


MINI REVIEW

Current nanotechnology advances in diagnostic biosensors

Ana I. Barbosa^{1,2}  | Rita Rebelo^{1,2}  | Rui L. Reis^{1,2}  | Mrinal Bhattacharya³ | Vitor M. Correlo^{1,2} 

¹3B's Research Institute on Biomaterials Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Universidade do Minho, Guimarães, Portugal

²CVS/3B's - PT Government Associated Laboratory, Guimarães, Portugal

³Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, Minnesota, USA

Correspondence

Mrinal Bhattacharya, Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN 55108, USA.

Email: bhatt002@umn.edu

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Abstract

Current diagnostics present challenges that are imposed by increased life expectancy in the worldwide population. These challenges are related, not only to satisfy the need for higher performance of diagnostic tests, but also to the capacity of creating point-of-care, wearable, multiplexing and implantable diagnostic platforms that will allow early detection, continuous monitoring and treatment of health conditions in a personalized manner. These health challenges are translated into technological issues that need to be solved with multidisciplinary knowledge. Nanoscience and technology play a fundamental role in the development of miniaturized sensors that are cheap, accurate, sensitive and consume less power. At nanometre scale, these materials possess higher volume-to-surface ratio and display novel properties (composition, charge, reactive sites, physical structure and potential) that are exploited for sensing purposes. These nanomaterials can therefore be integrated into diagnostic sensing platforms allowing the creation of novel technologies that tackle current health challenges. These nanomaterial-enhanced sensors are extremely diverse, since they use numerous types of materials, nanostructures and detection modes for a multitude of biomarkers. The purpose of this review is to summarize the current state-of-the-art of nanomaterial-enhanced sensors, emphasizing and discussing the diagnostic challenges that are addressed by the different engineering and nanotechnology approaches. This review also aims to identify the drawbacks of nanomaterial-enhanced sensors, as well as point out future developmental directions.

KEYWORDS

Biosensors, Diagnostics, Healthcare challenges, Nanomaterial, Nanotechnology

1 | INTRODUCTION

Rapid, reliable and easy access diagnostics can significantly improve early detection and treatment monitoring of high mortality diseases, such as cardiovascular diseases, cancer, diabetes, among others (Rebelo et al., 2019). The successfully diagnostics of a disease, even prior to the manifestation of any symptom, can be crucial to efficacious treatment and survival rate (Chamorro-Garcia & Merkoçi, 2016).

Biomarkers are frequently used to diagnose these diseases; however, they need to be quantified in a specific concentration range in physiological fluids, such as blood and urine, which is a time-consuming process that uses bulky and expensive laboratory equipment. Thus, there is a need for portable, rapid and reliable technologies that can simplify laboratory biomarker quantitation (Wu et al., 2017). In order to achieve this, a plethora of sensing mechanisms have been combined with biological elements, producing biosensor technologies, which enable the quantitation of analytes in mixtures.

Ana I. Barbosa and Rita Rebelo contributed equally to the work presented.

Biosensors are composed by a biological element, that will select the analyte from a mixture, a transducer, that transforms the signal resulting from analyte binding into something readable, and a reader, that shows the signal value (Mehrotra, 2016). Biosensors are usually classified according to their transducer nature into electrochemical, optical, piezoelectric, electronic and gravimetric (Leca-Bouvier & Blum, 2005; Liu & Jiang, 2017; Rebelo et al., 2019). Furthermore, all different types of biosensors present attributes such as sensitivity, accuracy, repeatability, precision or specificity that are measured during the optimization of a biosensor and reflect its performance (Rebelo et al., 2019; Turner, 2015; Figure 1). Although several biosensing technologies have been reported in the last 10 years, only a few have been successfully commercialized, such as discussed in detail in Section 4.

In order to improve the limitations of current biosensors, they have been integrated with systems creating a plethora of lab-on-chip miniaturized diagnostic device platforms for point-of-care and multiplex biomarker quantification (Liao et al., 2018). One such device is microfluidics enables the processing of very small sample volumes (pL-nL) in short times (min or s), improving the performance of diagnostic tests (Barbosa et al., 2018).

However, the demand for wearable and implantable technologies imposes new approaches that go beyond lab-on-chip devices (Barbosa & Reis, 2017; Rebelo et al., 2019). Nanotechnology allows the next level of miniaturization of bioanalytical systems, by integrating sensors, fluidics and signal-processing circuits, which can provide the large-scale integration of different biochemical reactions on a smaller footprint (Vashist et al., 2012; Zhang et al., 2009), contributing for biosensors to achieve current society healthcare needs. Nanomaterial-enhanced sensors are analytical devices capable of detecting a target analyte from a biological sample (blood, tissue, saliva, urine, etc. taken from a person), that integrate nanomaterial as a strategy for improving biosensors features. Materials at nanoscale have been shown to exhibit properties that are vastly different from the bulk material from which they are derived. Their small size, large surface area-to-mass ratio and high reactivity impart characteristics that provide biosensors with unique capabilities.

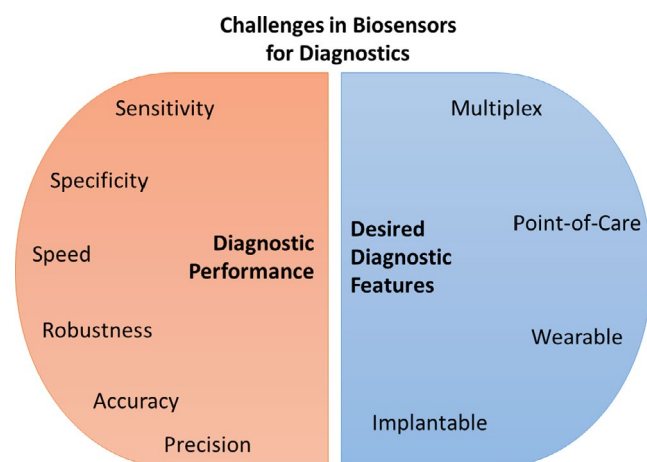


FIGURE 1 Challenges in diagnostic biosensors

This review intends to present the current state-of-the-art of nanomaterial-enhanced sensors, discussing how these nanomaterials can address the current technological challenges in clinical diagnostic biosensors. The limitations and future directions of nanomaterial-enhanced sensors are also explored.

2 | NANOMATERIAL-ENHANCED SENSORS FOR CLINICAL DIAGNOSTICS—AN OVERVIEW

Nanomaterial-enhanced sensors are miniaturized devices that use nanotechnology for improving the quantification of molecules within a mixture using specific immobilized receptors and transducers capable of transforming a physical-chemical mechanism into a readable signal. These sensors aim to tackle current diagnostic challenges in terms of performance, point-of-care applications, wearable/implantable capacity and multiplex monitoring (Figure 1). Nanomaterial-enhanced sensors present several advantages, when compared with macro scale sensors. These include low production cost due to miniaturization, high portability, lower-power consumption, high surface area and large pore volume per unit mass, high tunable size and shape-dependent characteristics and unique and tailorable surface chemistry. These unique properties, which can be achieved by the use of nanomaterial, lead to enhanced specificity and sensitivity of the biosensor, essential for clinical diagnosis since the concentration of biomarkers in blood is a millionth compared to the concentration of other blood proteins.

Nanomaterial-enhanced sensors can be produced by several techniques, being the most common top-down lithography, bottom-up fabrication and self-assembled nanostructures (Biswas et al., 2012). These fabrication techniques are combined with different approaches for nanomaterial synthesis, such as layer-by-layer, sputtering, emulsion, ball mixing and electrochemical (Malhotra & Ali, 2018). Therefore, several nanomaterial with unique electrical, optical, electrochemical and thermal properties have been developed to detect biomarkers with improved performance (Hou et al., 2016). Among those, nanoparticles (NPs), nanowires (NW), nanofilms (NF), quantum dots (QDs), nanocrystals (NC), nanorods (NR), nanobelts, nanotubes (NTs) embedded nanostructures and self-assembled nanomaterial stand out. Figure 2 represents several shapes of nanomaterial according to their dimensions. The exploration of these nanostructures with different functionalities is crucial for biosensor developments.

