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Review—Bio-Nanosensors: Fundamentals and Recent Applications

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Nanosensors to detect and measure biomarkers are increasingly pervasive in our daily lives. Nanostructured materials provide an unprecedented opportunity to measure ultra-low concentrations ($< \text{nM L}^{-1}$) of target analytes, which in turn enables improved understanding of complex biological processes. Sensors with low detection limits serve multiple roles, most notably for early detection of diseases, containment of infections and viral propagation, monitoring biodata, and food quality control, as well as elucidating high-resolution genome-phenome relationships. Here, we introduce the fundamental components of a bio-nanosensor, including the signal transduction elements and target-selective receptors (e.g., low molecular weight synthetic moieties, or organic molecules such as DNA, enzyme, antibody, etc). We focus on electronic, electromechanical, optical, and electrochemical transducers, and discuss how the advent of nanostructured materials, particularly 2D materials and nanoparticles, have enabled improved performance in terms of biocompatibility, sensitivity, selectivity, wearability, and limits of detection. We present an overview of recent progress in the development and application of nanomaterials for the detection and quantification of biomarkers, along with the pros and cons of the different transduction strategies, and close with a discussion of persisting challenges and opportunities in the field.

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In recent years, the development of nanosensors has gained significant attention in many fields of application, including but not limited to chemical, biological, medical, industrial, agricultural, thanks to their capabilities in real time detection and quantification of analytes.^{1–4} New strategies for designing sensors capable of measuring extremely low concentrations of different molecular species are fueled by the promise of lower costs of fabrication, ultrahigh sensitivity and selectivity, low-power consumption, real-time response, biocompatibility, in-vitro capabilities, and ease of integration with conventional electronic manufacturing processes.^{5–8}

A nanosensor is traditionally composed of three major parts: a probe, a transducer and a detector (Fig. 1).⁹ The design of a nanosensor depends on the targeted analyte's physical, or chemical property used to identify and quantify it. This particular property of interest is the base for the selection, design and configuration of the sensor's probe and transducer. The probe collects the signal through a physical or chemical interaction with the analyte and sends this information to the transducer.

The transducer must be selected according to the type of signal probed and how it will be converted into a measurable quantity (e.g. electrical signal, frequency, etc.), associated to the concentration of the analyte of interest. In the latter step, a detector is designed to filter the measurable quantity associated to the concentration and to provide a readable value that can be converted into the desired units. In Fig. 1, the probe or electrode collects the information related to analyte-light interactions in optical transducers, electron transport across analyte-mediated interfaces in electrochemical and field-effect transducers, and analyte-induced material deformations/vibrations in mechanical transducers. When an analyte binds with high affinity to the ligand, the probe produces a distinguishable output signal change, which is then translated into user defined units by the electronic/optical system. The translated signal represents a measurable value of analyte presence/concentration that is then quantified by an electronic/optical system to favor interpretation. Zeng et al. demonstrated a biometric-based tactile chemomechanical transduction strategy based on sponge electrode-modified barium titanate-polyppyrole-reduced graphene oxide (BTO-PPy-rGO) that allows a flexible tactile sensor to perceive bio/chemical signals. The sensor platform was designed for point-of-care immunoassay/aptasensor of antibiotics and provide high sensitivity, wide generality and

portability.¹⁰ Other examples of transduction technologies in biosensing can be found in Refs. 11, 12. Here we focus on sensors for detecting and measuring biological markers (e.g. metabolites, proteins, nucleic acids, etc.), hence we need to take into account these live in a solvated, thermally noisy and unspecific environment populated by many other molecules.¹³ Different techniques have emerged to avoid this problem by increasing the sensor's signal-to-noise ratio,¹⁴ including isothermal amplification (IA) for nucleic acid systems. IA relies on enzymatic amplification (e.g., DNA polymerases) to amplify a target sequence, without requiring temperature changes. Therefore, it offers several advantages over PCR, including: faster speed to diagnosis (continuous, exponential amplification independent of thermal cycling), reduced power requirements (ideal for portable point-of-care diagnostics), higher sensitivity (exponential amplification), and improved specificity (depending on primers). Some of the most common IA techniques today are: Helicase-dependent amplification that is based on the in vivo DNA replication by using helicase to unwind DNA, allowing primers to bind;¹⁵ Isothermal multiple displacement amplification that uses a strand-displacing DNA polymerase and multiple primer sets;¹⁶ Loop-mediated isothermal amplification that uses four to six primers and a strand displacing DNA polymerase;¹⁶ Rolling circle amplification that synthesizes long single-stranded DNA using a short, circular single-stranded DNA template and a single primer;¹⁷ Single primer isothermal amplification uses only one DNA-RNA chimeric primer along with RNase H and a DNA polymerase with strand displacement activity;¹⁸ and Strand displacement amplification that relies on a restriction enzyme (HincII) and an exonuclease-deficient DNA polymerase.¹⁹

A nanosensor's performance, is characterized by its sensitivity, selectivity, measurement range, measurement time, repeatability, and limit of detection.²⁰ Sensitivity refers to the lowest change to which the sensor can respond (Fig. 2) and selectivity to the affinity of the sensor to the particular analyte being detected in the presence of other materials. The fundamental concept of selectivity is described in Fig. 3, where an ideal case is shown in the measurement 1 plot. Here, only the target analyte is associated with the ligands on the probe's surface, which is traduced as a clean concentration signal (signal 1), i.e., without noise. In this case the sensor is highly selective. In the measurement 2 plot, the red analyte can have partial binding affinity to the ligand. In this case it introduces a measurable noise in the signal, leading to a variation in the sensor response ΔC (signal 2). Ideally, higher selectivity means ΔC tends to zero. In C_1 ,

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Figure 1. Diagrammatic representation of a nanosensor. Purple-spherical markers represent the target analyte, and red-cubic markers as unwanted analytes (noise).

Figure 2. Schematic representation of nanosensor's sensitivity, LOD, measurement range and time. Purple-spherical markers represent the target analyte. At C_1 one analyte-ligand interaction is represented on the probe's surface, which is equivalent to the lowest concentration that this sensor is capable to detect or LOD. At C_2 a second analyte-ligand interaction has taken place, in this case the change produced in the concentration value represent the minimum change that this sensor is capable to detect (sensitivity). At C_3 and C_4 additional analyte-ligand interactions are represented as equivalent changes in the analyte concentration, the sensor response to these changes is represented by A_3 and A_4 values.

for both measurement 1 and measurement 2 plots, one target analyte-ligand interaction is presented, leading to a LOD value. For C_2 , in measurement 1 the target analyte-ligand interaction produces an A_2 response value, whereas in measurement 2 the undesired target-ligand interaction produces a ΔC response value, which is so much lower than A_2 . This signal difference is represented as selectivity.

The measurement range defines the minimum and maximum limits of measurable analyte concentration, and measurement time refers to the minimum time at which the sensor can offer a response to changes in concentrations (Fig. 2). Repeatability indicates how

different measurements may be compared when they are performed at different times with the same type of sensor and for the same analyte concentration. Two different experiments are shown in the measurement 1 and 2 plots of Fig. 4. Four concentrations of target analytes are measured. For both experiments, the response signals (signals 1 and 2) are obtained with analyte-ligand interactions at different positions. The sensor's repeatability is represented as the similarity between each response value (LOD, A_2 , A_3 , A_4) at a certain analyte concentration, i.e., standard error between signal 1 and signal 2 tends to zero. The limit of detection or LOD, specifies

Figure 3. Schematic representation of nanosensor's selectivity. Purple-spherical markers represent the target analyte, and red-cubic markers represent undesired analytes.

the lowest analyte concentration that the sensor is capable to detect for a given volume (Fig. 2). The LOD and the sensitivity, are generally used to evaluate the performance between different nanosensors. In practice, the shelf-life stability is also used to specify the repeatability of the measurements in time (weeks, months, years).

Nanosensors can be categorized as optical, mechanical, chemical or electrical,²¹ or a combination thereof. Each category offers advantages and disadvantages, depending on the practical purpose required and the properties of the analyte of interest. Electrical nanosensors have recently emerged for the measurement of ultra-low concentrations of analytes thanks to their reliability, small form factor, high sensitivity, low LOD, low power consumption, ease of fabrication and low fabrication cost.²² Within this category, there are two representative technologies, field-effect-transistor (FET) and electrochemical nanosensors.

Recent reviews are focused on organic, 2D and other nanostructured materials for biosensors in diagnostics applications.^{23–30} This review is meant to introduce the current state of the art in

nanosensors development, to explain their fundamental principles of operation,³¹ their current and future fields of application, and to describe the main differences conveyed from optical, mechanical, field-effect and electrochemical transducers for the detection and measurement of biomarkers.³² It provides a detailed overview of the current state of the art, a comparison of capabilities, and a summary of some of the prevailing technological and scientific challenges, and opportunities, and concluding remarks.

