognition domains specifically bind to target molecules, forming an antigen-antibody immune complex. This complex can be detected using optical, electrochemical, or piezoelectric biosensors.

Monoclonal, polyclonal, and recombinant antibodies are all used in biosensing. Proper orientation of antibodies on the transducer surface can significantly enhance biosensor sensitivity.[20]

3. Aptamers

Aptamers are short synthetic nucleic acid sequences designed using the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process. They can selectively bind to ions, proteins, drugs, or even whole cells. Aptamers exhibit high stability against variations in pH, temperature, and ionic strength, making them strong alternatives to antibodies in many biosensors.[21]

4. Nucleic Acid-Based Biosensors

Nucleic acid biosensors, also known as genosensors, use stem-loop or linear probes to detect target sequences. Due to the specificity of nucleotide base pairing, these sensors can detect even single-base mismatches, which is crucial in genetic mutation studies. Recently, locked nucleic acids (LNA) and peptide nucleic acids (PNA) have been used to enhance the stability and selectivity of genosensors.[22]

5. Cells and Tissues

Cells and tissues are used in biosensors to detect various molecules and to monitor cellular physiology and mechanical behavior. In recent years, bacteriophages and phage-display systems have been employed to detect molecules based on protein-protein interactions.

Additionally, membrane receptors and non-catalytic soluble proteins that specifically bind to target molecules are also used as bioreceptors.[23]

6. Molecularly Imprinted Polymers (MIPs)

Molecularly imprinted polymers are synthetic polymers that contain specific binding sites tailored for target molecules. They are fabricated using a molecular template, and after the template is removed, cavities with the appropriate shape and chemical functionality remain. Currently, MIPs are primarily developed for detecting small molecules.[24]

5.2. Transducers

The transducer converts the biorecognition event into a measurable signal. Based on the type of transducer, biosensors can be categorized as optical, mechanical, or electrochemical.

5.2.1. Optical Biosensors

Optical biosensors detect changes in the phase, polarization, or frequency of an optical field associated with a biorecognition element upon interaction with an analyte. Depending on the transduction mechanism employed, optical biosensors can be classified into absorbance-based, fluorescence-based, and luminescence-based systems. Absorbance-based biosensors measure changes in light intensity when the sensing surface is irradiated in the presence of analytes.

These amplitude changes can be correlated with the analyte concentration in solution. When operating in the visible spectrum, such devices are referred to as colorimetric biosensors. The analyte concentration can be quantified using the Beer-Lambert law, as shown in Equation (1):

Equation (1): $\varepsilon C \Delta x = \log (I0I)$

Where:

- ***I*=intensity of the transmitted electromagnetic wave.**
- ***I0*=intensity of the incident electromagnetic wave**
- **ε=molar extinction coefficient**
- ***C*=analyte concentration**
- **Δx=change in optical path length**

For example, Mahato et al. developed a naked-eye colorimetric optical biosensor that detects the concentration of alkaline phosphatase in milk samples based on the enzyme's catalytic action on a chromogenic reagent, producing a measurable color change. In another study, a mobile phone was used to analyze color variations on the sensor surface corresponding to changes in alkaline phosphatase concentration. The researchers found that the red channel of the RGB color model provided the highest sensitivity due to its steeper response slope. Based on this observation, a paper-based kit was designed for detecting this enzyme in milk samples.

In recent years, optical biosensors have advanced to the point where they can detect single molecules or ultra-trace levels of analytes. Beyond light absorption, mechanisms such as Förster resonance energy transfer (FRET) and photobleaching recovery have also been employed. These mechanisms operate according to the Jablonski energy diagram, in which emission occurs at a lower energy level and is independent of the maximum absorption wavelength.

Optical biosensors are particularly important due to their high speed, excellent sensitivity, and outstanding selectivity in detecting biological molecules.

In another study, a labeled aptamer-based optical biosensor was introduced for the detection of thrombin. In this system, the fluorescence of the labeled molecules was initially quenched by a graphene oxide (GO) layer before thrombin binding. As thrombin progressively bound to the aptamer, the fluorescence intensity increased. The maximum fluorescence signal was observed at approximately 80 seconds, indicating the time required for target recognition. The subsequent gradual

decrease in fluorescence intensity was attributed to photobleaching over time.

Another optical method used in biosensing is chemiluminescence, which enables highly sensitive molecular detection without requiring an external light source, making it suitable for in vivo measurements.

Among other important optical techniques are surface plasmon resonance (SPR) biosensors. This method measures changes in the refractive index at the sensor surface, which correlate with the concentration of the target molecule. When polarized light strikes a conductive surface, such as a metal-dielectric interface, at a specific angle, collective oscillations of electrons are generated, causing a decrease in reflected light intensity at a characteristic angle known as the resonance angle. This angle depends on the mass accumulated on the sensor surface. Monitoring changes in reflectivity, angle, or wavelength over time allows quantification of the target analyte.