Nanomaterial is generally integrated in sensors to enhance their output signals. Their type and structure will depend on the transducing mechanism. The specific analyte receptors can be directly immobilized on a surface by adsorption (Baby et al., 2010; Qin et al., 2012), by covalent attachment (Song et al., 2011), or encapsulation within a coated layer of a permeable conductive polymer, or by using a cross-linking reagent (Gao et al., 2010; Janegitz et al., 2015; Liu et al., 2012; Unnikrishnan et al., 2012). In electrochemical sensors, a chemical reaction occurs between the receptor and

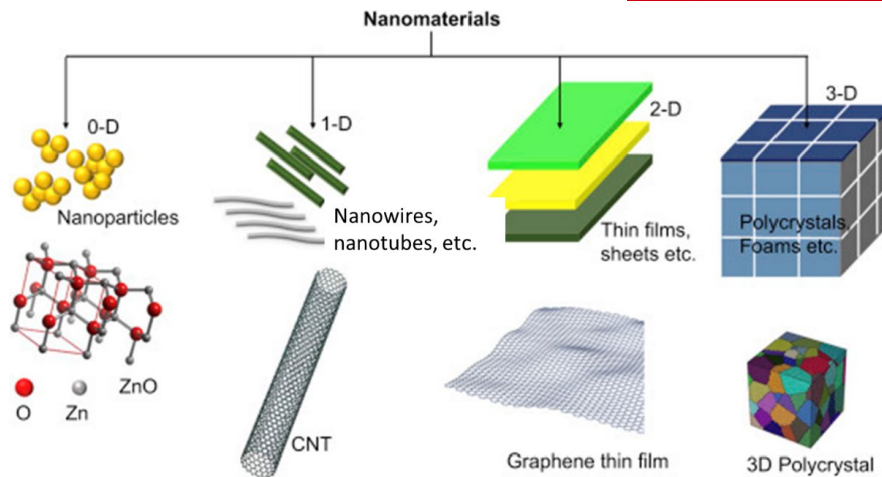


FIGURE 2 Classification of nanomaterial according to their dimensions (Malhotra & Ali, 2018). Figure adapted with permission from Elsevier

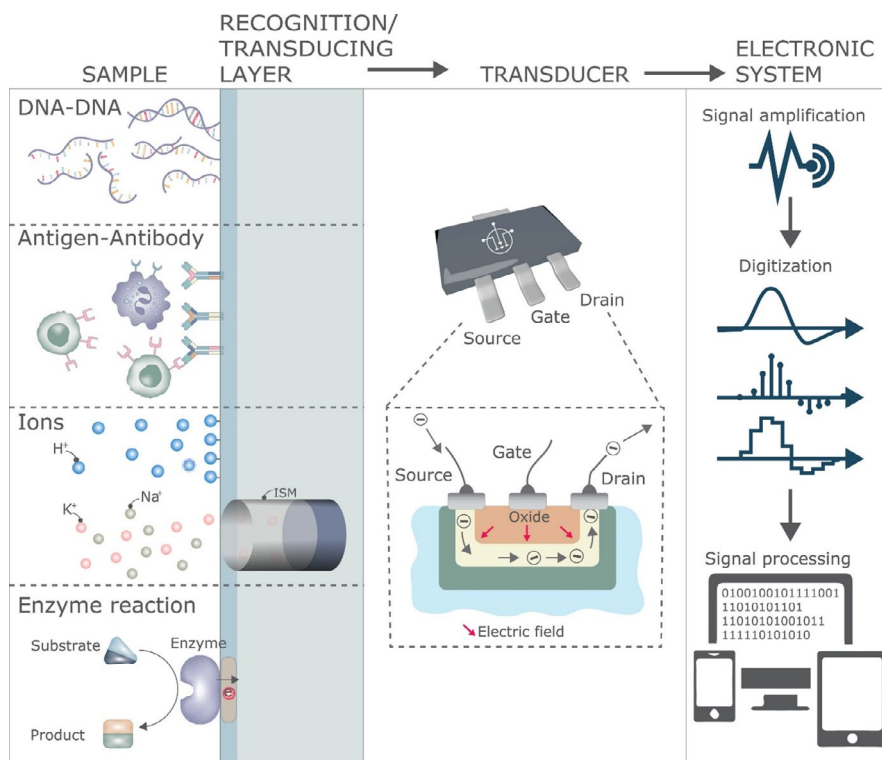


FIGURE 3 Schematic of a biological and chemical FET sensor (Kaisti, 2017). Figure adapted with permission from Elsevier

analyte producing the release, acceptance or consumption of ions. This generates a signal such as an electrical current or potential, and the magnitude of the change in the signal can be used to quantify the presence and concentration of the analyte/biomarker. There are many types of electrochemical detection techniques: potentiometry, voltammetry, impedance spectroscopy and conductometry. For example, field-effect transistors (FETs) use potentiometry to measure ions present in the gate electrode area of the FET (Figure 3). NW, with cross section in the nanometre scale and a length-to-diameter

ratio in the range of >1000 nm, have been used as transducers for FET detection. FET-based devices are composed of source, drain and gate electrodes. Most NW are made of silicon which rely on its semiconductor properties and is synthesized through bottom-up or top-down approaches (Biswas et al., 2012). The semiconductor nanowire bridges the source and the drain, while the gate electrodes modulate the conductance of the channel. FET sensors operate in the fM and nM range (Patolsky et al., 2006; Zhang & Ning, 2012). Sensitivity can be increased by reducing the diameter of the NW (Li

et al., 2005), controlling doping density (Nair & Alam, 2007). Further increases in sensitivity can be achieved by changing the method of detection (Rajan et al., 2013) (Ermanok et al., 2013) (Gao et al., 2010). The research group of CM Lieber (Gao et al., 2010, 2015; Patolsky et al., 2006; Patolsky et al., 2006; Zheng et al., 2005) were one of the first to use SiNW for detection of biomarkers. They were able to detect concentrations in the range of pM to fM range. Their devices also allowed the detection of multiple protein markers. Multiple NW were deposited in a single device (Figure 3) and functionalized for different protein markers, thus making these devices versatile. Carbon nanotube (CNT) NW has also been used for FET detection (Kong et al.), but show a lower detection limit (in pM) than their silicon counterparts. Nanowire sensors (Patolsky et al., 2006) have been demonstrated to detect cancer-associated in analytes in blood, tumour tissues and exhaled breath of cancer-infected patients (Shehada et al., 2015).

In optical biosensors, the reaction between the analyte and the receptor produces luminescence due to electromagnetic excitation at optical frequencies. Sensitivity is dependent on the detection mode such as fluorescence, Raman Spectroscopy, Surface Enhanced Raman scattering (SERS), refraction and others. Biological moieties attach to nanostructures affecting their light emitting characteristics. Changes that occur due to molecular binding affect the excitation of nanomaterial. This phenomenon can be exploited to detect an analyte of interest. For instance, surface plasmon resonance (SPR) sensors measure the change in refractive index of the medium at the sensing surface due to the binding of the molecules. There are two types SPR sensors: propagating SPR (PSPR) sensors and localized SPR (LSPR) sensors (Mulvaney, 2011; Figure 4). Both PSPR and LSPR have detection limits of less than 1 pM. Gold nanoparticles (AuNPs), often referred to as plasmonic NPs, are used to improve the sensing performance of LSPR sensors (Anker et al., 2008; Haes et al., 2005). These NPs are signal producers, exhibit strong absorption

in the visible and near infrared (NIR) wavelength regions and generate large electric fields due to LSPR on the surface of the particles (Swierczewska et al., 2012), increasing the sensitivity (Burda et al., 2005). The sensors require uniform nanomaterial to produce a narrow LSPR peak. A shift in the peak occurs when a biomarker attaches. Shifts in LSPR peak can be detected by absorption spectra in the visible light region by using colorimetric technique.

The colour of the particles is affected by the size and shape of the material and their dielectric constant. AgNPs displayed better sensing performance compared to AuNPs because of its higher dielectric constant. Aggregation of NPs enhances sensitivity by increasing the absorption coefficient. This makes the use of magnetic NPs an attractive choice (Elghanian et al., 1997; Lee et al., 2011; Medintz et al., 2005; Nie & Emory, 1997; Qian & Nie, 2008; Qian et al., 2008). AuNPs are the most widely used in SPR-based sensors as they are easy to synthesize and functionalize with various biological molecules. Furthermore, AuNPs with many biologically attached functional groups or antibodies have shelf life of around 18 months enabling long-term SPR detection. The combination of NPs with functionalized graphene (Georgakilas et al., 2012; Kuila et al., 2012; Yang, Bao, et al., 2017) and CNTs (Gao et al., 2012; Setaro, 2017) has resulted in increased SPR sensitivity. In addition to metal NPs, other NPs that have been used in SRS sensing including NPs made from latex (Mosier-Boss, 2017; Zeng et al., 2014) and liposomes (Lum et al., 2017; Zeng et al., 2014). Nanotechnology has enabled the ultrasensitive detection of biological analytes using SPR sensing techniques.