Optical Nanosensors

Optical nanosensors are based on the interactions between analytes and light.^{33,34} The most representative optical detection methods used in nano-sensors are: Surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), surface-enhanced Raman spectroscopy (SERS), fluorescent spectroscopy and colorimetry.³⁵

Surface plasmon resonance (SPR) sensors rely in measuring the amount of change in reflected light from a thin metal surface due to

Figure 4. Diagrammatic representation of nanosensor's repeatability. Purple-spherical markers represent the target analyte.

an absorbed analyte. The light source is polarized at a certain angle, known as the resonance angle. Typically, the metal surface is chemically functionalized to immobilize the specific analyte of interest. When light (electromagnetic radiation) excites the surface electrons of the metal (plasmons), a resonant oscillation occurs causing a change in the material's refractive index, which also depends on surface bound analytes. An optical detector is used for measuring the reflected light, which is proportional to the added mass on the metal surface. For example, Piliarik et al. reported a SPR biosensor for high-throughput screening of protein biomarkers in complex samples such as blood plasma.³⁶ It was used for the detection of protein biomarkers relevant to cancer diagnostics—human chorionic gonadotropin (hCG) and activated leukocyte cell adhesion molecule (ALCAM) both in buffer and in 10% blood plasma (Fig. 5a). The antibodies were immobilized on the sensing areas via hybridization of antibody–oligonucleotide conjugates to thiolated complementary oligonucleotides microspotted on the sensor surface (DNA-directed immobilization) achieving LOD as low as 45 ng ml^{-1} (ALCAM) and 100 ng ml^{-1} (hCG) in blood plasma samples.

Vaisocherová et al. reported a SPR biosensor for direct label-free detection of the Epstein-Barr virus (anti-EBNA).³⁹ The antibody detection was performed using the immunoreaction between anti-EBNA and a respective synthetic peptide (EBNA-1), which was conjugated with bovine serum albumin (BSA–EBNA) and immobilized on the sensor surface. This work reports hydrophobic interactions to immobilize the ligand, which showed a high compatibility with the non-polar structure of EBNA. Sensor response to anti-EBNA exhibited a high degree of reproducibility and the LOD for the direct detection of anti-EBNA in a diluted human blood serum was estimated to be 0.1 ng ml^{-1} . Other works related to SPR sensors using antibodies for biomarker detection can be found in Refs. 40, 41.

Novel SPR biosensors have been proposed in recent years aimed at improving the sensor's sensitivity or LOD. This specific two properties are important due to the proportional relation with the optical sensor capability to improve the signal to noise ratio under any configuration even for ultra-low concentration of target analyte measurements. Srivastava et al. proposed a novel SPR biosensor comprising of BK7 prism, the new family of 2D transition metal carbide/nitride materials known as MXene, transition metal

Figure 5. (a) Layout of the SPR bio-sensor with polarization contrast and internal referencing. The light path is divided into reference beams (A) and (C) and the image of the sensor surface (B). Detail of the sensor surface depicts the functionalization with a protein array and a low-fouling background.³⁶ Reprinted with permission from Elsevier. (b) Schematic diagram of silicon-WS₂/nanosheets-enhanced SPR biosensor. A gold thin film is attached at the bottom of the SF10 prism followed by silicon nanosheet and 2D MX₂ layer that is in directly contact with the analyte.³⁷ Reprinted with permission from Springer Nature. (c) Phase-sensitive Mach-Zehnder interferometer SPR biosensor. The SPR phase is extracted by comparing the phase difference between the interference signals of p polarization and s polarization, which allow to eliminate all common-path phase noise. The PTZ introduces a periodic linear phase shift. The refractive index change associated with the analyte concentration is measured.³⁸ Reprinted with permission from The Optical Society.

dichalcogenides (TMDCs), black phosphorus and gold(Au) as plasmonic materials.⁴² The sensitivity of this sensor is improved by 15.6% compared with conventional SPR bare metal film sensors. This result is achieved with the inclusion of the MXene material, which is characterized by important properties like high electrical conductivity, large surface area and high interlayer spacing. Ouyang et al. presented a sensitivity-enhanced SPR biosensor structure based on silicon nanosheet and 2D transition metal dichalcogenides (MX₂),³⁷ which have shown better performance than the conventional SPR scheme with pure Au thin film. Based on the Kretschmann configuration, the system consists of SF10 triangular prism, gold thin film, silicon nanosheet and 2D MX₂ film (Fig. 5b). Excessive MX₂ layers result in increased energy loss and reduced the sensitivity.

Wu et al. proposed an alternative SPR biosensor configuration based on the Mach-Zehnder interferometer for differential phase detection (Fig. 5c).³⁸ P-polarized light has an electric field polarized parallel to the plane of incidence, while s-polarized light is perpendicular to this plane. A Wollaston prism is used for the *p* and *s* phase quantities determination, where *s* is used as reference, and the differential phase between the *s* and *p* polarizations is measured. This design improves sensitivity because of its effectiveness in minimizing common-mode noise. One variant to SPR method, is the localized surface plasmon resonance (LSPR). Here metal nanoparticles are used, and the emitted light generates localized surface plasmons that oscillate on the nanostructure, with refractive changes that depend on the interactions between the ligands and the analyte. This technique improves the LOD in comparison with conventional SPR. El Barghouti et al. proposed a LSPR biosensor based on silver nanoparticles coated with a graphene film and deposited on a substrate. The plasmonic response of this configuration was very sensitive to variations in the thickness of the graphene film, i.e., the sensitivity increases with the thickness of the graphene layer.⁴³ Another alternative to the metallic

nanoparticles for this method is the inclusion of gold nanorods (GNRs). GNRs present a larger light absorption and cross section in comparison with gold nanoparticles. Wang et al. introduced a novel high sensitivity LSPR biosensor based on anisotropic GNRs for the detection of hepatitis B surface antigen (HBsAg) with a LOD of 0.01 IU (or index unit)/ml.⁴⁴ The surface of the GNRs was functionalized with monoclonal hepatitis B surface antibody (HBsAb) through physical adsorption (Fig. 6a). Further detail on LSPR biosensors can be found in recent reviews.^{45,46} The primary field of application for SPR and LSPR is biomarker detection of molecular binding events.^{47–49} Different configurations are possible by varying the metallic materials. Nanoparticles (NPs), and immobilization ligands using Gold NPs (AuNPs) are perhaps the most common, yet the use of carbon nanomaterials have seen a significant increase over the last few years, including the use of graphene and graphene oxide (GO) for the detection of human proteins.⁵⁰ For example, Wu et al. presented a SPR based graphene-on-gold biosensor where the sensor's sensitivity is proportional to the number of graphene layers. This increased sensitivity is explained due two fundamental properties of graphene: i) strong and stable absorption of biomolecules with carbon ring structures and ii) increased sensitivity to refractive index changes as a function of the SPR curves.⁵¹

Singh after an exhaustive analysis of SPR biosensing platforms showed that despite the important advantages of the current SPR and LSPR technology, the portability of this instruments has been the main disadvantage preventing it from reaching its full potential. Other limitations are related with selectivity in complex biological solutions, sensitive detection of membrane-associated species and lack adaptation for sensing elements for portable diagnostic devices.⁵³

In order to improve the LOD obtained with SPR and LSPR biosensors, the Surface-enhanced Raman scattering (SERS) method is implemented in the sensor design,^{54–57} which is based on the

Figure 6. (a) Schematic diagram of the GNR LSPR biosensor for detection of HBsAg in buffer, serum or plasma. Monoclonal HBsAb is labelled on the surface of the GNRs through nonspecific adsorption to detect HBsAg. The analyte binding events are monitored by changes of local refractive index in the LSPR process.⁴⁴ Reprinted with permission from Elsevier. (b.) Experimental setup of SERS biosensor for PSA detection. The system is based on three major parts: laser light source, spectrograph and fiber-optic probe. The light source is a HeNe laser and the fiber-optic probe uses a band-pass and long-pass filters for OD 6 and OD 4 rejection.⁵² Reprinted with permission from American Chemical Society.

classical Raman spectroscopy where inelastic scattering of photons is produced by the interaction between incident photons from an electromagnetic source and a specific analyte molecule.

The scattering and incident photons will have different frequencies because a shift produced by the energy transfer within the molecule. An enhanced variant of the Raman method uses metallic NPs to improve the scattering signal intensity, i.e., intensity will depend on the spatial location of the metal in comparison with the target molecule, showing that the maximum intensity is presented at the interstitial sites of the particle.^{58,59} This technique has been used for nanosensing in different fields such as biomedical,^{60,61} food science,^{62,63} and environmental monitoring.⁶⁴

For example, Grubisha et al. developed a SERS biosensor based on a novel reagent composed of AuNPs with molecular labels for low level detection in immunoassays of prostatic-specific antigen (PSA), achieving LOD of 1 pg ml^{-1} in human serum.⁵² The experimental setup of this SERS biosensor can be observed in Fig. 6b.

Another important work related with the SERS biosensor involves the direct detection of glucose. Glucose detection across SERS represent a challenge since it has a small normal Raman cross

section and adsorbs weakly or not at all to bare silver surfaces, nevertheless Shafer-Peltier et al. proposed the first step toward to solve this problem.⁶⁵ To avoid the absorption problem, glucose is partitioned into an alkanethiol monolayer adsorbed on the silver surface thereby preconcentrating it within the zone of electromagnetic field enhancement.