A typical SPR biosensor consists of an optical detector, a gold-coated chip, and a functional layer for immobilizing the bioreceptor. For this technique to work, at least one of the interacting molecules must be immobilized on the sensor surface. In the localized SPR (LSPR) variant, metallic nanoparticles, such as gold nanoparticles, are used for imaging, sensing, and even photothermal therapeutic applications.

Another group of optical biosensors includes optical waveguide interferometric biosensors (measuring phase differences), ellipsometric biosensors (which detect polarization changes), and surface-enhanced Raman scattering (SERS) biosensors. All of these are used for high-sensitivity detection of biomolecules and cellular processes.

In recent years, fiber-optic biosensors have been developed to enable rapid detection without the need for complex laboratory instrumentation.[25-29]

5.2.2. Mechanical Biosensors

In mechanical biosensors, changes in physical and mechanical properties, such as force, displacement, mass, or vibration, are measured following the interaction of the target molecule with the sensor surface. These biosensors offer several advantages compared to other sensing methods:

1. Extremely high mass resolution enables the detection of very small quantities of analytes. For example, nanoelectromechanical systems (NEMS) have achieved mass detection at the zeptogram scale.
2. Measurable displacement under applied forces, enabling the quantification of extremely small forces at the cellular scale.
3. Very fast response times, making it suitable for monitoring rapid biological events.
4. Operation in both liquid and gas phases, enhancing versatility.
5. Label-free detection, unlike many optical methods that require molecular labels.

Mechanical biosensors are generally classified into the following categories:

- Quartz Crystal Microbalances (QCMs)
- Surface Acoustic Wave (SAW) Sensors
- Nanomechanical Systems

These biosensors typically consist of a cantilever probe, a mechanical transducer, and a signal processor. The cantilever is functionalized with the target-specific molecule, and its properties, such as protein adsorption capacity or detection limit, depend on its geometry. Micro- or nanoscale cantilevers are preferred for achieving lower detection limits.

After the target molecule binds to the cantilever surface, its mechanical properties change proportionally to the added mass. These changes can be measured using two modes:

1. Static Mode

In this mode, the bending (deflection) of the cantilever caused by mass loading is measured. A reference electrode is required, and the deflection can be described using the Stoney equation, as shown in Equation (2):

Equation (2)

$$\Delta\sigma 1 - \Delta\sigma 2 = Et23\ (1-U)L2\Delta z$$

Where:

- **$\Delta\sigma 1$ and $\Delta\sigma 2$=Changes in surface stress on the upper and lower sides, respectively.**
- **Δz=Cantilever displacement**
- ***E*=Young's modulus**
- ***U*=Poisson's ratio**
- ***L* and *t*=cantilever length and thickness**

2. Dynamic (Resonant) Mode

In this mode, changes in the natural resonance frequency of the cantilever are measured. As mass accumulates on the cantilever, its resonance frequency decreases. This relationship is expressed in Equation (3):

Equation (3)

$$\Delta m = 10.96\pi 2\ (1f02 - 1f12)$$

Where:

- ***F0*=Resonance frequency before mass loading**
- ***F1*=Resonance frequency after mass loading**

Mechanical transducers are divided into displacement-based and piezoelectric-based systems.

- **Displacement-based systems use an optical detector that monitors the reflected laser beam from the cantilever surface.**
- **Piezoelectric systems use crystals that generate electrical signals in response to mechanical deformation.**

In these systems, target binding alters the mass, surface stress, effective stiffness, and viscoelastic properties of the sensor, all of which are measurable.

In one study, Wu et al. developed a silicon nitride-based mechanical biosensor capable of detecting PSA even in the presence of large amounts of nonspecific serum proteins.

In another study, Huber et al. introduced a nucleic acid-based mechanical biosensor capable of detecting mutations in a gene associated with skin cancer pathways without any labeling or genetic amplification, significantly improving detection speed and accuracy.

Cantilever-based mechanical biosensors have been successfully used to detect a wide range of biomolecules—including protein biomarkers and cancer cells—in various laboratory environments.[30-34]

5.2.3. Electrochemical Biosensors

According to the International Union of Pure and Applied Chemistry (IUPAC), an electrochemical biosensor is defined as "an integrated, self-contained device capable of providing specific quantitative or semi-quantitative analytical information." These biosensors operate by measuring changes in current, potential, conductivity, or electric-field effects resulting from the interaction between the target molecule and the biorecognition element on the sensing surface.

Electrochemical biosensors are preferred over many other biosensing platforms due to their stability, selectivity, high sensitivity, and reliable analytical performance. They typically operate using a three-electrode configuration, consisting of:

- **Working electrode: the primary signal-transduction surface where nanoscale modifications and surface engineering are applied**
- **Counter (auxiliary) electrode: completes the electrical circuit and stabilizes current**
- **Reference electrode: provides a constant and stable potential during measurement.**

Selecting an appropriate working electrode is one of the most critical steps in designing an electrochemical biosensor, as all surface modifications and signal-enhancing nanomaterials are applied to this electrode.