Another optical detection strategy often used in nanomaterial-enhanced sensors is fluorescence and other forms of luminescence. QDs exhibit useful properties for fluorescence sensing and labelling. A quantum dot is arranged in binary (e.g. CdSe, CdTe, GaAs, InAs, SiC) or ternary compounds (e.g. InGaN, InGaP, InGaAs) consisting of few hundred to thousands of atoms with size ranging from 1 to 20 nm (Guzelian et al., 1996). They display properties intermediate between bulk semiconductors and discrete atoms, and their properties depend on their size and shape (Allen & Bawendi, 2008; Bruchez et al., 1998; Kairdolf et al., 2008; Chan & Nie, 1998; Xie et al., 2007). The shift in energy level, otherwise (increase in the QD band gap), increases as the QD size decreases (Norris & Bawendi, 1996; Norris et al., 1994, 1996). Larger diameter sized QD's (5–6 nm) emit longer wavelengths, while smaller diameter sized QD's (2–3 nm) emit shorter wavelengths, though the specific colour emitted is affected by their composition. A comparison of QDs to the traditional fluorophore's is discussed in details elsewhere (Resch-Genger et al., 2008). QD have several advantages over conventional dyes as they have high brightness, good photostability, a broad spectrum of excitation and long fluorescence lifetime (Jaiswal et al., 2003; Resch-Genger et al., 2008). NIR (near infrared) (650–1350 nm) emission is highly desirable for biomedical imaging due to its reduced light scattering and low tissue absorption. This has prompted researchers to make use of the NIR optical window to conduct deep-tissue optical imaging and sensing with the aid of QD, which are essentially zero-dimensional nanomaterial. QD can be synthesized in non-polar

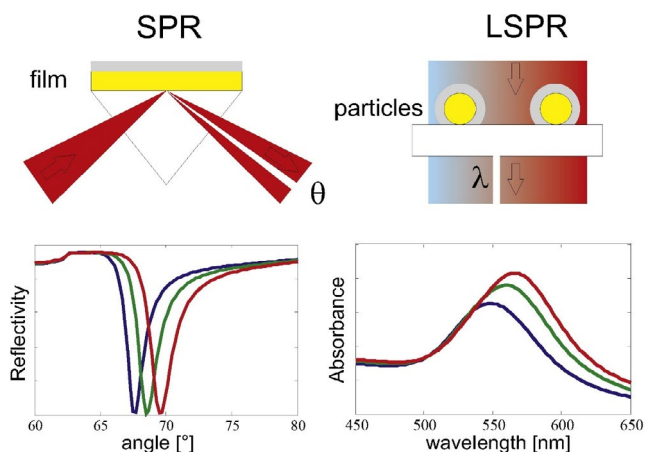


FIGURE 4 Diagrams showing the sensing principle of the studied SPR (left) and LSPR (right) systems. The graphs display the calculated shift of dip/peak upon adsorption of different thickness molecular film with a refractive index of 1.48 in water (Jatschka et al., 2016). Figure reproduced with permission from Elsevier

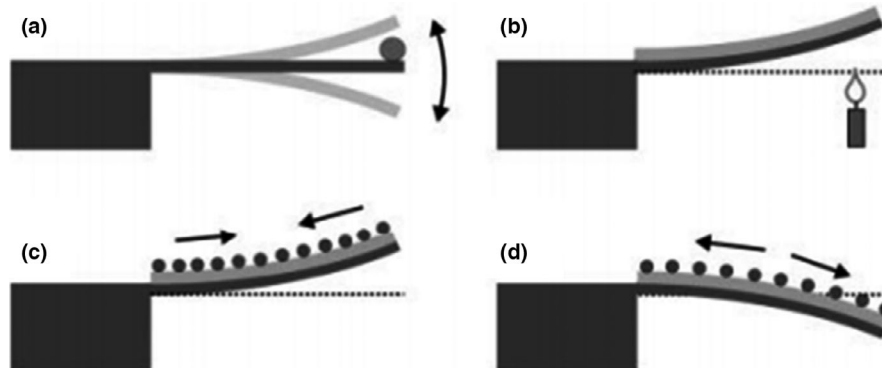


FIGURE 5 Cantilever sensor operation mode: (a) detecting mass variations on the cantilever by deviations in resonance frequency; (b) bimetallic mode detecting temperature variations by a static bending; and (c, d) surface stress mode, where asymmetric molecular binding to the cantilever's top or bottom surface leads to an overall cantilever bending. For example, adsorption on the top surface can either cause tensile stress (c), bending the cantilever upwards, or compressive stress (d), bending the cantilever downwards (Fritz, 2008). Figure reproduced with permission from The Royal Society of Chemistry

solvents (Crouch et al., 2003; Murray et al., 1993), aqueous solutions (Alivisatos, et al., 2004), or on a solid substrate (Bhattacharya et al., 2004). Their excellent fluorescent properties make them an outstanding fluorescence resonance energy transfer (FRET) donor. The brightness makes them attractive for sensors with high sensitivity, and their photostability enables repetitive imaging applications (Chen & Periasamy, 2006; Shi et al., 2015). QDs can also be designed with nanoscaffold purposes. They present a large surface area as solid substrate for molecule adsorption, and multiple acceptors that can be conjugated to a single QD, allowing signal amplification and sensitivity increment (Resch-Genger et al., 2008). QD form a core-shell structure, for example CdSe (Cadmium selenide) QD with ZnS (Zinc sulphide) shell, which enhances QD luminescence by improving its quantum yield and narrowing the emission spectrum, minimizes the toxicity of core materials by preventing leach outs.

Mechanical sensors detect forces and deflection due to mass changes caused by the adsorbed analyte. These mass variations can be transduced by frequency, temperature and stress discrepancy. For example, the attachment of an analyte to a sensor can be studied using cantilevers. These cantilevers can be integrated in microelectromechanical system (MEMS) or nanoelectromechanical systems (NEMS). MEMS-based devices were initially based on solid-state semiconductor like silicon (Doll et al., 2009; Park et al., 2010; Villanueva et al., 2008) and its derivatives like silicon nitride (Hyun et al., 2006; Venkatasubramanian et al., 2012) and silicon dioxide (Huang et al., 2013; Yang et al., 2008). Because of clean room requirements and large initial equipment costs, alternate materials with matching performance but reduced material and fabrication costs were sought after. In this respect, synthetic polymers are an attractive choice. Various polymers such as parlene, polypropylene, SU-8, fluoropolymer, polyethylene terephthalate, polystyrene and others have been explored to realize miniaturized devices, and studies highlighting the optimal performance of cantilever sensors based on structural characteristics and material selection have been reported (Mathew & Ravi Sankar, 2018). Cantilever-based sensors

are classified as surface stress-based sensors (due to cantilever beam deflection or static mode), dynamic-mode based sensors (due to change in resonant frequencies) and bimetallic mode based sensors (due to change of thermal expansion of cantilever metallic layer) (Figure 5).

When an analyte binds, it adds mass to the device and develops a surface inducing deflection of the cantilever. The deflection can be quantified by optical methods such as laser beam deflection (Figure 6), though electrical readout has also been employed (Backmann et al., 2005; Fritz et al., 2000; Mertens et al., 2008; Wu et al., 2001; Zhang et al., 2006). Using optical methods, the resolution of cantilever deflection is in nanometres (Binnig et al., 1987) and mass detection sensitivity is in pictogram (Chen et al., 1995). In the dynamic-mode method, the sensor oscillates with resonance frequency, and this frequency changes when a mass is attached to the cantilever. The dynamic-mode method is less sensitive and can lead to erroneous result due to the damping effect of the fluid. When detection in static mode is made using piezoresistive readout, the performance of the device can be enhanced by amplifying the stress generated by cantilever bending, thus improving electrical sensitivity (Ansari et al., 2012).

In mechanical sensors, it is generally desirable to reduce the dimensions of the device, since nanoscale increase mass resolution. The ability of devices to be displaced or deformed greatly increases with a reduction in its dimensions. There are several challenges in miniaturizing these cantilever-based sensors. Detection using optical means becomes difficult as device dimensions' scale below optical wavelength. Also, sensitivity using electrostatic detection and piezoresistive devices decreases at nanoscale, not only due to detection modes, but also due to other interfering factors that are enhanced at nanoscale. For example, the non-specific binding of other species rather than the target analyte is amplified at nanoscale, resulting in most of the sensor interactions and increasing the possibility of error by introducing noise in the data. Thus, the detection limit and sensitivity is not only depended on the sensor design, but also

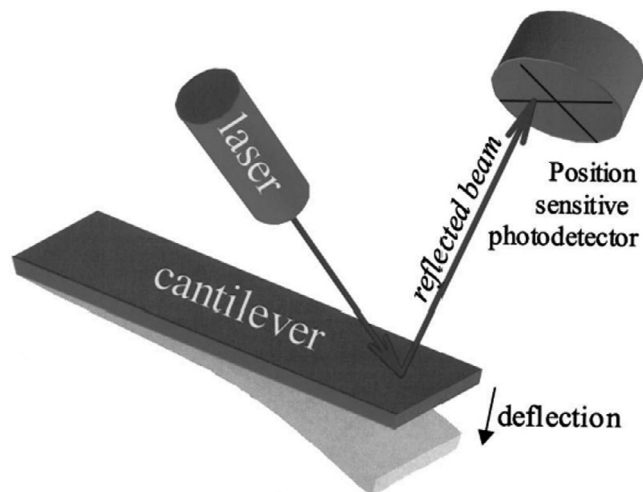


FIGURE 6 Optical recognition of cantilever deflection (Lavrik et al., 2004). Figure reproduced with permission from AIP Publishing

in other factors, related to diagnostic assays (Swierczewska et al., 2012).