Recent works have showed that using carbon-based materials improves the SERS detection.⁶⁶ For instance, He et al. developed a gold-decorated graphene SERS biosensor where the graphene film works as a high efficiency SERS-active substrate for DNA detection, achieving a LOD as low as 10 pM .⁶⁷ Further detail on SERS biosensors can be found in a recent review.⁶⁸ Compared to the large size of conventional SPR biosensors, Quang et al. developed a portable SERS sensor for detection of dipicolinic acid (DPA) and malachite green (MG), hazardous materials, in a microfluidic channel. This sensor could overcome the poor reproducibility in the SERS detection by using a micropillar array channel to achieve efficient mixing between silver nanoparticles and hazardous trace materials. The sensor LOD was $200\text{--}500 \text{ ppb}$.⁶⁹

Yet another optical biomarker sensing platform consists of the use of fluorescent signals emitted upon external radiation in order to

Figure 7. (a) Chemical structure of the QDs-ConA- β -CDs-AuNPs FRET biosensor. The sensing mechanism is based on the switching off of FRET through the highly specific recognition of ConA by glucose. In the presence of glucose, the AuNPs- β -CDs segment of the bio-nanosensor is displaced by glucose which competes with β -CDs on the binding sites of ConA, resulting in the fluorescence recovery of the quenched QDs.⁷⁸ Reprinted with permission from John Wiley and Sons. (b) Chemical structure of the QDs/DNA/AuNPs N-SET biosensor. The red and blue ssDNA strands are complementary. In absence of Hg^{2+} the probes are not capable of hybridization and are dispersed in the aqueous solution due to the electrostatic repulsion from the negatively charged ssDNA attached on the nanoparticle surfaces. When Hg^{2+} ions are present hybridization occurs leading to a quenching of the fluorescent emission of the QDs due to the close proximity between QDs and AuNPs.⁸⁰ Reprinted with permission from American Chemical Society.

improve the sensing distance range between the device and the analyte.^{70,71} The fluorescent method is a photo emission/absorption process based on the ability of certain substances, known as fluorophores or dyes, to emit/absorb photons; the former, when excited by electromagnetic radiation. This property can be used for labeling target molecules and to detect binding events of interest.⁷² Two major fluorescent techniques exist today: fluorescence resonance energy transfer (FRET) and NP-based surface energy transfer (N-SET). FRET is a nonradiative energy transfer process between two fluorophores, a donor and an acceptor. The emission spectra of the donor must overlap the absorption spectra of the acceptor. When used for sensing, different functional configurations can be used, for example, a donor fluorophore binding to an acceptor analyte of interest, leads to fluorescent emissions that are quenched as the acceptor analyte approaches the donor. The efficiency of this process

is inverse to the distance between donor and acceptor, with optimum performance in the range of 1–10 nm. On the other hand, an N-SET process is based on the same principle but in this case the acceptor is a NP, typically metallic, capable of quenching the fluorescence emissions from a nearby donor. The advantage of the N-SET over the SET process, comes from an increased distance range between the donor-acceptor pair.⁷³ These fluorescent methods are widely used in areas of biology and medicine.^{74–77} Tang et al. presented a novel high sensitivity and selectivity assembled FRET biosensor based on quantum dots (QDs), concanavalin A (ConA), β -cyclodextrins (β -CDs) and gold nanoparticles (AuNPs) (QDs-ConA- β -CDs-AuNPs) for glucose detection in human serum samples with a LOD as low as 50 nM (Fig. 7a).⁷⁸ Here, the sensing mechanism is based on the fluorescence interaction between concanavalin A (ConA)-conjugated quantum dots (QDs) as an energy donor and thiolated

β -cyclodextrins (β -SH-CDs)-modified AuNPs as an energy acceptor. An important strategy to overcome the distance limitations in FRET sensing platforms is related to design the sensor through a multi-stage conformation as is described in Medintz et al.,⁷⁹ where a two-step FRET quantum dot-*Escherichia coli* maltose-binding protein (QD-MBP) sugar-sensing assembly with a fluorescent dye acting as a relay station was designed and prepared surpassing inherent QD donor-acceptor minimum interaction distance. An ultrasensitive and selective QDs/DNA/AuNPs N-SET biosensor was developed by Li et al. for Hg^{2+} detection in water (Fig. 7b), a LOD as low as 0.4 ppb was achieved in buffer solution and the use of QD/Au optical assay eliminates the photobleaching with organic dyes.⁸⁰ DNA, used as molecular probe, offered great stability in a nonphysiological solution in comparison with other biological probes.

Last, but not least, the colorimetric techniques are based on the detection of color changes (shifting frequencies) resulting from the interaction of light and a material or analyte of interest.^{81–84} The concentration of a sample analyte in solution, is calculated from the intensity of light before and after it passes through the sample by using the Beer–Lambert law, which relates the attenuation of light to the properties of the solvated analyte through which the light is travelling. Colorimetry is less sensitive in comparison with the other methods described here, but it has been successfully used for cancer detection with AuNPs.^{85,86} Mollasalehi and Shajari developed a rapid and non-invasive nano-biosensing method for simple diagnosis of prevalent lethal cancers based on a colorimetric nanosensor with non-crosslinking hybridization of gold nanoprobe capable to detect extracellular miRNAs.⁸⁷ This novel biosensor allowed cancer screening test to early and effective diagnosis and treatment. Optical nanosensors are characterized by their high sensitivity, especially when nanoparticles are used. Nevertheless, selectivity can still be a challenge in complex biological solutions. A summary of seminal work associated to optical bio-nano sensors is given in Table I.

Mechanical Nanosensors

Mechanical nanosensors are based on the measurement of forces, displacements and mass changes at the molecular scale, commonly using a mechanical element known as cantilever (Fig. 8).⁹⁸ Mechanical sensors are classified based on the underlying physical principle used, as surface-stress or dynamic mode based. In surface-stress systems quasistatic deflections are produced due to molecular binding events at the surface of the cantilever, which is transduced via reflecting laser beams, piezoresistivity or capacitance. In the dynamic mode methods, mass changes at the surface causes resonance frequency shifts that are measured and related to the concentration of an analyte of interest. Other mechanical based sensing technologies, such as quartz crystals microbalances (QCM)⁹⁹ and whispering-gallery microcavity (WGM)¹⁰⁰ are also widely used. QCM measure changes in inertial mass and WGM changes in analyte absorption. Multiple practical application using these kinds of sensors in biology and food monitoring are found in the recent scientific literature.^{101–103} Lim and Lee developed a sensitive and selective detection technology of MicroRNA-21 (miR-21) using a QCM biosensor. The miR-21 act as an important biomarker for cancer.¹⁰⁴ The LOD achieved by the sensor was 0.87 pM.

An Au-coated dynamic piezoelectric cantilever sensor for real-time detection of microRNA in buffer and human serum was developed by Johnson and Mutharasan.¹⁰⁵ The microRNA levels present in the sample was quantified by measuring the resonant frequency response as molecular binding occurs on the cantilever. A LOD as low as 4 fM was achieved. Mechanical biosensors have also been demonstrated in the study of cancer. For instance, Shan et al. developed an aptamer-based QCM biosensor for the selective and sensitive detection of leukemia cells (Fig. 9a).¹⁰⁶ The principle of operation of this sensor is based on the immobilization of an aptamer on the sensor's surface, which selectively capture the target analyte;

then, aminophenylboronic acid-modified gold nanoparticles (APBA-AuNPs) are used to label the cells followed by a silver enhancement for signal amplification. The resonant frequency change of the QCM caused by the deposition of silver metal is monitored in real time.

Furthermore, Vollmer et al. presented a WGM biosensor for detection of label-free molecules (Fig. 9b).¹⁰⁷ This sensor uses optical resonances in a dielectric microparticle as the WGM mechanism. The resonances are generated when light, confined by total internal reflection TIR, orbits near the particle surface and returns in phase after each revolution. Binding of proteins on the microparticle surface is measured from a shift in resonance wavelength.

A list of selected studies on mechanical biosensors is summarized in Table II. Mechanical nanosensors offer fast response times in the detection of biomarkers but are often affected by thermal noise and non-specific adsorption (lowering selectivity).

Field-Effect-Transistor (FET) Nanosensors

Field-effect-transistors (FETs) are one of most promising technologies in electrical nanosensing due to its simple construction, direct, and fast detection and measurement of analyte concentrations. FETs have the advantage of direct conversion of analyte activities in proportional electrical signals, including current or conductance (or transconductance). A nano FET for biomarkers in solution is typically composed of two electrodes (drain and source), a semi-conducting channel sitting on a thin dielectric layer, and a third electrode (preferably a back gate) coupled to the other side of the dielectric layer (Fig. 10). Analytes binding to the surface of the semiconducting channel causes changes in the surface potential, which are measured as changes in the channel's conductance. The gate potential sets the accumulation or depletion of charge-carriers in the channel.¹¹⁶ There are different nano-FET configurations for sensing, and each of one depends of the properties of the target analyte and the material used for the conducting channel, among which we can find as ion selective membranes (ISFET),¹¹⁷ nanowires (Nw-FET),^{118–120} carbon based FETs,^{121–125} nanoribbons (NR-FET),^{126–128} nanotubes based FETs (CNTFET),^{129,130} and other FETs based on 2D materials, like phosphorene.^{131,132}

The ion sensitive FET (ISFET) is based on the MOSFET configuration, except that the metal gate is replaced by an ion selective membrane (ISM), with an electrolyte solution and a reference electrode that sets the gate and test solution potentials. We will discuss the ISM principle of operation in the electrochemical sensor's section. ISFET typical applications are related to pH sensing.^{133–137} When the ISFET is configured to be sensitive to ions different than H^+ they are also referred to as ChemFETs.^{138,139} Chang and Lu developed an ISFET biosensor implemented in a 0.35- μm CMOS process for direct detection of DNA hybridization.¹⁴⁰ This work demonstrated the excellent capabilities of CMOS ISFET to provide highly sensitive and label-free detection. A novel ISFET-based immunosensor capable to detect low concentrations of tumour necrosis factor alpha (TNF- α) in saliva to monitoring patients with heart diseases was developed by Alcacer et al.¹⁴¹ Here, the immunosensor achieved a LOD of 5 pg mL^{-1} and was implemented as a IoT-based system to facilitate its operativity and portability. Liu and Cui fabricated an ISFET biosensor based on a layer-by-layer (LBL) self-assembly technique, where one multilayer film embedded with nanoparticles ($\text{PSS}/\text{In}_2\text{O}_3$) operates as the channel, and the other multilayer film (PDDA/SiO_2) serves as the gate dielectric (Fig. 11a).¹⁴² This configuration offers a high sensitivity and good repeatability for pH sensing. Nw-FETs are commonly used as ultrasensitive detector of label-free analytes and its principle of operation is based on changes in the channel electric field due the binding of charged molecules on the Nw surface. Vu et al. proposed a novel strategy to overcome the limitations in Debye length, charge distributions and target analyte orientations on silicon nanowire field-effect transistors (SiNWFETs) by employing an aptamer as a bio-amplifier for quantifying Amyloid

Table I. Selected studies on optical nanosensors.