The operating principle of electrochemical biosensors is based on the kinetics of ion or electron transfer from the reaction site to the electrode surface. In both label-free and label-based systems, increases or decreases in electron transfer are recorded as measurable signals.

In biosensors employing catalytic bioreceptors, such as enzymes, the biocatalyst initiates a reaction that enhances electron transfer to the electrode surface, producing a detectable signal.

Based on the type of transducer used, electrochemical biosensors are classified into three major categories:[35]

1. **Amperometric and voltametric methods**
2. **Potentiometric**
3. **Impedimetric and conductometric Methods**

3. Surface Engineering Using Nanomaterials

Nanomaterials are materials with at least one characteristic dimension within the 1-100 nm range. However, according to the British Standards Institution, nanomaterials may include structures up to 1000 nm. They are widely used in fields such as drug delivery, photothermal therapy, and biosensing. Their exceptionally high surface-to-volume ratio makes them particularly valuable in sensor design.

Nanomaterials in Biosensors

To convert a biological event into a readable electronic or optical signal, an active element is often required. Common signal-generation mechanisms include electrochemical and optical methods. Electrochemical approaches are especially favored due to their high sensitivity, reliability, portability, and ease of integration.

This section discusses several emerging nanomaterials that can be incorporated into biosensing devices to facilitate the coupling between the substrate and bioreceptors and to enhance the conversion of biorecognition events into electrical or optical signals.

3.1.1. Conductive Polymers

Conductive polymers constitute a broad class of organic materials characterized by conjugated electron systems within their molecular structures. This unique arrangement enables properties such as:

- **Tunable conductivity achieved through doping**
- **Redox activity**
- **Simultaneous transport of ions and electron**

Their physical and chemical properties can be modified through doping, thereby enhancing their processability.

One well-known example is poly (3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT: PSS). PEDOT, synthesized chemically or electrochemically, exhibits high conductivity and excellent electronic stability in biological environments. Although PEDOT is initially insoluble, doping it with the negatively charged, water-soluble PSS disperses it uniformly in aqueous media, making it highly suitable for organic bioelectronics.

Most conductive polymers can physically entrap biomolecules or provide functional groups (e.g., carboxyl, amine) for covalent attachment. This capability enables the immobilization of enzymes, antibodies, and nucleic acids, thereby giving conductive polymers broad utility in biosensor fabrication.

After four decades of development, polymers such as polythiophenes, Polyaniline (PANI), Polypyrrole (PPy), poly(p-phenylene vinylene), and their derivatives have been widely used in biosensors. In particular, PEDOT: PSS is employed in flexible polymer electrodes and organic transistors for detecting bacteria, biomarkers, glucose, and nucleic acids due to its tunable conductivity, intrinsic flexibility, biocompatibility, and low cost.

In one study, a flexible biosensor was fabricated using metal electrodes and a PEDOT: PSS active layer on an ultrathin polymer substrate, enabling the detection of glucose and uric acid in saliva. Another PEDOT: PSS-based biosensor, combined with an ionic gel, was used for lactate detection.[36]

Polyaniline

Polyaniline (PANI) nanostructures have been widely used in biosensor development due to their favorable electrochemical properties. PANI is a p-type semiconducting polymer composed of repeating units in both reduced and oxidized states. Among its three oxidation states, the emeraldine form is the most conductive and stable at room temperature, making it the most widely used in biosensing applications.

The sensing performance of PANI arises from its high surface-to-volume ratio and stable redox pairs, which facilitate electron transfer among the enzyme, the conductive polymer, and the electrode. However, a major challenge is the loss of conductivity under physiological conditions. This occurs because PANI is typically synthesized in highly acidic, proton-rich environments, and in biological media, it gradually loses protons, reducing its conductivity.[37]

Polypyrrole

PPy was the first conductive polymer introduced for biosensing applications. Due to its ease of functionalization, tunable properties through dopants, ability to entrap enzymes via mild electrochemical deposition, stability, aqueous processability, and biocompatibility, PPy has become one of the most widely used materials in biosensor fabrication.

PPy is synthesized through the oxidative polymerization of the pyrrole monomer, a process that enables the entrapment of enzymes within the polymer matrix. PPy has been engineered into various morphologies, including nanoparticles, nanotubes, nanospheres, and core-shell structures, to enhance its performance and stability.

For example, an amperometric biosensor was fabricated by electrochemically depositing PPy onto carbon or metal electrodes, followed by the immobilization of horseradish peroxidase. This system was successfully employed for the detection of hydrogen peroxide.[38]

3.1.2. Metals

Ductile metals, particularly gold and silver, have long been considered primary candidates for constructing biosensor electrodes. These metals exhibit excellent conductivity, strong mechanical durability under bending and stretching, and well-established protocols for physicochemical functionalization with various biorecognition elements.

Metallic electrodes with specific structural configurations, such as helical, mesh, or wavy geometries, can maintain outstanding electrical performance even under mechanical deformation. Typically, thin metal films are adhered to flexible substrates after depositing a conductive adhesion layer, such as chromium. Microcracks that form in the metal layer during deformation help distribute strain elastically across the film network, thereby preserving electrical conductivity.