The particular combination of sensors materials, fabrication technologies and detection modes will dictate which diagnostic current challenges can be addressed and therefore the medical application of the biosensors. Different nanomaterial-enhanced sensor approaches can be taken in order to improve the diagnostic performance or add point-of-care, wearable and implantable diagnostic features. For example, the effect of graphene in vivo applications depends on how the graphene was synthesized and purified (Pinto et al., 2013). Therefore, for wearable and implantable sensors, graphene nanomaterial production process needs to follow specific criteria. Graphene high conductivity, electrochemical stability and flexibility make this material an ideal candidate for biosensors with electrochemical and optical detection modes. Graphene microelectrode arrays and graphene field-effect transistors have been used for neural stimulation (Thunemann et al., 2018). Wet-spun reduced graphene oxide have been used for free-standing penetrating electrodes (Apollo et al., 2015).

The combination of different nanomaterial with particular nanostructures in sensor devices and the interaction of these nanomaterials with specific detection modes will produce biosensors with unique features capable of addressing current clinical diagnostic needs.

3 | CURRENT DIAGNOSTIC CHALLENGES: NANOMATERIAL-ENHANCED SENSORS APPROACHES

Biosensors are promising tools for clinical diagnostics; however, they still face current technical challenges (Figure 1) that pushes them away from becoming a reality and serving the worldwide population

healthcare needs. These challenges are the improvement of diagnostic performance, the capacity for multiple detection, the production of point-of-care quantitative devices and the production of wearable and implantable technologies for biomarkers detection. Several strategies have been reported that integrate nanomaterial in biosensors, providing some insights in how these challenges can be solved. Table 1 presents the state-of-the-art of nanomaterial-enhanced sensors in clinical diagnostics, with reported literature from 2017 to present, and provides a clear connection to which technical challenge is being addressed by the nanotechnology approach. The discussion and exploration of Table 1 are done along Section 3 of this review.

3.1 | Nanomaterial for diagnostic performance demands

In general, a diagnostic test measures a specific analyte in complex samples, such as blood, urine or saliva. In order for the technology to be successful, the test needs to be sensitive, have a low detection limit, have a wide dynamic range, be precise and accurate (Figure 7). These performance parameters are essential for accurate diagnostics and subsequent clinical treatment decisions. Therefore, finding ways to overcome current diagnostic technologies performance is one of the main challenges in the biosensors field. Nanotechnology has for sure given great contributions towards performance improvement in biosensors as described below.

Of all the parameters mentioned above, the sensitivity and detection limit are the most frequently cited parameters of interest in biosensors literature. These two parameters are at times used interchangeably; however, they are different terms, and it is important to understand their differences. Analytical sensitivity, a term that is indicative of the capacity of the method to differentiate between two very close signal points, is usually given by the slope of the calibration curve. The detection limit, on the other hand, describes the minimum signal that a device/test can quantify with a specified precision and reproducibility (Armbruster & Pry, 2008; Barbosa & Reis, 2017; Shrivastava & Gupta, 2011).

Several nanomaterials have been used in different biosensor technologies to improve the analytical sensitivity of diagnostic tests and lower their detection limits. These are summarized in Table 1. These nanomaterials can be applied in different manners, like NPs, nanocrystal, QDs, among others, depending on the biosensor detection mode and biochemical assay involved. Nevertheless, in general, nanomaterials enhance the efficiency of probe immobilization, due to their high surface area and chemistry (Barbosa et al., 2017, 2019), providing signal amplification, improving analyte-transducer contact and promoting efficient catalysis in case of electrochemical sensors. The combination of these effects enables the quantification of low analyte concentrations in assays with improved performance, which is highly desirable in diagnostics. References presented in Table 1 and several published reviews (Kerman et al., 2008; Luo et al., 2006; Wongkaew et al., 2019; Xu et al., 2020; Zhu et al., 2015) reveal that

TABLE 1 State-of-the-art of diagnostic biosensors challenges addressed by nanomaterial-enhanced sensors

Addressed challenge	Structure	Materials	Fabrication method	Detection Mode	Target Analyte	Sample type	Reference
Performance	NPs	Gold	Electrodeposition	Electrochemical	cTnI	Buffer	Jo et al. (2017)
		Copper/platinum	Synthesis		PSA	Buffer	Feng, Li, et al. (2017)
		Graphene/Gold	Synthesis		Dopamine	Buffer	Li, Wang, et al. (2017)
		Silver	Electrodeposition		Glucose	Blood	Wang et al. (2017)
		Iron oxide	Hydrothermal reaction		Nucleic acids	Buffer	Altay et al. (2017)
		Iron oxide	Synthesis		Acetylcholine	Serum	Chauhan et al. (2017)
		Silica	Stöber process	Optical	PSA	Serum	Kong et al. (2017)
		Silver	Electrochemical		MMP-9	Blood	Zhao et al. (2019)
		Silica	Commercial		H ₂ S	Buffer	Liu et al. (2020)
		Carbon	Commercial	Electrochemical	Glycerol	Human urine	Ramonas et al. (2019)
NTs	Carbon	Carbon	Commercial	Piezoelectric	Microbes	Blood	Shi et al. (2017)
		Carbon	Commercial	Electrochemilluminescence	PSMA	Buffer	Juzgado et al. (2017)
		Carbon	Commercial	Electrochemical	Glucose	Blood	Ahmad et al. (2017)
NR	Zinc oxide	Sputtering& Hydrothermal Method	Electrochemical				
		Hydrothermal & chemical vapour deposition					
QDs	Carbon	Carbon	Acid attack	Optical	Desmin	Serum	Li et al. (2017)
		Carbon	Hydrothermal carbonization		Copper (Cu(II))	Buffer	Ghosh et al. (2019)
		Poly arginine-graphene	Layer-by-layer	Electrochemical	MDA	Buffer	Hasanzadeh et al. (2017)
		Carbon	Synthesis		Growth factor receptor-2 & MCF-7 cells	Serum	Gu et al. (2019)
NPs & Nanosheets	Nanoclusters	Gold/Graphene	Hydrothermal process	Optical	L-Cysteine	Buffer	Thirumalraj et al. (2018)
		Silver/Gold	Synthesis		HAase	Serum	Liu et al. (2019)
NanoPs & QDs	NanoPs & QDs	Gold	Synthesis	Electrochemilluminescence	Telomerase activity	Buffer	Feng, Zhou, et al. (2017)
		Cadmium sulphide	Reverse micro-emulsion		p53	Serum	Heidari et al. (2019)
NC& NPs	Nanohorn & Nanocubes	Cadmium sulphide					
		Gold	Synthesis		N-terminal pro-brain natriuretic peptides	Serum	Liu et al. (2017)
Flower-like nanocomposites & NTs	Flower-like nanocomposites & NTs	PdCu & Carbon	Commercial	Electrochemical	Glucose	Serum	Wang et al. (2019)
		Nickel(II)-terephthalic acid					
NTs& NC	NTs& NC	Carbon	Chemical Vapour Deposition		CEA	Serum	Tran et al. (2018)
		Carbon					

(Continues)

TABLE 1 (Continued)