Target Analyte	Detection Method	Detection Material	LOD	Pros	Cons	References
Creatine Kinase (CK-MB)	SPRml	Creatine Phosphate	0.209 ng ml ⁻¹	Improved sensitivity, high affinity and low cost	Not reported	88
β -lactam antibiotics	SPRml	Penicillin-binding protein PBP 2x	<2 ng ml ⁻¹	Improved Sensitivity	Selectivity affected by non-specific binding.	89
SARS-CoV-2	SPR	Spike (S) glycoprotein	Not Reported	Improved sensitivity and excellent linear detection range.	Selectivity analysis not reported.	90
Biotin-Streptavidin (SA)	LSPR	Biotin-functionalized Ag-nanotriangles	1 pM	Ultrasensitive detection	Sensitivity affected by size and shape of NPs.	91
miR-17 (miRNA)	LSPR	DNA hairpin-Ag nanoparticles	1pM	High specificity and selectivity	Full measurement procedure take approximately 1 h	92
Kanamycin	SERS	Anti-kanamycin/Fe ₃ O ₄ -NPs	2 pg ml ⁻¹	Extremely sensitive detection	Nanoparticles synthesis and stabilization.	93
17 β -estradiol	Fluorescence	β -estradiol 6-(O-carboxy-methyl) oxime-BSA	2.1 nM	High specificity, selectivity and reusable properties	Performance affected by interferences of real environmental sample matrix.	94
MicroRNA-155 (miRNA-155)	Fluorescence	La(III)-MOF,Ag NPs and DNA aptamer probes	5.20fM	Rapid, highly sensitive and selective	Sensors performarce depends on pH concentration of miRNA-155 and temperature	95
<i>E. coli</i> O157:H7	Fluorescence	<i>E. coli</i> -specific RNA-cleaving DNzyme)	1.57 CFU ml ⁻¹	High-efficient detection capability and sensitivity	Low specificity and selectivity of in vitro screening and detection	96
FIt-1	Colorimetry	Peptide-CB[8]	0.2 nM	High protein selectivity	Peptide dependence performance.	97

Figure 8. Surface-stress nano-sensor using a cantilever. Purple-spherical markers represent the target analyte. When the analyte-ligand interactions take place in the cantilever channels, the surface mass change depending on the analyte concentration. These surface mass changes lead to a resonance frequency shift due to the oscillations given on the cantilever.

Figure 9. (a) Diagrammatic representation of aptamer-based QCM biosensor for leukemia cells detection. Target leukemia cells were selectively captured by the aptamer immobilized on the QCM sensor surface. APBA-AuNPs are used to label the cells followed by silver enhancement, and the resonant frequency change of the QCM caused by the deposition of silver metal is monitored in real-time.¹⁰⁶ Reprinted with permission from Elsevier. (b) Experimental setup. Optical resonances of a spheroidal glass microparticle are excited by coupling the spheroid evanescently to an eroded single mode optical fiber.¹⁰⁷ Reprinted with permission from AIP Publishing.

beta oligomers (A β 1–42). Next, signal amplification was optimized in combination with self-assembled monolayers (SAMs).¹⁴³ A double-gate Nw-FET for label-free electrical detection of biomolecules was proposed in Ref. 144 (Fig. 11b). This sensor is composed of

two gates (G1 and G2) vertically standing on the silicon nanowire that allow independent voltage control to modulate channel potential and offers an improved sensitivity in comparison with conventional single gate Nw-FETs. Similarly, Tamersit and Djeflal employed a double

Table II. Selected studies on mechanical nanosensors.

Target Analyte	Detection Method	Detection Material	LOD	Pros	Cons	References
Alfa fetoprotein (AFP)	Surface-stress	AFP antibody-AuNP conjugates	21 pg ml ⁻¹	Linear detectable range, good reproducibility and high sensitivity	AuNPs-antibody conjugate's pH must be constant (7.5)	108
Hep62 cells	Surface-stress	Hep62 cells-specific aptamers	300 cells ml ⁻¹	Simple detection and high stability	Sensitivity influenced by aptamer	109
DNA	Dynamic mode	Complementary DNA	Not Reported	High mass per-area sensitivity	Not reported	110
<i>Staphylococcus aureus</i>	QCM	<i>S. aureus</i> antibody-protein A-iron nanoparticles	145 µg ml ⁻¹	Good sensitivity, selectivity and repeatability	High nanoparticles price affects the practical of the proposed sensor	111
Paraoxon	QCM	AChe enzyme-nanofiber	4.57 × 10 ⁻⁸ M l ⁻¹	Good performance and response time in and highly selective	Sensor response depends on nanofiber surface homogeneity	112
HER2/neu	QCM	HER2/neu Monoclonal antibody	10 cells ml ⁻¹	Novel highly sensitive QCM biosensor to detect tumor cells (SKB3)	Sensor response can be affected by thermal stress	113
H5N1	QCM	Aptamer ssDNA hydrogel	0.0128HAU (HA unit)	Reduced detection time	No real infected objects were tested	114
Acetylcholinesterase (AChE) and its inhibitors	WGM	5CB microdroplets	0.1 p g ml ⁻¹ -1 pg ml ⁻¹	High sensitive and real time monitoring	Lab-on-the-Chip not miniaturized	115

Figure 10. Field-Effect Transistor (FET) configuration for nanosensing.

Figure 11. (a) The schematic diagram of the nanoparticle thin film based ISFET. Au is used for source and drain electrodes and Chromium is used as adhesive material between the gold and the insulating substrate. The PDDA/PSS layers serve to enhance the adsorption of nanoparticle layers and also contribute to form the polyelectrolyte/nanoparticle multilayers.¹⁴² Reprinted with permission from Elsevier. (b) Schematic of the double-gate nanowire FET with immobilized biomolecules. Anti-AI (antibodies, Y-shape) are diffused through a solution and selectively bound to its antigens, which are immobilized onto the opened channel at the top of the nanowire.¹⁴⁴ Reprinted with permission from American Chemical Society. (c) (A) Experimental set up for detecting *Salmonella Infantis* with a network of CNTFETs functionalized with anti-*Salmonella* antibodies. (B) Antigen-antibody interaction of *S. Infantis* with a SWCNT functionalized with anti-*Salmonella* antibodies and protected with Tween 20.¹⁴⁶ Reprinted with permission from Elsevier.

gate graphene NR-FET for high-performance in DNA and gas detection.¹⁴⁵ Here, the dielectric modulation technique is used for DNA detection and the work function modulation technique is used for gas detection. In comparison with other FET-based sensors, the proposed sensor offers high sensitivity and better electrical and scaling performance.

Recently, Bao et al. discussed a top-down approach offers more advantages for silicon NR-FET fabrication over bottom-up approaches, due to the possibility of precise device confinement over width and length, seeking to control its electrical properties.¹⁴⁷ The fabricated silicon NR-FET is functionalized with 3-aminopropyltriethoxysilane (APTES) for carcinoembryonic antigen detection offering a sensitivity

of detection as low as 10 pg ml^{-1} . Villamizar et al. reported a single-walled CNT-FET for the selective detection of *Salmonella Infantis* (Fig. 11c).¹⁴⁶ Anti-*Salmonella* antibodies were adsorbed onto the SWCNTs and subsequently the SWCNTs were protected with Tween 20 to prevent the non-specific binding of other bacteria or proteins. Further detail on nanofETs can be found in recent reviews.^{148–150}

Table III provides a summary of recent biomarker sensing devices based on FET. This technology offers important improvements in sensitivity, limit of detection and robustness against thermal noise and non-specific adsorption. Nevertheless, their measurement range tends to be limited by saturation of the transistor, which depends on the channel's material.

Table III. Selected studies on FET nanosensors.