Metallic nanostructures, such as gold and silver nanowires or microarrays, are widely used not only as traditional electrodes but also as active sensing platforms. Although they generally exhibit lower sheet resistance (Ω^{-1}>50) compared to graphene-based nanowires (several hundred Ω^{-1}), their charge-transport behavior is comparable. Complex networks of metallic nanowires create rough, high-surface-area interfaces that facilitate efficient functionalization with biorecognition elements.

In recent years, a broad class of non-graphene two-dimensional materials with mono- or few-layer structures has emerged. These include transition-metal oxides, transition-metal dichalcogenides, and metal chalcogenides, each possessing unique electrical and mechanical properties.

Two-dimensional metal-oxide-based materials have attracted significant attention in biosensor design due to their suitable conductivity, mechanical robustness, and desirable semiconducting properties. Additionally, conductive polymers with conjugated electron systems, such as PANI and PPy, have been combined with non-noble metal oxides like zinc oxide, tin oxide, and gallium oxide to form active sensing layers. When arranged as thin films or engineered nanostructures, these composites exhibit high conductivity, good flexibility, and excellent stability.[39]

3.1.3. Carbon-Based Nanomaterials

Carbon nanostructures, such as graphene and carbon nanotubes (CNTs), are among the most promising nanomaterials for biosensor development. Graphene, first introduced as a two-dimensional sheet only one atom thick, has had a transformative impact on electronics, materials science, and sensing technologies due to its exceptional properties. It can be produced as thin films, inks, gels, or

nanoscale structures and can interact directly or indirectly with biomolecules while maintaining biocompatibility.

Beyond graphene, other carbon nanomaterials, such as carbon nanofibers and carbon nanospheres, offer high surface-to-volume ratios, mechanical flexibility, stability under diverse conditions, biocompatibility, and low cost, making them excellent candidates for biosensing applications.

Graphene is particularly attractive for flexible electrodes due to its extremely high electrical conductivity, high carrier mobility, mechanical flexibility, optical transparency, and mechanical strength. Among its derivatives, GO and reduced graphene oxide (rGO) have received the most attention.

Graphene and its derivatives can be synthesized using dry methods, such as chemical vapor deposition (CVD) and transfer printing, as well as wet methods, including aromatic polymerization, electrochemical deposition, and physical or chemical exfoliation of graphite. Their final properties depend not only on the synthesis method but also factors such as doping, dimensions, number of layers, and edge structure. For example, bilayer graphene exhibits a tunable bandgap, whereas monolayer graphene lacks a bandgap, which limits certain applications.

Reduced GO has been used to fabricate microelectrode arrays on plastic substrates. These arrays, composed of small disks, enable highly sensitive detection of dopamine without the need for mediators or additional functionalization. Their performance remains stable under mechanical deformation, long-term storage, and exposure to interfering substances.

Graphene-based materials have also been used to fabricate thin-film transistors and field-effect transistors. For instance, a transparent and flexible transistor was fabricated using rGO patterns on a polymer substrate. The source and drain electrodes were formed from GO layers deposited on a modified polymer substrate, and the conductive channel was created using microfluidic techniques. Following chemical reduction, the transistor maintained its conductivity even after 5000 bending cycles. The channels were then functionalized with linker molecules, enabling the successful detection of fibronectin.

CNTs, conceptualized as rolled graphene sheets, exist in single-walled and multi-walled forms. Their hexagonal carbon-ring structure allows them to form highly porous three-dimensional networks, providing excellent conductivity, high flexibility, and abundant binding sites for bioreceptors, thereby enhancing biosensor sensitivity.

The properties and performance of CNTs are highly dependent on the synthesis method. In one study, CNTs were directly grown on a flexible polymer substrate at a low temperature, and the resulting biosensor quantitatively detected human serum albumin using impedance spectroscopy.[40]

3.1.4. Light-Sensitive Elements

Optical biosensing systems convert biological interactions into signals such as luminescence, fluorescence, reflection, and absorption. While many biosensors rely on electrochemical transduction, optical systems deliver signals through light by changes in amplitude, intensity, polarization, frequency, wavelength, or phase, resulting in visually detectable analyte responses.

Advantages of optical biosensors include:

- Reduced interference from buffer pH and electromagnetic noise
- Multiplex detection capability
- No electrical insulation required

Optical biosensors can be classified into two types direct and indirect:

- Direct sensors rely solely on changes in the optical properties of the transducer upon analyte binding (e.g., SPR, reflectometry, interferometry, photoluminescence).
- Indirect sensors rely on labeling agents such as fluorescent quantum dots, gold or silver nanoparticles, or organic dyes.

Early optical biosensors included optical fibers and waveguides made from dielectric materials such as plastic or glass. Fiber-optic biosensors offer excellent flexibility, chemical stability, multiplexing

capability, low weight, and small size. The sensing probe can be precisely engineered at the fiber tip to detect changes in wavelength, frequency, phase, intensity, or polarization.