Addressed challenge	Structure	Materials	Fabrication method	Detection Mode	Target Analyte	Sample type	Reference
	NR& NPs	Zinc oxide Gold	Hydrothermal process and sputtering		CA-125	Buffer	Gasparotto et al. (2017)
	Nanocubes & NPs	Cobaltic oxide Ceric dioxide Gold	Synthesis Electrodeposition		Squamous cell carcinoma antigen	Serum	Li, Zhang, et al. (2017)
	NPs film composite	Graphene/Au	Synthesis		Glycated haemoglobin	Blood	Jain & Chauhan (2017)
	NPs Nanofibres	Gold & Poly(vinyl alcohol)/poly(ethyleneimine) Carbon & Nickel	Electrospinning Synthesis		Glucose	Buffer	Sapountzi et al. (2017)
	Multiwalled NTs & NPs		Synthesis		Glucose	Serum	Başkaya et al. (2017)
	Nanoneedle	CuO/nitrogen reduced graphene	Synthesis		Glucose	Serum	Yang et al., 2017
	Nanocage & Nanotubes	Gold & Carbon	Layer-by-layer		MCF-7 breast cancer cells	Buffer	Yang et al. (2018)
	NPs Nanocomposite	Gold & CuFe2O4/reduced graphene oxide	Synthesis		L-cysteine	Buffer	Atacan (2019)
Point-of-care	Nanosheet QDs	Graphene Paper	Synthesis by Acetobacter xylinum & coating	Optical	Human-IgG protein <i>E. coli</i>	Serum	Cheeveewattanagul et al. (2017)
	NPs	Gold		Optical	HIV-1 p24 antigen	Serum	Kosaka et al. (2017)
		Carboxyle-terminated magnetic	-	Optical Electrochemical Electrochemical	PSA	Buffer	Suaifan & Zourob (2017)
Wearable	Nanofibrils	Collagen	Self-assembled	Piezoelectric	Pressure	Skin	Ghosh & Mandal (2017)
	NPs	Platinum	Synthesis	Electrochemical	Glucose	Perspiration	Abellán-Llobregat et al. (2017)
	NTs & Nanosheets	Carbon Gold	Layer-by-layer	Electrochemical	Glucose & pH	Sweat	Oh et al. (2018)
	Nanofibre membrane Nanowire	Poly(vinylidene fluoride) Silver	Layer-by-layer	Pressure	Physiological signals	Respiration / Skin	Yang, Li, et al. (2018)

(Continues)

TABLE 1 (Continued)

Addressed challenge	Structure	Materials	Fabrication method	Detection Mode	Target Analyte	Sample type	Reference
Multiplex	NR & Nanoporous	Graphene p-AHNSA	Synthesis	Electrochemical	Dopamine and 5-hydroxytryptamine	Plasma	Raj et al. (2017)
	Nanosheet & Nanoporous	Gold/zinc oxide polyamide	E-beam cryo-evaporator & Sputtering	Electrochemical	Glucose	Sweat	Munje et al. (2017)
	Dots	Carbon	Synthesis	Optical	<i>E. coli</i> , <i>D. desulfuricans</i> , <i>S. sciuri</i> , <i>L. monocytogenes</i> , <i>S. aureus</i> and <i>P. aeruginosa</i>	Buffer	Zheng et al. (2019)
Implantable	Nanobeads & QDs	Iron oxide	Commercial	Optical	EV71-related microRNAs	Serum	Wang et al. (2020)
	NTs	Carbon	Synthesis	Electrochemical	Ascorbic Acid	Rat	Zhang et al. (2017)
	NPs	Iron oxide	Ultrasonication	Optical	HE4	Mice	Williams et al. (2018)
			Synthesis	Optical	HE4	Tissue/Urine	Kwon et al. (2017)

CA-125, cancer antigen 125; CEA, carcinoembryonic antigen; cTnI, cardiac troponin I; *D. desulfuricans*, *Desulfovibrio desulfuricans*; *E. coli*, *Escherichia coli*; EV71, Enterovirus 71; H₂S, hydrogen sulphide; HAase, hyaluronidase; HE4, human epididymis protein 4; *L. monocytogenes*, *Listeria monocytogenes*; MDA, malondialdehyde; MIMP9, matrix metalloproteinases; *P. aeruginosa*, *Pseudomonas aeruginosa*; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; *S. aureus*, *Staphylococcus aureus*; *S. Sciuri*, *Staphylococcus sciuri*.

engineered nanomaterials are attractive for sensing applications, as electrochemical biosensors, as their conductive properties can enhance catalysis and electron transfer. Table 2 summarizes the performance parameters of nanomaterials enhanced sensors further discussed in this section.

3.1.1 | Nanoparticles

Nanoparticles are by far the most used nanomaterial in the field, due to their varied properties that can be easily tuned by changing parameters as size, shape and composition (Capek & Capek, 2006; Zamborini et al., 2012). NPs provide interesting properties for sensing applications like enhanced electrical conductivity, high reactivity, large surface area-to-volume ratio, improved optical effects, magnetic properties and quantum confinement effects. They are also easily synthesized in the laboratory or commercially available which facilitates their usage in research settings. 1D nanoscale structures have gained great attention due to their properties of high surface area-to-volume ratio, ultrasmall scale and important applications in nanoscale device generation. These structures are commonly used in microfluidic chips and sensing devices, since they provide several advantages over bulk approaches, like reduced sample needs, operate in the laminar flow regime in the microchannels and controlled self-assembly of nanostructures (Xing & Dittrich, 2018).

Nanoparticles are used for high sensitivity and detection limit improvement in electrochemical diagnostic sensors. By increasing the surface area of the working electrode, there is an acceleration in the electron transfer at the electrode interface and an increment in the area available for probe immobilization (Barbosa et al., 2019). This increment in the surface area is usually translated in signal amplification, and therefore, lower biomarkers concentration can be detected. For instance, a working electrode made of screen-printed carbon was covered with electrodeposited AuNPs and 5,2':5'2''-terthiophene-3'-carboxylic acid (TTCA). This sensor presented excellent analytical performance, quantifying cardiac troponin I (cTnI) with a dynamic range of 1–100 pM (0.024–2.4 ng/ml) and a detection limit of 1.0 pM (24 pg/ml), which is lower than the existing cut-off values (40–700 pg/ml). The chronoamperometric sensor performance is due to AuNPs deposition, that increases the surface area, enabling a broad dynamic range, and TTCA deposition that allows immobilization of biomolecules, in addition to exhibiting good electrical properties. This higher performance in cTnI detection is extremely useful since cTnI has shown high sensitivity and selectivity towards acute myocardial infarction (Jo et al., 2017). In another example, an electrochemical biosensor was developed for dopamine detection. Abnormal levels of dopamine are linked to Alzheimer's disease, depression, Parkinson's disease, hyperactivity disorder and schizophrenia. The developed biosensor consisted of AuNPs supported by graphene oxide functionalised by an ionic liquid coated onto a glassy carbon electrode. The loading of AuNPs significantly enhanced the catalytic performance of the sensor complexes measured with cyclic voltammetry, due to their excellent electron transfer properties



FIGURE 7 Analytical parameters for validation of biosensor performance

and electrocatalytic capacity for dopamine oxidation (Li, Wang, et al., 2017). Moreover, the application of NPs as labels in electrochemical measurements has also been reported with performance improvements, since the labels also act as signal amplifiers. PtCu bimetallic NPs loaded on 2D/2D reduced graphene oxide/graphitic carbon nitride (PtCu@rGO/g-C₃N₄) provided large surface area and biocompatibility, for immobilization of secondary antibodies. These NPs acted as signal amplifiers in electrochemical impedance spectroscopy measurements, since they present good activity for the reduction of hydrogen peroxide (H₂O₂), with a linear range of 50 fg/ml to 40 ng/ml, and LOD of 16.6 fg/ml (S/N = 3) for prostate-specific antigen (PSA), which is often used for prostate cancer screening (Feng, Li, et al., 2017).

Liu et al. (2020) reported the development of a nanosensor for ratiometric luminescence detection of hydrogen sulphide (H₂S) in aqueous solution and live cells using mesoporous silica nanoparticle. The change in the level of H₂S in the body is associated with several diseases, such as Alzheimer's disease, Down's syndrome, diabetes, liver cirrhosis and even cancers (Lin et al., 2015). As an example, the H₂S level is directly implicated with the inflammation of the cancer cells. But there is no simple and quick determination of this marker (Szabo, 2016). As shown in Figure 8, the sensors containing mesoporous silica nanoparticle (MSN) present a reference signal due to conjugated fluorescein isothiocyanate (FITC) and an H₂S responsive unit with embedded ruthenium(II) (Ru(II)) complex. The synthesized Ru@FITC-MSN emit luminescence emission bands centred at 520 nm (FITC) and 600 nm (Ru(II) complex). The emission of Ru(II) complex when quenched upon addition of copper ion (Cu²⁺), forms an in situ RuCu@FITC-MSN nanosensor. When reacted with H₂S in HEPES buffer solution, turn on the emission of Ru(II) complex showing the ratiometric luminescence response to H₂S in solution (Liu et al., 2020).

Nanoparticles have also been highly used to improve the performance of optical biosensors either by serving as labels for signal amplification or as immobilization substrate. For instance, constructed silica-coated Ag SERS nanotags were used to detect matrix metalloproteinases 9 (MMP-9), whose early detection can be essential

for the survival rate and recovery evaluation of stroke patients (Figure 9a). The biosensor performed in the range up to 100 ng/ml with a detection limit of 1 pg/ml, in unprocessed blood samples. The AgNPs (Figure 9b) shown strong enhancement effects from individual particles due to their ability to localize the surface electromagnetic fields through the hot spots in their structures. The AgNPs were modified with polyvinylpyrrolidone (PVP) to enhance stability which is more suitable for biological monitoring (Zhao et al., 2019). In another study (Kong et al., 2017), positively charged amino-functionalized SiO₂NP's worked as nanocapturer by electrostatically adsorbing PSA aptamer to form SiO₂NP-aptamer nanocomposite which was capable of adsorbing negatively charged tetraphenylethylene derivative 3 (TPE3) to form aggregation-induced fluorescence emission-SiO₂NPs nanocomposite.