Target Analyte	ml	Detection Material	LOD	Pros	Cons	References
Arginine	Ion selective	Urease	0.05 mM	Good reproducibility	Lysine and cysteine inhibitory effect	151
Dengue Serotype2 (DEN-2)	SiNW	Peptide nucleic acid (PNA)	<10fM	Highly sensitive and fast response	Short measurement range	152
H1N1, H5N1	SiNW	Peptide nucleic acid (PNA)	1fM	Ultrahigh sensitivity and specificity	Sensitivity affected by size of the SiNws	153
Dehydroepiandrosterone sulfate (DHEA-S)	Graphene	Aptamer complementary DNA	44.7 nM	High sensitivity and affinity	EDL formation in aqueous media	154
Opioids (naltrexone)	Graphene	μ -opioid protein	10 pg ml ⁻¹	Enhanced solubility and stability un aqueous solutions	Slow response time	155
ATP	Graphene	DNA aptamer	10pM	Highly sensitive	Non-reusable sensor	156
Odorant amyl butyrate (AB)	Graphene	Olfactory receptor 2AG1 (hOR2A61:OR)	0.04fM	Ultrasensitive and highly selective	Sensitivity is affected by structural resistance	157
UDP-glucose	GNR-FET	Sucrose	<pM	Highly sensitive and fast response	Difficult implementation	127
Glucose	In ₂ O ₃ nanoribbon	Glucose oxidase/chitosan/SwCNT	10 nM	Highly sensitive detection in body fluids	Nor reported	158
SARS-CoV-2	CNT	Spike protein antibody (Sab)	0.55fg ml ⁻¹	Good sensing performance in terms of sensitivity, specificity and detection time	Not reported	159

Figure 12. Electrochemical nanosensor with three electrodes configuration. Purple-spherical markers represent the target analyte. A redox type reaction occurs on the surface of the working electrode due to the interaction between the functionalized electrode and the target analyte. As result of this chemical process, a current flow through the working and counter electrode. If the analyte is reduced at the working electrode the current is a cathodic current, while the analyte oxidation produces an anodic current. The potentiostat ensures the required voltage conditions on each electrode and measure the faradaic current.

Electrochemical Nanosensors

Electrochemical methods are used to measure the presence of an analyte from the detection of changes in potential, current, resistance or conductivity produced by a chemical redox-type (reduction-oxidation) reaction that takes place inside an electrochemical cell. The analyte of interest is contained in an electrolyte solution that interacts (conventionally) with three electrodes, which act as the sensor's transducer (Fig. 12). Two key aspects define the electrochemical nanosensor: 1) the working electrode and 2) the electrochemical technique. The working electrode types are grouped as mercury drop electrodes, carbon-based electrodes, glassy carbon electrodes, diamond electrodes, carbon paste electrodes, screen-printed electrodes and paper-based electrodes.

Currently, the most common electrochemical techniques are potentiometric, conductometric, voltammetric, amperometric and impedancimetric.^{160,161} Recently, unlike these traditional techniques, photoelectrochemical (PEC) biosensing has drawn tremendous attention from researchers in the field of biosensing. PEC sensors are based on a photo-to-electric conversion process, where a photocurrent is obtained under the excitation from an external light source, which improves the sensitivity of the method compared with the traditional techniques.^{162,163} Zeng et al. developed an advanced photoelectrochemical sensing platform for the determination of Kana antibiotic by coupling palindromic molecular beacon based strand-displacement recycling amplification with bismuth oxychloride (BiOCl-Au)-CdS quantum dot (QDs)-based Z-scheme photoinduced reaction system.¹⁶⁴ This sensor used for the first time the palindromic molecular beacon as nucleic acid based amplification technique improving sensitivity and LOD (29 fM). Similarly, Lv et al. developed a novel NaYF₄:Yb,Tm@ZnO-based PEC biosensor for carcinoembryonic antigen (CEA) determination assisted by DNA walker with a homemade 3D printer device to perform a portable detection.¹⁶⁵ The overall sensitivity of the system is enhanced by the 3D tracks of DNA walker. The achieved detection limit was 0.032 ng ml⁻¹.

Traditional electrochemical techniques and electrode types are discussed next.

Electrochemical techniques.—*Potentiometric* methods are based on the measurement of charge potential at the working electrode. This potential results from the chemical reactions that take place inside the sample solution due to the analyte concentration at the reference electrode, and under condition of non-current flow. The

Figure 13. The schematic diagrams of the potentiometric arrayed AA biosensor based on MBs-AO/GO/ZnO. (a) top view and (b) cross-section. Silver layer acts as the reference electrode and conductive wires, the ZnO layer is used as the sensing matrix, the GO layer increases the number of surface active site, MBs provides the high surface to volume ratio for enzyme immobilization onto the membrane and AO is used as the biometric layer for AA catalysis.¹⁷⁰ Reprinted with permission from Creative Commons Attribution 4.0.

measured potential corresponds to the separation of charge across an interface that directly depends on the electrode type and can be described using the Nernst equation, which allows the calculation of ionic activities or effective concentration as a function of the electrode potential and a standard potential.¹⁶⁶ Lv et al. used a sensor of this type for detection of prostate-specific antigen (PSA) and proposed an in situ signal-amplification strategy to overcome weak electrical readout.¹⁶⁷ This strategy consists of using oligonucleotides and detection antibody-labeled gold nanoparticles as the signal-generation tags on anti-PSA capture antibody-modified glassy carbon electrode with a sandwich-type assay mode. The results indicated an improved performance of the sensor characterized by high sensitivity and a low detection limit (13.6 pg ml⁻¹).

There are two types of electrodes commonly used in potentiometric methods: metallic and ion selective electrodes. Metallic electrodes work as a direct indicator of the metal's cation in solution, through a redox reaction that develops an electrical potential depending on the equilibrium between the cation and the electrode surface. Ion selective electrodes (ISE) on the other hand, are capable of selectively measuring the activities of a specific ion using a permeo-selective membrane or ISM and the resulting potential depends on an ion transport process between an inner membrane solution containing the ion of interest at a fixed activity and the outer solution with the target analyte, resulting in charge separation or a phase-boundary potential across the membrane. The membrane can be composed of silicate glass, inorganic salts, viscous organic liquids or polymers, and must be non-soluble in the analyte solution, electrically conductive and highly selective to the analyte of interest. In practice, the membrane interface is not only permeable to a specific ion, which causes undesired ions to interfere and produce additional charge. In these cases, the Nernst equation can be derived in the Nikolsky-Eisenman equation, which allows the estimation of a sensor's selectivity and LOD.^{168,169} The increasing interest in the use of potentiometric sensors for biological applications has given rise to the development of novel models. For example, a sensitive potentiometric ascorbic acid (AA) biosensor was introduced in Ref. 170 (Fig. 13). It uses a magnetic beads-ascorbate oxidase/graphene oxide/zinc oxide (MBs-AO/GO/ZnO) membrane-based screen-printed electrode to improve sensitivity,

selectivity and LOD for AA, in comparison to other AA sensor models. Recently, Silva et al. reported the use of a paper-based strip electrode in a label-free potentiometric immunosensor for *Salmonella typhimurium* detection, showing two different immunosensing interfaces assembled on the electrode surface to control an ionic flux through a carboxylated PVC membrane.¹⁷¹ An anti-body like ionophore layer was used as a carrier molecule between the solution and membrane in the potentiometric detection of CA 15–3 in serum.¹⁷² Isildak et al. showed that 4-hydroxybenzo-15-crown-5 ether can be used as ionophore in PVC membrane beryllium ISE for the detection of Be^{2+} in solutions.¹⁷³ Enzymatic biocatalytic precipitation and nanogold labeling techniques have been used to improve the sensitivity in potentiometric immunosensors response.¹⁷⁴ The logarithmic dependence between the chemical potential and the analyte activity, as expressed by the Nernst equation, leads to a low sensitivity in potentiometric ion sensors. To overcome this, a recently coulometric transduction method for solid-contact ion selective electrodes (SCISEs) was introduced, which lowers membrane resistance and improves response time.¹⁷⁵

Voltammetric methods involve a time dependent potential applied to the working electrode. The changing potential leads to a current flow –involving a chemical redox reaction in the sample solution– between the working and reference electrodes, which provides quantitative and qualitative information associated to the target analyte. Depending on the oxidation or reduction process, the current flows through the working to the counter electrode (also known as a faradaic current). If the analyte is reduced at the working electrode, the faradaic current is a cathodic current, while the analyte oxidation produces an anodic current. The magnitude of this current is determined by the rate of the redox reaction, which is proportional to the rate of the mass transport to and from the electrode and electron kinetics.¹⁷⁶ The mass transport represents the rate at which the involved chemical species move toward or away the electrode surface and can be expressed through diffusion, migration and convection modes. The diffusion mode is based on the movement of ions or molecules, due to the different analyte concentrations on the electrode surface and the bulk solution. In convection, the motion occurs when the solution is mechanically mixed. During the migration mode, reactant and products move due to the electrode surface charge. Voltammetric processes generally occur in the region where the diffusion is the only significant influencing mode in the mass transport. The rate at which electrons move in the redox process, i.e., electron kinetics, also affects the current. If the electron kinetics are fast, the redox process is reversible and at equilibrium. If the electron kinetics are slow, the redox process is irreversible, and the chemical reaction is not described by the Nernst equation. In addition to the faradaic current, other non-faradaic or background currents exist that can affect the voltammetric measurements. This current is typically related to the redox reaction of impurities, at the bulk solution and the electrode surface charge that leads to the formation of a structured electrode solution interface also known as electrical double layer (EDL).