A low-power optical transducer on a plastic foil was developed for colorimetric gas sensing. This system employs a planar optical waveguide coated with an ammonia-sensitive film for gas detection. Alongside flexible substrates such as PDMS and paper, a new generation of optical biosensors has emerged based on luminescent organic dyes, quantum dots, phosphorescent nanoparticles, and carbon dots for point-of-care diagnostics.

In one such system, fluorescein was immobilized within microfluidic channels to capture and analyze helper T-lymphocytes in blood samples. Fluorescent proteins, such as green fluorescent protein (GFP), are also used as optical labels for monitoring cellular activity in living cells. However, organic dyes often suffer from low photostability, susceptibility to photobleaching, and pH-dependent behavior.

To overcome these limitations, semiconductor quantum dots have been introduced as superior alternatives. Their optical and electronic properties can be precisely tuned by adjusting their size and shape, and they exhibit significantly higher photostability than organic dyes. More recently, graphene quantum dots have been developed, offering high biocompatibility, size-dependent emission, and ease of functionalization.[41]

2.3. Functionalization of Nanomaterials

Surface engineering based on nanotechnology plays a crucial role in designing biosensor probes, as it directly determines the integrity, stability, and performance of the active sensing layers. Biosensor probes typically include a nanobiological interfacial layer where biorecognition elements are immobilized to interact with the target analyte and generate a measurable signal.

The attachment of biorecognition elements to nanomaterials must be stable, compatible, and reliable to ensure consistent and reproducible signals under various biological conditions. An appropriate functionalization strategy must achieve the following objectives:

- Controlled density of biomolecules on the surface
- Correct orientation of biorecognition elements
- Proper spacing between biomolecules and the nanomaterial
- Tunable binding affinity
- Preservation of biological activity
- A reproducible immobilization method compatible with diverse biomolecules

These principles form the foundation of surface engineering in biosensors.

Main Categories of Biomolecule Immobilization on Nanomaterials

1. Covalent Interactions (Stable Chemical Bonds)

These methods involve forming strong chemical bonds between nanomaterials and biomolecules. Common reactions include:

- Amide Bond Formation
- Cross-linking Reactions
- Click Chemistry
- Activation of functional groups

A key example is the binding of gold nanoparticles to thiol-containing molecules, forming highly stable Au-S bonds that are ideal for immobilizing biorecognition elements.

Among covalent methods, three approaches are most widely used:

a) Glutaraldehyde Cross-linking

Glutaraldehyde acts as a crosslinking agent between hydroxyl groups on nanomaterials and amine groups on biomolecules.

b) Activation of the Carboxyl Group

Carboxyl groups on nanomaterials are activated and subsequently form amide bonds with the amine groups of proteins. This method is advantageous because it:

- Works under physiological conditions.
- Occurs in aqueous environments.
- Produces highly stable bonds.

c) Strong Biotin-Avidin Interaction

One of the strongest non-covalent interactions in nature.

Process:

- **The nanomaterial surface is coated with avidin or streptavidin.**
- **A biotinylated biomolecule binds strongly to the surface.**

This method is ideal for affinity-based biosensors.

2. Noncovalent(Physical) Interactions

These include:

- **Electrostatic interactions**
- **Hydrophobic interactions**
- **Van der Waals forces**
- **Hydrogen bonding**
- **Physical entrapment within polymer matrices**

Although simple, these methods are sensitive to pH, ionic strength, temperature, and surface conditions, and are generally less stable than covalent approaches.

3. Surface Modification Using Silane Molecules

Silane-based molecules bind to hydroxyl-containing surfaces and form a stable layer for biomolecule attachment. This method is particularly suitable for metal-oxide nanomaterials.

4. Advanced Bio-Immobilization Techniques

Recent innovations have introduced highly sensitive and reproducible immobilization strategies, including:

- **Electrochemical activation and immobilization**
- **Thermal activation immobilization**
- **Controlled methods for protein stabilization**
- **High-throughput and multiplexed microfluidic systems**

These approaches enable the development of biosensors with ultra-high sensitivity and point-of-care applicability.[42-44]

4. Analytical Studies

After probe fabrication, biosensors are evaluated based on a set of standard analytical performance parameters. Preliminary studies of the sensing surface assess its sensitivity and selectivity toward the target analyte.

A regression (calibration) curve is constructed by plotting the sensor output signal against various analyte concentrations. This curve serves as a reference for subsequent analytical measurements.

Based on the calibration curve, the following analytical parameters were evaluated:

a) Sensitivity

Sensitivity is defined as the slope of the regression line, representing the change in the output signal relative to changes in analyte concentration.

Equation (8):

Sensitivity=YQ−YPXQ−XP

Where:

- ***Xp*** **and** ***X_Q*****=two analyte concentrations.**
- ***Yq*** **and** ***Y_Q*****=corresponding sensor signals.**

A steeper slope indicates a more sensitive biosensor.

b) Limit of Detection (LOD)

LOD is the minimum analyte concentration that produces a detectable signal.