The binding of the aptamer to the target PSA resulted in the release of the aptamer from the surface of SiO₂NPs, which made the TPE3 aggregate on the SiO₂NPs surface thus, emitting high fluorescence. This method lowered the detection limit of PSA to 0.5 ng/ml (Kong et al., 2017).

3.1.2 | Nanotubes/Nanowires

In addition to NPs, NTs and NW offer large surface area/unit mass, and depending on their chemical composition, they can offer conductivity improvements. Existing screening procedures for prostate cancer in clinical practice are unable to detect low serum PSA levels. Nanowire-based have contributed to the detection of protein cancer markers (Gao et al., 2015; Puppo et al., 2016; Tzouvakaki et al., 2016; Zheng et al., 2005). A real-time method for detection of PSA using n-type SiNW FET (silicon nanowire field-effect transistor) biosensor has been reported whereby an immobilized PSA antibody on the SiNW surface was used to recognize the PSA. This ultrasensitive method (below 1 fg/ml) could be attained by adjusting the dimension of the SiNW and the doping concentration of the Si channel (Zhang & Ning, 2012). An integrated microfluidic purification device system and a SiNW FET array was used to analyse prostate as well as breast cancer markers (Stern et al., 2010). The purification device was used to pre-isolate the target molecules from whole blood by binding them to specific antibodies immobilized on the channel and subsequently cleaved by the photochemistry. The purified target molecules were then transferred to the NW cell for real-time sensing. PSA at a concentration of 2.5 ng/ml and CA 15.3 (carbohydrate antigen-15.3) (30 U/ml) were detected from whole blood (Stern et al., 2010).

Carbon NTs based nanosensor have also been used. CNTs show a unique combination of mechanical, electrical and electrochemical properties, that make them ideal components in diagnostic biosensors. As biosensors, single-walled CNTs (SWCNTs) hold promises for detecting key biological analytes, including nitric oxide (NO), glucose, H₂O₂ and others (Heller et al., 2009; Iverson et al., 2015; Kim et al., 2009; Ulissi et al., 2014; Zhang et al., 2011). DNA-SWCNTs conjugates can show molecular recognition that are DNA

TABLE 2 Performance parameters of nanomaterial-enhanced sensors that reported performance improvements

Nanomaterial	Target	Dynamic range	Limit of detection (LoD)	Analyte response Time	Recovery	Reproducibility (RSTDV)	Precision (RSTV)	Reference
NPs	cTnI	1–100 pM (0.024–2.4 ng/mL)	1 pM (24 pg/mL)	5 min	-	-	-	Jo et al. (2017)
	PSA	50 fg/mL to 40 ng/mL	16.6 fg/ml	50 min	-	1.9%	-	Feng, Li, et al. (2017)
	Dopamine	7 nM to 5 μ M	2.3 nM	70 sec	98% to 102%	3.1%	2.8%	Li, Wang, et al. (2017)
	Glucose	0.15 to 13 mmol/L	0.02 mM	5 sec	94.2% to 103.4%	4.5%	3%	Wang, Wang, et al. (2017)
	DNA	5–25 μ g/ml	1.15 μ g/ml	-	-	-	-	Altay et al. (2017)
	ACh	4.0 nM to 800 μ M	4.0 nM	<4 sec	95.7 \pm 0.2 and 97.6 \pm 0.3%	4.65%	4.3%	Chauhan et al. (2017)
NTs	PSA	1 to 50 ng/ml	0.5 ng/ml	25 min	95.5 \pm 1.71 to 103.6 \pm 3.82%	-	-	Kong et al. (2017)
	MMP-9	< 100 ng/ml	1 pg/ml	-	-	-	-	Zhao et al. (2019)
	H ₂ S	0.5–4 μ M	0.36 μ M	-	-	-	-	Liu et al. (2020)
	Glycerol	0.05 to 1.0 mM	18 μ M	41 \pm 4 s and 0 \pm 3 s	92 to 105%	-	-	Ramonas et al. (2019)
NRs	Microbes	-	10 and 100 CFU/mL	-	-	-	-	Shi et al. (2017)
	PSMA	2.6–12 ng/ml	0.88 ng/ml	-	-	-	-	Juzgado et al. (2017)
	Glucose	< 8.45 mM	0.40 μ M	<2 s	96%	4.6%	-	Ahmad et al. (2017)
	Glucose	0.1 mM to 10 mM	1 mM	100 s	-	-	-	Chung et al. (2017)
QDs	Desmin	0.714–4.286 ng/mL	1 ng/ml	-	-	-	-	Li, Yan, et al. (2017)
	Copper (Cu(II))	-	0.01 μ M	-	-	-	-	Ghosh et al. (2019)
	MDA	0.001–0.2 M	0.329 nM	-	-	6.16%	-	Hasanzadeh et al. (2017)
	Growth factor receptor-2 & MCF-7 cells	0.001–10 ng/ml 1 \times 10 ² –1 \times 10 ⁵ cell/mL	19 fg/ml 23 cell/ml	-	95.7% to 108.3%	3.4%	<5%	Gu et al. (2019)
NPs & Nanosheets	L-Cysteine	0–400 nM	0.51 nm	-	-	-	-	Thirumalraj et al. (2018)
Nanoclusters	HAase	0.5–37.5 U/ml	0.3 U/ml	-	95.1% to 101.9%	1.09%–2.81%	-	Liu et al. (2019)
	NanoPs & QDs	Telomerase activity	0 to 32000 cells/mL	-	-	-	-	Feng, Zhou, et al. (2017)
NC& NPs	p53	20 to 1000 fg/ml	4 fg/ml	-	93.5% to 107.1%	-	<4.82%	Heidari et al. (2019)
	Nanohorn & Nanocubes	NT-proBNP	0.1 pg/ml to 25 ng/mL	0.05 pg/ml	94% to 95%	2.73% to 3.48%	-	Liu, Wang, et al. (2017)

Nanomaterial	Target	Dynamic range	Limit of detection (LoD)	Analyte response Time	Recovery	Reproducibility (RSTDV)	Precision (RSTV)	Reference
Flower-like nanocomposites & NTs	Glucose	20 μ M to 4.4 mM	4.6 μ M	<5 s	93.3%–100%	1.9%	-	Wang et al. (2019)
NTs & NC	CEA	0.025–25 ng/ml	0.5 pg/ml	-	107.2–108.3%	6.0%	-	Tran et al. (2018)
NR & NPs	CA-125	-	2.5 ng/ μ l	-	-	-	-	Gasparotto et al. (2017)
Nanocubes & NPs	Carcinoma antigen	100 fg/ml to 80 ng/mL	33 fg/ml	100 s	96.4% to 100.7%	2.68%	<5%	Li, Zhang, et al. (2017)
NPs film composite	Glycated haemoglobin	0.3 to 2000 μ M	0.2 μ M	-	96.0% to 97.5%	3.62%	4.42%	Jain & Chauhan (2017)
NPs Nanofibres	Glucose	10–200 μ M	0.9 μ M	-	-	<4%	-	Sapountzi et al. (2017)
Multiwalled NTs & NPs		0.05–12.0 mM	0.021 μ M	100 s	96.4% to 98.7%	3.7%–4.6%	-	Başkaya et al. (2017)
Nanoneedle		0.5–639 μ M	0.01 μ M	100 s	98% to 100%	6.18%	-	Yang, Li, et al. (2017)
Nanocage & Nanotubes	MCF-7 cells	1.0×10^2 to 1.0×10^6 cells/mL	80 cells/ml	-	-	-	-	Yang, Fu, et al. (2018)
NPs Nanocomposites	L-cysteine	0.05–0.2 mM	0.383 μ M	-	-	4.2%	-	Atacan (2019)