The different existing voltammetry techniques are uniquely classified based on the applied time-based potential characteristics.¹⁷⁷ The most common techniques include linear sweep and cyclic voltammetry. Each one results in a plot or voltammogram of current vs applied potential, where the current is a function of the target analyte's concentration. In linear sweep voltammetry, a linear potential ramp is applied to scan either oxidation or reduction reaction, whereas in cyclic voltammetry positive and negative potentials are used to scan both analyte oxidation and reduction process. In the cyclic process, the current response has separate current peaks for oxidation and reduction processes. If an irreversible redox reaction takes place just one of these peaks is defined.¹⁷⁸

Aptamer-based voltammetric biosensors have attracted great attention in early disease diagnosis. Linlin Li et al. introduced a novel voltammetric sensor for *Mycobacterium tuberculosis* (MTB) early detection using the ESAT-6 antigen secreted by the bacteria as

target analyte, and a glassy carbon electrode (GCE) with reduced graphene oxide with metal-organic framework (MOF-rGO) deposited on the surface.¹⁷⁹ Toluidine Blue (TB) was immobilized on the GCE and platinum/gold core/shell (Pt@ Au) was used to assemble thiolated ESAT-6 binding aptamer on the electrode and further response amplification. The sensor performance exhibited excellent specificity and favorable spike recovery for ESAT-6 detection. In related study, N. Li et al. used MPT64 antigen as target analyte, where the voltammetric sensor is based on the dual-aptamer (APT-I and APT-II), and the hybrid signal-amplifying nanoprobe, which resulted in good performance over a wide linear range, high selectivity and a low LOD.¹⁸⁰ Wang et al. demonstrated early detection and monitoring of thrombin, which is an important indicator for thrombotic diseases, using a novel regenerable and ultrasensitive voltammetry biosensor based on G-quadruplex/heim/HRP/AuPd/poly(o-phenylenediamine) bioconjugates immobilized on the surface of modified glassy carbon electrode, AuPd nanoparticles are used for signal amplification by synergistic catalysis (Fig. 14).¹⁸¹ The sensor exhibits a wide linear range, good selectivity and low LOD. For voltammetry immunosensors in health care, a novel signal amplification strategy is introduced in Ref. 182. It is based on two current changes produced by the synergism between the degradation of methylene blue and non-conductive calcium carbonate spheres, which is used for CA199 detection and improves the overall sensor sensitivity. Other fields of application have been successfully demonstrated using voltammetric techniques. For example, in food monitoring applications, the sensitive detection of heavy metals, such as mercury ions, is important to food safety.

Recently, a voltammetric sensor was proposed by H. Jin et al. for Hg^{2+} detection, based on the Hg interaction with thymine-thymine base pairs. A T-rich thiolated DNA (S1) is immobilized on a gold electrode for enabling binding reactions with T-rich biotin-DNA (biotin-S2) through T-Hg(II)-T interaction. RGO@AuNR-TH-SA, linked to the gold electrode, binds to biotin-S2 via SA. The electrochemical response is proportional to the Hg^{2+} concentration. This sensor shows high selectivity, reproducibility, and repeatability, and a low LOD. In pharmaceutical analysis, voltammetry biosensors performance have been improved by using carbon paste electrodes. For instance, Vieira Thomaz employed a nanostructured TiO_2 /carbon graphite matrix in the development of a laccase (Lcc) carbon paste biosensor for paracetamol detection, improving the sensor response with low LOD and good sensitivity.¹⁸³

The **Amperometric** technique is similar to the voltammetric one, albeit the applied electrical potential to the working electrode is constant and the current is measured as a function of time compared to the reference electrode. The oxidation or reduction reactions occur at the electrode surface. This method has a higher sensitivity when compared with the potentiometric technique. The amperometric technique is commonly used in biocatalytic and affinity sensors, due to its simplicity and low background current effect.¹⁸⁴ Recently, Cordeiro et al. introduced the use of coated tungsten (W-Au) microelectrodes functionalized with permeo-selective membranes, for biocatalytic sensors.¹⁸⁵ This microelectrode improved the sensitivity and selectivity of glucose monitoring in human brain.

Multiplexing to the simultaneous detection of several analytes is an important capability in biomarker sensing. Printed multiplexed amperometric biosensors have been demonstrated on nanostructured conductive hydrogels in.¹⁸⁶ Moreover, Kucherenko et al. presented a biosensor array for the simultaneous determination of glutamate, glucose, choline, acetylcholine, lactate and pyruvate, by immobilizing selective enzymes on the surface of amperometric platinum disc electrodes, demonstrating good selectivity and reproducibility capabilities.¹⁸⁷

In health monitoring, Guerrieri et al. presented a novel highly selective amperometric biosensor for choline determination in patients with renal diseases based on choline oxidase immobilized by co-crosslinking on a Pt electrode with a novel anti-fouling and anti-interferent membrane, that aim to improve the interference rejection, stability and selectivity of the device.¹⁸⁸ Furthermore,

Figure 14. (a) Synthesis procedures G-quadruplex/hemin/HRP/AuPd/poly(o-phenylenediamine) probes. (b) Schematic illustration of the preparation process of the biosensor. The thiolated thrombin aptamer is immobilized on the surface of AuNP/GCE through Au-S interaction. The aptamer can recognize and capture thrombin after being incubated with thrombin. The G-quadruplex/hemin/HRP/AuPd/poly(o-phenylenediamine) nanoprobe is immobilized on the electrode in the presence of the captured thrombin. The peak current is related to the amount of nanoprobe on the AuNP/GCE surface, i.e., it reflects the amount of thrombin immobilized on the AuNP/GCE surface.¹⁸¹ Reprinted with permission from Springer Nature.

Vargas et al. proposed an amperometric biosensor for miRNA-21 detection for diagnosis or prognostic in patients with breast cancer.¹⁸⁹ The sensor is based on a competitive DNA/RNA hybridization format, implemented on the surface of magnetic microbeads (MBs) and screen-printed carbon electrodes (SPCEs), and offers low LOD, wide linear range, high sensitivity and selectivity. In the precision agriculture field, pesticide control is an important topic for environmental safety. Recently, a novel amperometric biosensor was presented by B. Jiang et al. for organophosphorus pesticides determination using an ionic layer-by-layer self-assembly technique, colloidal AuNPs and a p-aminobenzenesulfonic acid-modified glassy carbon electrode with immobilized diazo-resins (DAR) on the surface.¹⁹⁰ This sensor exhibited improved precision, stability and low LOD.

In *conductometry*, changes in conductivity are measured from the analyte or medium involved in chemical reactions. Conductivity is expressed as the ability to conduct a current. If the media is a solution containing ions, its conductivity depends on the ion's mobility between the electrodes, where the bulk solution acts as an electrical conductor. Also, the immobilization of species on the electrode surface can produce changes in the conductivity of the electrode when detecting binding events with the target analyte. This technique is characterized by its simplicity and the no dependence on a reference electrode, and faradaic effects.¹⁹¹ Conductometry has been widely used for biosensors in environmental monitoring and clinical analysis applications. A novel conductometric immunosensing platform for highly sensitive detection of prostate cancer marker is developed in Ref. 192 by using tetracyanoquinodimethane (TCNQ)-doped thin films of copper-MOF and $Cu_3(BTC)_2$, assembled on gold screen-printed electrodes. Target specific antibodies related to the prostate cancer antigen (PSA) were immobilized on the electrode surface. The doping process improved the overall conductance reaching low LOD and good linearity. Furthermore, Liang et al. introduced a conductometric immunoassay sensor for detecting a liver cancer biomarker, alpha-fetoprotein (AFP), in human serum samples, using enzyme-conjugate nanometer-sized enzyme-doped silica beads.¹⁹³ The arginase-labeled anti-AFP antibodies were

Figure 15. Schematic illustration of the bienzyme-based conductometric immunoassay for target alpha-fetoprotein (AFP) on monoclonal mouse anti-human AFP antibody (mAb)-coated microplate by using L-arginase-coated urease-encapsulated nanometer-sized silica beads: (A) immunoreaction protocol for target AFP between mAb and polyclonal rabbit anti-human AFP antibody-arginase conjugate (Arg-pAb)-labeled urease-functionalized silica nanoparticle; (B) conductometric measurement; and (C) bienzyme-based hydrolytic reaction.¹⁹³ Reprinted with permission from Royal Society of Chemistry.

immobilized on the surface of the synthesized nanoparticles (Fig. 15). The sensor sensitivity, LOD and linear range is achieved in comparison with other techniques. In the environmental monitoring field, a recent study by Kolahchi et al. presented a sensitive miniaturized conductometric biosensor for phenol detection.¹⁹⁴ The sensor was developed by immobilizing *Pseudomonas* sp. (GSN23) bacteria on the surface of gold interdigitated microelectrodes.

Last but not least, the electrochemical impedance spectroscopy (EIS) technique is based on a small AC potential signal applied to the working electrode, in order to measure the alternate current resulting from the chemical reaction.^{195,196} The impedance response can be analyzed from the relation between the applied voltage and output current and has both magnitude and phase. The applied signal frequency is varied over a wide range for obtaining the impedance spectrum. Then, the equivalent resistance and capacitance can be determined by the in-phase and out-phase current responses, i.e., current can be i) in phase, with the voltage signal in a resistive behavior, and ii) out of phase, if a capacitive behavior is presented. The resistive components include the sample solution resistance, charge transfer resistance and mass transfer resistance. The capacitive components are defined by the electrical double layer (EDL) on the electrode-sample solution interface. The charge and mass transfer resistance are related to the electron kinetics and diffusion mass rate at the chemical redox reaction, respectively.