Equation (9):

LOD=3 SDBlankSlope

Where:

- SD_{Blank}**=standard deviation of blank measurements.**
- ***Slope*****=sensitivity of the probe.**

c) Limit of Quantification (LOQ)

LOQ is the lowest concentration of an analyte that can be reliably quantified.

Equation (10):

LOQ=10 SDBlankSlope

LOQ is always equal to or greater than LOD.

d) Selectivity

Selectivity refers to the ability of a biosensor to distinguish the target analyte from other molecules and produce a signal only in response to the target.

e) Response Time

The time required for the biosensor to generate a measurable signal after exposure to the analyte.

f) Recovery Time

The time required for the biosensor to return to its baseline state and become ready for a second measurement cycle.[45]

5. Recent Trends in Biosensor Research

5.1. Calibration-Free Sensors

Calibration-free biosensors are designed to operate in complex systems without any on-site calibration or sample preparation, enabling semi-skilled users to operate them easily. To ensure accuracy, signal generation errors in calibration-free sensors must be minimized, and various strategies have been developed to achieve low error percentages.

Vicentini et al. developed a calibration-free immunosensor for detecting human leukocyte antigens in serum. Li and colleagues introduced a calibration-free, aptamer-based biosensor in which the signal change caused by analyte binding was not affected by variations in the sensor surface structure. The ratio of responsive to non-responsive square-wave voltammetric signals was used to quantify cocaine, doxorubicin, and kanamycin.

The same group also developed a calibration-free phenylalanine biosensor, where specific aptamer binding to phenylalanine and a rhodium-based receptor was monitored using a redox indicator and square-wave voltammetry.

Surface fouling, caused by the adsorption and deposition of biological or chemical molecules, remains a major limitation in developing truly calibration-free sensors. Significant technological improvements are still required for biosensors to become fully calibration-independent.[46]

5.2. Anti-Fouling Surface-Based Sensors

Unwanted adsorption, deposition, or polymerization of biological or chemical molecules, such as proteins, lipids, or cells, on the sensor surface is one of the most critical challenges in biosensing, especially for in vivo applications. This phenomenon, known as surface fouling, leads to:

- High noise levels
- Reduced selectivity
- Interference with target-receptor interactions
- Non-specific signal generation

To address this issue, several strategies have been developed, including:

- Hydrophilic Coatings
- Controlled release of nitric oxide
- Novel designs for reference electrode

Anti-fouling layers not only improve reliability but also significantly extend sensor lifetime.

Materials used for anti-fouling layers include:

- Hydrophilic polymers, such as polyethylene glycol
- Conductive hydrogels
- Engineered peptides
- Nucleic-acid-based ternary layers

These materials create physical or chemical barriers that prevent non-specific adsorption.

In one study, porous structures formed by cross-linking bovine serum albumin with glutaraldehyde and containing embedded gold nanorods were used to reduce sensor fouling. This system successfully detected an inflammatory signaling molecule even after one month of electrode exposure to human serum. The porous architecture blocked large interfering molecules while allowing specific antibody-target interactions.[47]

5.3. Nanozymes

Nanozymes are nanomaterials that exhibit enzyme-like catalytic activity, enabling them to catalyze various chemical reactions. Although natural enzymes offer superior specificity and catalytic efficiency, nanozymes have rapidly gained attention due to their:

- Tunable catalytic activity
- Low cost
- High stability
- Resistance to harsh conditions
- Ease of functionalization

Since the first report of enzyme-like activity in nanoparticles, numerous nanozymes have been developed, leveraging unique physicochemical properties such as:

- Luminescence
- Electrical conductivity
- Paramagnetic behavior

Nanomaterials used as nanozymes include:

- **Carbon-based nanomaterials (CNTs, graphene, GO, carbon dots)**
- **Metal nanoparticles (e.g., gold nanoparticles)**
- **Metal-oxide nanomaterials (e.g., cerium oxide)**

Nanozymes fall into two major families:

1. **Redox family: enzymes catalyze electron-transfer reactions and are widely used in electrochemical and optical biosensors.**
2. **Hydrolase family: enzymes catalyze hydrolytic bond-cleavage reactions and are useful for detecting specific biomolecules.**

Due to their stability, tunability, scalability, and low cost, nanozymes have become key components in next-generation biosensors.[48]

5.4. Smartphone Interfaces

With approximately 1.75 billion smartphone users worldwide, leveraging smartphone capabilities for biosensing offers unprecedented advantages. Smartphones can function as:

- **Detectors**
- **User Interfaces**
- **Data-Processing Units**

High-resolution cameras can be used directly for optical detection methods such as microscopic imaging, fluorescence, and colorimetric biosensing, or they can be integrated into advanced optical systems.

Smartphones have also been used for SPR detection by measuring refractive index changes at the sensor surface, enabling highly sensitive analyte detection.