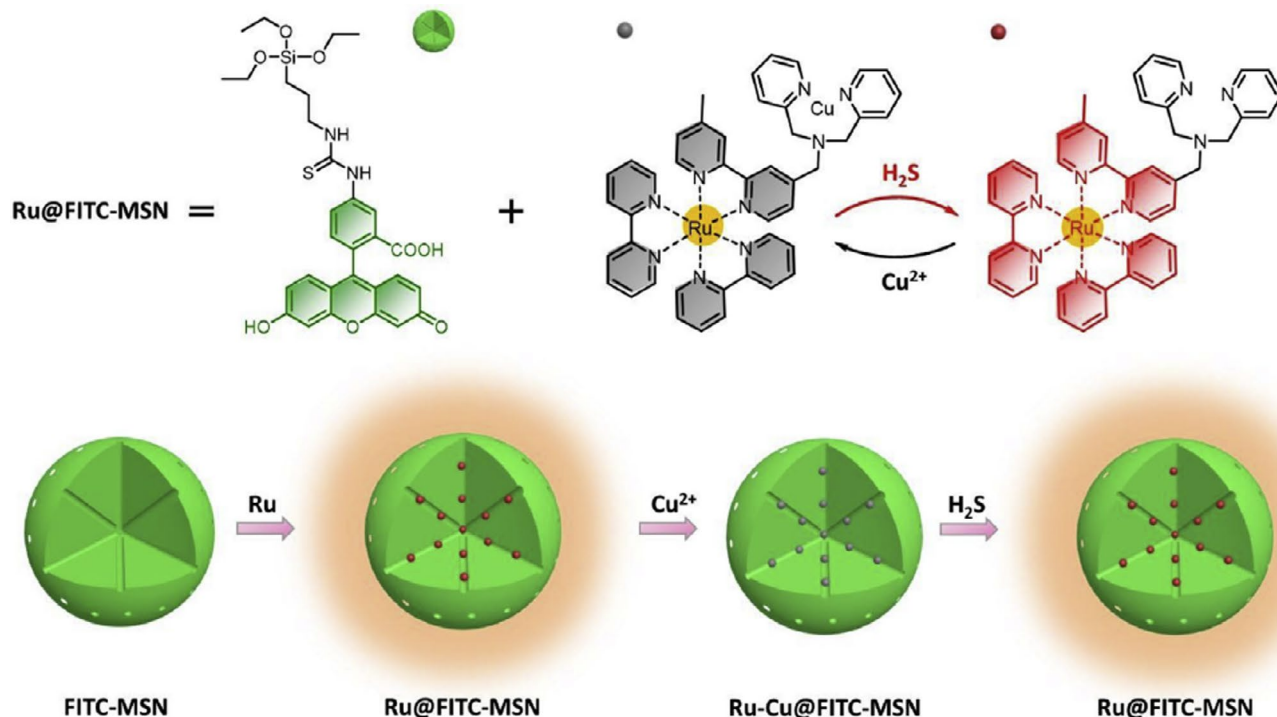


FIGURE 8 Schematic of nanosensor for H₂S detection (Liu et al., 2020). Figure reproduced with permission from Elsevier

sequence-dependent. For example, (AT)15-wrapped as specific NO sensors (Ulissi et al., 2014; Zhang et al., 2011) and (GT)15-wrapped SWCNTs as H₂O₂ sensors in the biological system (Heller et al., 2009). SWCNTs show distinctive Raman and photoluminescence properties in the nNIR spectral region. These features make them suitable for bio-imaging, due to minimal optical scattering and absorption in the NIR range (700–2,500 nm) (Smith et al., 2009). It has been demonstrated (Heller et al., 2009; Jin et al., 2010) that the single-stranded DNA (GT) 15 wrapped around the SWCNTs surface is a very effective approach to detect H₂O₂ presenting excellent specificity, and sensitivity to single-molecule, in addition to high spatial and temporal resolution from cells. The H₂O₂ is detected by fluorescence quenching of SWCNTs wrapped with (GT)15 (Jin et al., 2010). Moreover, SWCNT's Raman signals have also been applied to tissue labelling (Liu et al., 2008; Zhou et al., 2016). Yu et al. (2006) also an electrochemiluminescence (ECL) immunosensor based MWCNTs (multiwalled CNTs) equipped with multiple enzyme labels for the detection of PSA in serum and tissue lysates. The detection of PSA in human serum samples was compared to the standard ELISA technique and showed the high accuracy of immunosensors, with a LOD (Limit of Detection) of 4 pg/ml in serum.

Kwon et al. (2010) combined conducting polymer (polypyrrole) NTs with aptamers, bonded to the NTs, to develop a biosensor for the detection of VEGF (Vascular endothelial growth factor). VEGF is a signalling protein that promotes the growth of new blood vessels, playing a fundamental role in angiogenesis, and therefore cancer development. Two carboxylated polypyrrole carbon NTs (CPNTs) of differing diameters were synthesized. The first ranged between 190–220 nm and the second between 100–130 nm. Although both

CPNTs displayed a high degree of sensitivity in detecting VEGF, the smaller diameter exhibited approximately two fold higher sensitivity, emphasizing the role of tube size (Kwon et al., 2010). This is similar to the effect of wire diameter on the sensitivity of FET devices (Li et al., 2005). The improved sensitivity of the smaller tube diameter transducer was attributed to better conductivity as a result of the enlarged surface area exposed. In addition, rapid real-time detection adds advantages to the system, since there is no need for labelling samples (Chen et al., 2011). Kwon et al. (2010) were able to detect unmatched levels of VEGF, which can have massive repercussions in the early detection of several cancers. Furthermore, the biosensor system developed is reusable, a very attractive property in diagnostic platforms.

Prostate-specific membrane antigen (PSMA), a glycoprotein expressed in the prostatic epithelium endowed with enzymatic activity, is a promising diagnostic marker (Burger et al., 2002). Juzgado et al. (2017) reported on a ECL ELISA-like sensor based on carbon NTs combined with a specific sandwich immunoassay for the PSMA detection. The use of CNTs for an immunoassay ECL sensor for the detection of 4 different prostate cancer biomarkers was presented (Kadimisetty et al., 2015). They functionalized the CNT-based sensor transducer surface and to immobilize the recognition unit (e.g. the antibody).

The fabrication of an ECL immunosensor, based on the use of functionalized MWNTs (f-MWCNTs, Figure 10a), for the detection of PSMA in cell lysates has been reported (Juzgado et al., 2017). Amino groups were introduced onto the sidewalls of oxidized CNTs using a combination of amidation and diazonium radical coupling reactions. The amino groups at the CNT sidewalls were then derivatized