EIS is capable of sampling electron transfer at high frequency and mass transfer at low frequency.¹⁹⁷ If the overall impedance response of the electrochemical system is determined by the mass transfer behavior, the chemical redox reaction is interpreted as reversible. In contrast, if the electron transfer behavior predominates, the chemical redox reaction is irreversible. The EIS technique is insensitive to environmental disturbance and offers a good signal to

noise ratio. Commonly, impedance applications are related to affinity sensors designs.¹⁹⁸

A novel EIS biosensor was developed by Bahner et al. for the detection of doxorubicin, a pervasive cancer treatment drug.¹⁹⁹ The sensor is based on the daunorubicin-binding aptamer, used as recognition element, and is immobilized with mercaptohexanol on gold. The overall sensor performance is characterized by a linear relationship between the charge transfer resistance and the doxorubicin concentration, resulting in high sensitivity, selectivity and low LOD. Moreover, a novel diamond-based biosensor was introduced by Nidzworski et al. for the early detection of the influenza virus.²⁰⁰ The sensor uses a nano-scale boron-doped diamond (BDD) electrode, functionalized with 4-aminobenzoic acid self-assembled monolayer (SAM), and anti-M1 antibodies (Fig. 16). A bovine serum albumin (BSA) solution is used to eliminate unspecific immobilization. The target analyte binding events change the electrochemical impedance spectra and a low LOD can be achieved as result of the experiment.

In the food monitoring field, Elshafey and Radi presented an EIS biosensor for the determination ofalachlor (ALA) in human food.²⁰¹ The sensor uses a molecularly imprinted polymer (MIP), immobilized onto a glassy carbon electrode by electrodeposition of o-phenylenediamine (o-PD). Finally, a novel screen-printed cell-based sensor was developed in Ref. 202 to assess N-acylhomoserine

Figure 16. Schematic illustration of biosensing system. (A) Throat swab culture acquisition. (B) Boron-doped diamond electrode surface modification with polyclonal anti-M1 antibodies, which then serve to identify the universal biomarker for influenza virus, the M1 protein.²⁰⁰ Reprinted with permission from Springer Nature.

lactones (AHLs) from freshwater fish. The sensor is based on a screen-printed carbon electrode (SPCE), modified with multi-walled carbon nanotubes (MWNTs) for improving the device's sensitivity, selectivity and LOD. Then, rat basophilic leukemia (RBL-2H3) receptors, encapsulated in alginate/graphene oxide (NaAgI/GO), were immobilized on the electrode surface, observing a decrease in EIS response with the concentration of N-3-oxododecanoyl homoserine lactone ($3OC_{12}$ -HSL).

Electrochemical electrodes.—*Mercury drop electrodes* are widely used in electrochemical techniques such as voltammetry. Mercury electrodes continuously drops into the solution through a capillary tube, thereby constantly renewing the electrode surface, hence guaranteeing reproducible and repeatability current-voltage curves. Unfortunately, this electrode has been restricted for in-vitro biological applications due to toxicity considerations.²⁰³

Carbon-based electrodes are established as a good alternative due to their biocompatibility and useful properties, as chemically inertness, reduction of background currents, broad potential windows, optimal surface chemistry and high stability against corrosion.^{204,205} Ryu et al. fabricated a carbon-based electrode by plasma-enhanced chemical vapor deposition (CVD) of vertically aligned nanotubes on a graphite foil substrate, which showed high chemical active area and electrocatalytic affinity for high performance in electrochemical applications (Fig. 17i).²⁰⁶

Glassy Carbon electrodes, also known as vitreous carbon electrodes, offer similar properties to the carbon-based electrodes, yet they are impermeable to gases, have extremely low porosity, are compatible with all common solvents, and are not chemically inert under electrochemical oxidation at high anodic potentials.²¹⁰ Yi et al. showed that the performance of a glassy carbon electrode under electrochemical oxidative conditions causes morphology changes on the electrode's surface (Fig. 17ii).²⁰⁷

Diamond electrodes have attracted great attention in recent years for electrochemical sensing applications, given that they can work under extremely high anodic potentials and exhibit robustness to aggressive chemical media in comparison with other electrodes. Furthermore, diamond has unique properties, including a high atomic density, good optical response (i.e. transparency), chemical inertness, and high thermal conductivity, albeit it is an electrical insulator. To improve its electrical conduction, diamond is doped with boron (BDD) showing an extremely high structural stability, while being insensitive to oxygen evolution, enabling low signal to noise ratios and an enhancement of the potential window (Fig. 17iii).^{208,211,212} BDD sensors have been demonstrated in the early detection of influenza²⁰⁰ and other related applications.^{213–215}

Carbon paste electrodes are the result of combining graphene powder with insoluble organic binders. This type of electrodes exhibit fast surface renewal, low background current, polarizability, modifiable structure and high reproducibility, although the organic solvent can affect the organic binder, decreasing the overall electrode's performance (Fig. 17iv).^{209,216}

Screen-printed electrodes are based on the screen-printing technology of graphite powder, or any other ink onto a polymer, ceramic or an inert substrate. This type of electrode is usually designed for single-use, has a small form factor, and provides important analytical features, albeit its kinetics are slow (Fig. 18).^{217–220}

Paper-based electrodes are composed of cellulose fibers. These exhibit high mechanical flexibility, have reduced thickness and provide a porous open structure capability. Printing techniques allows different materials to be deposited on paper, including carbon nanomaterials, with improved electronic transport response.²²¹ Preston et al. reported a novel silver nanowire (Ag NW) paper hybrid electrode with stable optoelectronic properties, high transmittance and low sheet resistance due to its greater optical haze (Fig. 19).²²²

Each one of the above electrodes can be used in any electrochemical technique. Generally, the choice depends on the analyte of

Figure 17. (i) SEM image of (a) surface morphology of carbon nanotubes-based electrode; (b) cross-sectional SEM image of carbon nanotube based electrode.²⁰⁶ Reprinted with permission from Creative Commons Attribution 4.0. (ii) SEM image of (a) pristine glassy carbon electrode; (b) oxidized in acid; (c) and alkaline; (d) media operated at 1.5 kV with secondary electron.²⁰⁷ Reprinted with permission from Creative Commons Attribution 4.0. (iii) SEM image of (a) microcrystalline BDD electrode; (b) polished surface of microcrystalline BDD electrode; (c) nanocrystalline BDD electrode; (d) ultra-nanocrystalline BDD thin film.²⁰⁸ Reprinted with permission from Royal Society of Chemistry. iv) SEM image of (a) polished carbon pasted electrode; (b) ground carbon pasted electrode with particles $<210\ \mu\text{m}$.²⁰⁹ Reprinted with permission from Elsevier.

Figure 18. SEM image of (a) gold nanostructured screen-printed electrode; (b) gold CNT hybrids screen-printed electrode; (c) gold-graphene hybrid screen-printed electrode. Magnification 1×104 .²¹⁸ Reprinted with permission from Springer Nature.

Figure 19. (a) Ag NW transparent paper electrode; SEM image of (b) Ag NW electrode with diameter of 100 nm; (c) cross-section; (d) top view.²²² Reprinted with permission from Royal Society of Chemistry.

interest, its chemical properties, and the sensing requirements. The most used electrochemical sensors are three-electrode systems: with working, reference and counter electrodes. The working electrode handles the chemical reactions of the target analyte, the reference electrode provides a stable (unchanging) potential during the reaction processes at the working electrode (typically metallic). The faradaic currents from chemical reactions flows through the working electrode to the counter electrode, which is usually made of a highly conductivity materials, such as platinum.

Biocatalytic and affinity electrochemical sensors.—Electrochemical biosensors can use recognition elements, such as, antibodies, nucleic acids, proteins, enzymes, tissue slices, etc., and can be grouped into two important categories: biocatalytic and affinity sensors.²²³

Biocatalytic sensors are commonly used for protein detection using enzymes as the principal recognition element, due to their high biocatalytic activity, affinity to biorecognition and signal amplification, and substrate specificity. These unique properties offer a biological detection process that is cheap and easy to implementation.²²⁴ For these sensors, the probe or working electrode must be coated with a thin layer of an immobilized enzyme that has

to be replaced periodically according to the electrode's lifespan. Common immobilization techniques include enzyme entrapment, encapsulation, incorporation, adsorption, cross-linking, and covalent attachment.^{225–227}