Simultaneously, a new generation of miniaturized electrochemical biosensors has emerged, equipped with internal circuits and wireless communication modules. These sensors can detect multiple biomarkers simultaneously and are ideal for point-of-care diagnostics due to their small size, low power consumption, and smartphone connectivity.

Smartphone-based applications ("apps") significantly improve user accessibility and on-site diagnostic capability.[49]

5.5. Wearable Biosensors

Wearable biosensors provide continuous, real-time monitoring of physiological parameters using non-invasive samples such as sweat, tears, saliva, or interstitial fluid. Recent advances focus on integrating:

- **Flexible electronics**
- **Wireless Data Transmission**
- **Skin-implantable systems**

In one study, Gao et al. developed a fully integrated wearable biosensor for monitoring sweat composition. The system simultaneously measured glucose, lactate, sodium, and potassium, transmitting data wirelessly to a smartphone for real-time display.

In another study, Alshareef et al. developed a hydrogel-based contact-lens biosensor capable of real-time glucose monitoring in tears, an entirely non-invasive method for diabetes management.

Additionally, tattoo-based biosensors have been introduced using color-responsive dyes that change in response to pH or other biomarkers. Yatisen and colleagues developed a minimally invasive colorimetric tattoo biosensor sensitive enough to detect physiological glucose variations.[50]

5.6. Lab-on-a-Chip Systems

Lab-on-a-chip (LOC) platforms are among the most important miniaturized diagnostic technologies. These millimeter- to centimeter-scale chips integrate multiple analytical processes and can analyze extremely small sample volumes in a short time.

Microfluidics is the core of LOC systems, enabling:

- **Transportation**
- **Separation**
- **Reaction handling**

Based on this technology, paper-based microfluidics, centrifugal chips, and digital nucleic-acid detection chips have been developed for detecting ions, pathogens, and various diseases.

In one study, Sakin et al. designed a microfluidic system capable of measuring chloride, zinc, and sodium levels in sweat using a smartphone. Fluorescent molecules produced color changes upon interacting

with the target analytes, which were quantified directly using the phone camera.[51]

5.7. Biomedical Applications

A wide range of biosensors is now routinely used in medicine. These devices detect analytes in tissues, microorganisms, organelles, cell receptors, enzymes, antibodies, and nucleic acids.

Biosensors have significantly advanced medical diagnostics by enabling the precise detection of various biological markers:

- **Antibody Interactions**
- **Enzyme Activity**
- **Glucose Thresholds**
- **Microbial Infections**
- **Tumor Growth**
- **Pathogens and Toxins**

In the current landscape, nanomaterial-based biosensors are widely used for detecting COVID-19.

Table 1. Summarizes Various Biomedical Applications of Biosensors

No.	*Purpose*	*Description*	*Source*
1	*Monitoring biological abnormalities*	*Numerous biosensors have addressed major challenges in healthcare by tracking vital signs and detecting biological abnormalities. Since early prevention and intervention reduce treatment costs, monitoring risk factors is essential. Effective time-management is a key goal, enabling patients to improve outcomes through communication with clinicians. Medical biosensors can rapidly detect physiological changes that require early treatment. Thanks to recent technological advances, monitoring systems can now be deployed at a much lower cost than hospital-based care.*	*52*
2	*Heart-rate monitoring*	*A biosensor—such as a smartwatch or fitness band—continuously tracks heart rate and transmits physiological data. Advanced sensors can detect specific biomarkers to support clinical diagnosis. A bioreceptor senses the function or concentration of a biomarker and generates electrochemical or optical signals. The transducer converts these raw signals into electrical outputs. These technologies act as evolutionary algorithms in biomedical applications and are key components of Industry 4.0.*	*53*
3	*Continuous monitoring of chemical changes in the human body*	*An implantable sensor placed beneath the skin enables continuous tracking of a patient's biochemical profile. This technology supports diabetes monitoring and exercise-related metabolic assessment. It has transformed remote patient-health monitoring at home.*	*54*
4	*Dietary monitoring*	*Individuals can now analyze food molecules using dietary sensors, which can also track caloric intake. For people with life-threatening allergies, heightened food awareness is critical. Healthcare systems use advanced nanosensors to analyze breath acetone levels in clinical settings.*	*55*
5	*Air-quality monitoring*	*Advanced sensing technologies allow easy monitoring of air quality. These systems continuously track daily air conditions and measure core body temperature, providing valuable information to users and clinicians about potential disease indicators. Wireless technologies enable precise monitoring of internal temperature, sleep patterns, exercise physiology, clinical diagnostics, and various hospital applications.*	*56*
6	*Glucose monitoring*	*Electrochemical test strips are widely used for glucose monitoring in diabetes management. Traditionally, finger-prick blood samples were required; however, researchers are developing wearable biosensors for continuous glucose monitoring. These biosensors are suitable for routine monitoring, such as diabetes management, as well as diagnostic tests, including pregnancy and fertility assessments.*	*57*
7	*Accurate results*	*Biosensors provide new, immediate, and more accurate results. They*	*58*

	and decision-making	*have been studied for decades in decision-making processes. Recent research has demonstrated their role in critical daily decisions linked to muscle activation. Multimodal biosensing tools are valuable assets in medical applications, enabling early diagnosis, disease monitoring, and treatment planning. These technologies provide significant advantages for the future of healthcare systems.*	
8	*Continuous cardiac-rhythm monitoring*	*The number of possible applications has increased significantly due to screen-printed electrodes that enable smaller wearable biosensors with more precise data. Cardiac-rhythm monitoring can now be performed rapidly. This technology can track stress levels in service workers, law-enforcement personnel, miners, firefighters, and others. Additionally, it can assess athletic fitness both before and after physical activity.*	*59*
9	*Patient-status monitoring in healthcare settings*	*Wearable biosensors assist children, athletes, the elderly, and many other populations. Advances in healthcare systems have driven rapid progress in biosensor technology. These devices have long been used in hospitals to monitor patient status by tracking biological processes and providing analytical insights. They minimize invasiveness for measurements such as blood-oxygen levels. Demand for wearable biosensors has increased due to conditions such as sleep apnea, peripheral artery disease, and pulmonary obstruction.*	*60*
10	*Disease management*	*Healthcare depends on effective diagnosis and disease management through the use of biosensors. Modern medicine emphasizes rapid point-of-care diagnostics. Many clinical disorders—including infections and cancers—can be detected using biosensor technologies. The demand for improved sensors continues to grow. Biosensors with enhanced sensitivity and accuracy are used in medical devices, smart cities, IoT systems, and wearable technologies. They can analyze various human features such as heart rate, activity patterns, sleep habits, personality traits, and more.*	*61*
11	*Symptom detection*	*Biosensors allow clinicians to detect symptoms more rapidly than standard approaches. A biosensor consists of a sensor, a transducer, and an electronic unit. Developers focus on enhancing healthcare performance. Hospitals use biosensors to regulate blood pressure, glucose levels, and cardiac rhythm. Real-time diagnostic testing offers significant benefits. Additionally, modern biosensors incorporate digital pill integration. The extensive data generated by biosensors facilitates the adoption of wearable smart technologies in medical institutions.*	*62*
12	*Tracking biological data*	*Medical patches can track biological data such as skin temperature and respiratory rate. Home-monitoring kits have expanded rapidly, enabling patients to track their health on a daily basis. Many diseases, including cancer, can be monitored at home. Biosensors also help clinicians verify medication adherence. Their widespread use in hospitals and home care makes them accessible in most healthcare facilities, improving patient experience and personalized care.*	*63*
13	*Tracking cellular proteins*	*Biosensors are used to track live-cell proteins in real time without labeling. A microfluidic system, composed of a cell module, and a biosensor module enables this functionality. Secreted proteins are delivered directly to the adjacent biosensor module. Continuous spectral monitoring allows real-time tracking of secretion dynamics. This microfluidic platform supports multiplexed, label-free detection within a compact footprint, making it suitable for lab-on-a-chip integration.*	*64*
14	*Biomolecular detection and quantification*	*Biosensors are essential analytical tools for detecting and quantifying biomolecules. They allow real-time monitoring of oxygen levels in biological systems and support organ-mimicking models. These systems help researchers develop new drugs by simulating organ-specific functions—such as oxygen transfer from air to*	*65*

		blood—on a microscale.	
15	*Post-surgical treatment monitoring*	*Biosensors improve post-surgical recovery by using pressure sensors for the brain and sensors that monitor implant-related infection and inflammation. Accurate processing, storage, and communication of this data are essential for improving patient outcomes. Effective biosensor use in healthcare is vital for building public trust in patient-data management.*	*66*

6. Future Outlook and Conclusion

Biosensors, as advanced bioanalytical tools, play a pivotal role in the rapid, accurate, and cost-effective detection of biological molecules. Recent advances in nanotechnology, surface engineering, and advanced material design have enabled the development of next-generation optical, mechanical, and electrochemical biosensors with high sensitivity, excellent selectivity, and the ability to operate with extremely small sample volumes.

Nanomaterials significantly enhance performance by increasing the effective surface area, improving charge-transfer kinetics, and providing robust platforms for bioreceptor immobilization. Furthermore, advanced surface-engineering strategies and anti-fouling layers have greatly improved long-term stability and enabled reliable operation in real-world environments.

Despite these advancements, several challenges remain, including the following:

- Stability of nanomaterials under physiological conditions
- Improving the signal-to-noise ratio
- Developing low-cost, fouling-resistant sensing surfaces
- Enhancing the efficiency of enzyme-based biosensors

Additionally, the growing demand for portable, wearable, and flexible-electronics-integrated with biosensing systems continues to shape future research directions.

Overall, this review demonstrates that the integration of nanotechnology, surface engineering, and advanced material design can pave the way for biosensors that are suitable not only for laboratory environments but also for everyday applications, personalized medicine, and real-time health monitoring. This research trajectory provides a promising outlook for the development of highly accurate, stable, and reliable biosensors in the next generation.

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