Affinity Sensors are based on strong and selective binding events between a biological recognition element, such as antibodies (Abs), membrane receptors, and oligonucleotides, and the target analyte. Here, the recognition process depends on size and shape complementarity, between the binding site and the target analyte.^{228–230} Depending on the biorecognition element that is employed, affinity sensors can be grouped as DNA hybridization and immunosensors. The DNA hybridization-based sensors detect the interaction of a DNA or RNA aptamer with certain proteins or base-pairing sequence complementarity to immobilized DNA/RNA on the electrode's surface. This sensor is commonly used in medical diagnosis for cancer, viral infections and genetic diseases.^{231–234} Immunosensors, on the hand, are based on the non-covalent reaction between an antigen (Ag) and an antibody (Ab). This process is characterized by high specificity and affinity. An Ag is any molecule recognized as foreign in an organism, whereas Ab molecules are produced by specialized cells of the immune system. The Ab must be immobilized on the electrode's surface, using a specific, high

catalytic organic label. The most common labels used are glucose oxidase (GOx), alkaline phosphatase (ALP) and β -galactosidase (β -Gal). Generally, Ab immobilization techniques includes covalent binding, adsorption and biotin-streptavidin linkages.^{235–237} Huang et al. designed a novel and low-cost self-powered temperature sensor based on Seebeck effect transduction for photothermal-thermo-electric coupled immunoassay of α -fetoprotein (AFP).²³⁸ Here, glucose oxidase (GOx)-conjugated detection antibody is immobilized onto a microplate resulting in a significant increase in the thermoelectric voltage. The temperature change was read out in an electrical signal by the flexible thermoelectric module in a 3D-printed integrated detection device. The achieved detection limit was 0.39 ng ml^{-1} . Recently, Yu et al., proposed a novel, low-cost and portable pressure-based point-of-care (POC) testing strategy for the rapid detection of carcinoembryonic antigen (CEA) via a flexible pressure sensor constructed by three-dimensional (3D) polypyrrole (PPy) foam.²³⁹ The pressure change caused by the decomposition of H_2O_2 was selected as the detection signal to improve sensitivity, measurement time and detection limit (0.13 ng ml^{-1}). Moreover, Zhu et al. developed a double ion-exchange reaction-based photoelectrochemical (PEC) immunoassay for sensitive split-type detection of PSA.²⁴⁰ Here, CuO nanoparticles are used as labels of detection antibody in sandwiched immunocomplexes, released Cu^{2+} ions that are transferred to PEC detection cell for double-ion exchange reaction with cadmium sulfide and nickel sulfide (CdS@NiS) nanocomposites. This configuration reduces the photocurrent and avoids the damage of light radiation to biomolecules. The achieved detection limit was 2.9 pg ml^{-1} . Luo et al. developed a near-infrared light activated non-enzymatic signal-off photoelectrochemical (PEC) immunoassay by coupling with the synergistic effect of dual-purpose copper ions for the ultrasensitive detection of α -fetoprotein (AFP) based on branched polyethylenimine (BPEI)-modified upconversion nanoparticle (UCNP@CdTe quantum dot (QD) nanohybrids.²⁴¹ In this novel approach, the released copper ions could initially absorb near-infrared incident light and then weaken the upconversion luminescence and reducing the generation of photogenerated electron-hole pairs. The sensor demonstrated a good performance in terms of sensibility, stability and specificity. The achieved detection limit was 1.2 pg ml^{-1} .

A key factor that affects the sensitivity of the immunosensors is the nonspecific binding (NSB) interaction between Ab and undesirable molecules. Design strategies to prevent this involves the usage of specific blockers, such as, BSA. Immunosensor applications include food safety, detection of biological toxins, bacteria and virus,^{242–244} as well cancer-specific drugs.²⁴⁵ Table IV presents a summary of different electrochemical sensor techniques applied to biosensing.

Conclusions

The use of sensors for biomarker detection continues to attract a growing interest, therefore the search for novel designs with higher sensitivity and selectivity, lower cost and ease of implementation and operation continues. The design of bio-nanosensors focuses on improving two major aspects: i) selectivity of target analyte/s and ii) detection technique. The former involves all information related with the analyte and its interaction with the experimental environment. Designers must establish the best possible signals (measurable and distinguishable with high SNR) associated to the interaction between a probe and a given target analyte. On the other hand, the detection method, or transducer, guides costs, ease of implementation, power consumption, reliability, and capabilities for in vivo or ex-vivo measurements, etc. A significant part of this effort remains on materials' choices that seek to improve the overall biosensor's sensitivity, LOD, selectivity, measurement range, measurement time and repeatability.

State-of-the-art approaches show that the most common bio-nanosensors today are based on optical, mechanical, electrochemical and FET principles. Each type with its own advantages and disadvantages depending on the application, as summarized in Table V.

In the medical field, the electrochemical bio-nanosensors are preferred for protein detection due to its simpler implementation, high sensitivity (fM-nM) and selectivity, low cost and fast chemical response. Moreover, bio-functionalized electrochemical devices can detect a wide range of biomarkers by chemical affinity between the analyte and the electrode's surface material. However, the sensor's performance may be compromised by the type of electrode and its response within the electrochemical technique that is employed. Therefore, the electrode material and functional groups need to be chosen accordingly.

For pH measurement and environmental applications, FETs and optical bio-nanosensors are commonly used due to its ultra-high sensitivity (fM-10mM), real-time monitoring, ultra-low LOD ($<1 \text{ nM}$) and on-site measurement. The ISFETs offer easy implementation and low-cost manufacturing process for pH and ion detection. One of the most salient advantages of FETs bio-nanosensors is related to their high capability of integration in electronic systems, which offers the opportunity for highly portable IoT devices. Furthermore, sensitivity capabilities can be improved by controlling the electronic transport properties of the channel's material, although this limited by the devices' geometry, which also constrain its measurement range.

Optical bio-nanosensors are usually preferred for applications that demand robustness against difficult environmental conditions as well as high temperatures. FRET and SET sensors offer high sensitivities ($<1 \text{ nM}$) and sensor stability, but the portability of this type of sensors is limited by the measuring instruments and accuracy setups that have to be accomplished during the measurements. Additionally, sensitivity can be improved by employing nanoparticles, whose optical properties are useful for overcoming device limitations, such as shorts measurement ranges and detection of complex biological molecules.

Mechanical bio-nanosensors offer high stability, repeatability and are commonly used in biological or food monitoring applications. These sensors are characterized by a high robustness, but demand extra effort in terms of calibration, whose performance is also constrained by the geometry of the cantilever, directly related to the device's LOD. On the other hand, sensitivity capabilities are proportional to the molecular mass of the analyte. Then, it is not possible to measure molecular species whose mass is under the detection threshold.

Current research and development efforts on nanosensors, seek to overcome the inherent design and manufacturing limits at the nanoscale, by exploiting new techniques, materials, and implementation technologies. Continued research in the field is expected to enable higher levels of technology integration, new bottom-up and self-assembled synthesis, improved portability and power efficiency, higher accuracy and resolution, and faster real-time monitoring capabilities. Nanosensors will soon become an integral part of handheld or embedded devices, e.g. for real time health monitoring, early disease detection, improved drug delivery systems in disease treatments; for humans, animals and plants alike. Furthermore, nanosensors will become essential tools to improve our understanding of, and to optimize the response of living organisms to biotic and abiotic stresses, i.e. to elucidate complex genomic-phenotypic relationships to correlate gene function with gene expression, under different conditions. Nanosensors (bio-nanosensors in particular), will offer new ways to study and understand a wide range of biological processes, over multiple length and time scales.

Table IV. Selected studies on electrochemical nanosensors.

Target Analyte	Electrochemical technique	Functionalized probe	LOD	Pros	Cons	References
Dopamine	Voltammetry	Graphite/naion-SPCE	0.023 μM	Good selectivity and stability	Constrained sensitivity	246
Beta-amyloid (A β) peptide	Voltammetry	AuNPs/ITO electrode	50 nM	In-vitro highly sensitive sensor	Specificity depends on redox properties	247
TNF- α	Voltammetry	TNF- α antibodies	2fg ml ⁻¹	High sensitivity, selectivity, stability and reusability	Low portability	248
SARS-CoV-2	Voltammetry	Spike antibody-graphene oxide (f-GO) modified glassy carbon electrode	1ag ml ⁻¹	High specificity, sensitivity and fast detection time.	Low potability	249
Prostatic specific antigen (PSA)	Amperometry	Ab-AuNPs-HRP	0.2 ng l ⁻¹	Rapid and sensitive detection	Non repeatability consideration	250
HER2	Amperometry	CD24c/AuNPs-GO/GCE	0.23 nM	Early analyte detection	Non-specific current appearance	251
Cardiac troponin I (cTnI)	EIS	Anti-cTnI-PtNPs/Graphene-MwCNTs/GCE	1pgm l ⁻¹	High sensitivity and selectivity	No reported	252
SARS-CoV-2	EIS	Spike (S)	1.065fg/ml	High sensitivity and selectivity, low-cost, portable, rapid diagnosis	Electrode repeteability.	253
Testosterone	EIS	Molecularly imprinted polymer/graphene-oxide (MIP/GO)	0.4fM	Good selectivity and long-term stability	No reported	254

Table V. Summary of bio-nanosensor platforms.

Sensing platform	LOD range	Sensitivity	Pros	Cons	Preferred applications
Optical	1 pg ml^{-1} – $100 \text{ } \mu\text{g ml}^{-1}$	Can be improved using nanoparticles (NPs)	Ultrasensitive analyte detection	Selectivity decreases in complex biological solutions	Environmental biomedical and pH measurement
Mechanical	4 fM - $20 \text{ } \mu\text{g ml}^{-1}$	Proportional to the molecular mass of the analyte	Mass per area sensitivity can be improved by cantilever geometry	Detection signal can be affected highly affected by non-specific adsorption	Biological and food monitoring
FET	0.04 fM - 20 mM	Can be controlled by the channel's electronic transport properties	Ease integration with electronic and portable systems	Short measurement ranges that depend on channel geometry and electrical properties	Environmental measurement, ion detection and IoT devices
Electrochemical	0.4 fM - $0.023 \text{ } \mu\text{M}$	Depends on the relation between the chemical affinity among the species and electrode properties	Ease implementation, setup. measurement and characterization	Non-specific binding molecules decreases signal to noise ratio and specificity depends on redox properties	Food Monitoring, pharmaceutical and biomarker detection

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