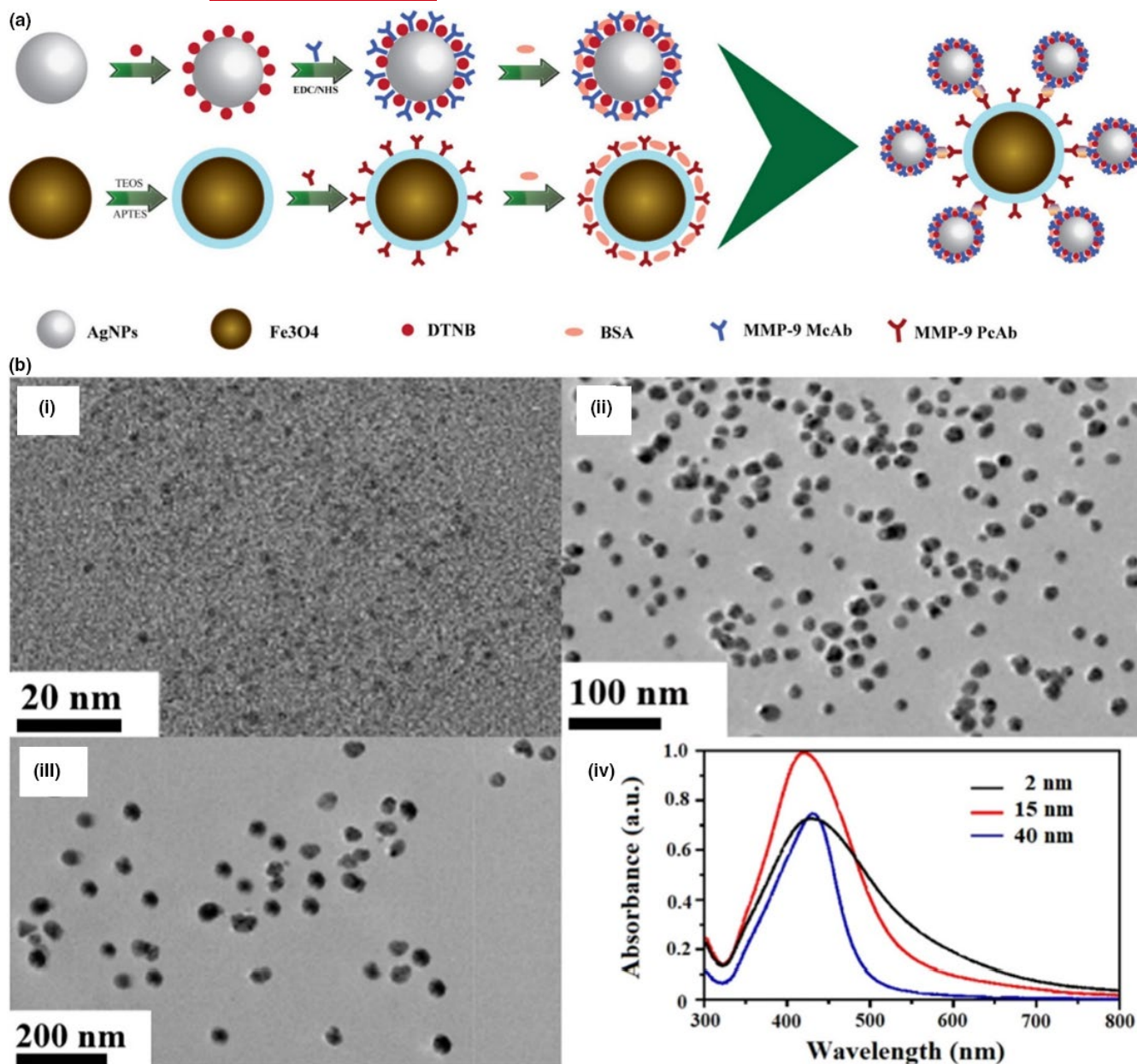


FIGURE 9 Detection of MMP-9 protein by SERS Immunoassay. (a) Schematic of SERS immunoassay: fabrication of SERS tags and of MMP-9 PcAb-conjugated magnetic beads, in addition to the resulting sandwich for SERS detection. (b) TEM images of (i) 2 nm AgNPs and (ii) 15 nm AgNPs and (iii) 40 nm AgNPs prepared by electrochemical method. (iv) UV-Vis absorption spectrum of AgNPs (Zhao et al., 2019). Figure reproduced with permission from Elsevier

to covalently bind the anti-PSMA monoclonal antibody (Ab 7E11c) used, while the maleimidic groups were exploited to anchor the immunoconjugate onto the surface of the electrode.

In order to highlight the specific role played by CNTs in the immunosensor performance, a second type of surface was prepared where the electrode modified with the electrografted PNSA (poly-(N-succinimide acrylate) film was directly incubated with the monoclonal antibody 7E11c, that is, without the intermediate CNT (Figure 10b). For the preparation of the control immunosensor, the same concentrations of 7E11c antibody as in the case of f-MWCNT@mAb were used. In this instance, significantly lower ECL signals were observed at all PSMA concentrations when compared to the device

containing CNT. This enhancement in the transduction signal intensity is the result of an increase of the surface coverage for the CNTs-based sensor.

Additionally, CNTs have also been used to improve the performance of graphite rod electrode in glycerol detection. These nanostructures increased the effective surface area of the working electrode, for enzyme immobilization and electron transfer, and in conjugation with tetrathiafulvalene the designed biosensor demonstrated a very high sensitivity ($29.2 \pm 0.9 \mu\text{A}/\text{mM}\cdot\text{cm}^2$) towards glycerol (linked to diabetes type II). The device displayed low LOD ($18 \mu\text{M}$), a linear range from 0.05 to 1.0 mM, high selectivity and satisfactory stability (Ramonas et al., 2019). In another example,

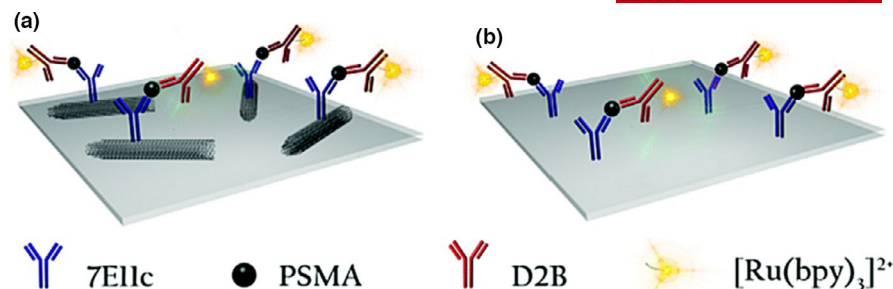


FIGURE 10 CNTs-based immunosensor. A schematic representation of the f-MWCNT@mAb immunosensor (a) and the control antibody-based immunosensor (b) developed in this work. Immunosenors are anchored to an optically transparent electrode covered by a polymer layer. PSMA antigen is specifically recognized by the capture antibody (7E11c, mAb) linked to the NTs or directly grafted onto the polymer surface. The quantification is performed by using the detection antibody (D2B, mAb@Ru) (Juzgado et al., 2017). Figure reproduced with permission from Royal Society of Chemistry

SWCNTs were used to improve the performance of microbe detection on a piezoelectric quartz crystal sensor, by acting as the electronic transducer and cross-linker for the immobilization of pleurocidin, an antimicrobial peptide. The sensor was able to detect microbes in 15 minutes. (Shi et al., 2017).

3.1.3 | Nanosheets

Graphene is the most used two-dimensional (2D) nanomaterial, which play an important role in the biosensing devices (Feng et al., 2012; Sadlowski et al., 2018; Wang et al., 2017) because of its exceptional physical, optical, electrochemical and magnetic properties (Klukova et al., 2016; Liu et al., 2012; Morales-Narváez & Merkoçi, 2018). Various kinds of graphene materials have been used in biosensors including pristine graphene, functionalized graphene such as graphene oxide (GO) (Yu et al., 2020), reduced graphene oxide (rGO) (Pei & Cheng, 2012) and graphene-based QDs (GQDs) (Moriyama et al., 2009). Graphene-based biosensors are organized in two main groups. The first, where functionalized graphene materials including GO and rGO (Peña-Bahamonde et al., 2018; Suvarnaphaet & Pechprasarn, 2017) are assembled onto the biosensor surface [electrode, FETs channel, etc.] to create an interface for improved assembling of molecular receptors. The second, where graphene materials are used for the construction of novel nanocomposites (Krishnan et al., 2019; Mao et al., 2010) to be used as electrodes. In this group, biosensor signal amplification and unique catalytic/chemical activity are used for sensitive protein biomarker analysis (Li et al., 2011, 2016).

In graphene sheets, the strong cross-linking between carboxylic acid groups on graphene materials and the amine groups of antibodies (COOH-NH_2) was used for immobilizing antibodies on novel biosensor interfaces (Mao et al., 2010; Zhang et al., 2013). The inclusion of graphene materials increased the loading amount, orientation and antibody binding capacity. For example, a graphene-modified sensor platform with increased surface area was developed, with immobilized antibodies assembled through COOH-NH_2 in combination with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and

N-hydroxysuccinimide (NHS), that achieved a low detection limit of 2 pg/ml (Li et al., 2011).

Graphene materials have been applied in FET biosensors, for the construction of 2D nano-FET biosensors (Ray et al., 2018; Xu et al., 2019), with several advantages such as higher amount of receptor biomolecules, low noise and high sensitivity, compared with 1D FET biosensors (Mukherjee et al., 2015). Graphene-based biosensors have been used for the detection of microRNA-21 (Kilic et al., 2015; Yin et al., 2012), and graphene-modified electrodes were used in ECL biosensor (Xu et al., 2011, 2015) for PSA detection. Carbon electrodes modified with a thionine-graphene composite were used to develop an electrochemical immunoassay sensor for the quantification of Bax protein in tumour cells (Xu et al., 2013) performance and easy handling into varied structures (flexibility) (Yang et al., 2018).

3.1.4 | Nanorods

Nanorods have also been used for improvement of biosensors performance, due to their unique properties and shape. Ahmad et al. (2017) reported grown vertically aligned ZnO NR decorated with CuO, on fluorine doped tin oxide (FTO) electrodes. This unique CuO-ZnO NR hybrid offers a large surface area and a penetrable substrate, thereby enabling improved electrochemical properties in glucose oxidation (Figure 11a). As a result, these fabricated electrodes (Figure 11b) exhibit high sensitivity ($2961.7 \mu\text{A}/\text{mM}\cdot\text{cm}^2$), linear range up to 8.45 mM, low LOD ($0.40 \mu\text{M}$), and short response time (<2 s) and applicability for glucose detection in human serum samples (Ahmad et al., 2017). In another study, ZnO NR powder surface-coated with carbon material was used as a working electrode for non-enzymatic glucose detection with low detection limit of 1 mM and a linear range from 0.1 to 10 mM was attained. The thin amorphous carbon layer (1 nm) is fundamental for the improving of sensing electrons and promoting the oxidation-redox reaction, since is the carbon decoration that makes ZnO react directly with glucose in the non-enzymatic detection. Therefore, is inferred that ZnO accelerate the electron transfer, while carbon film offers binding sites and electron transportation platforms for glucose (Chung et al., 2017). Taking into account the NR' advantages in electrochemical

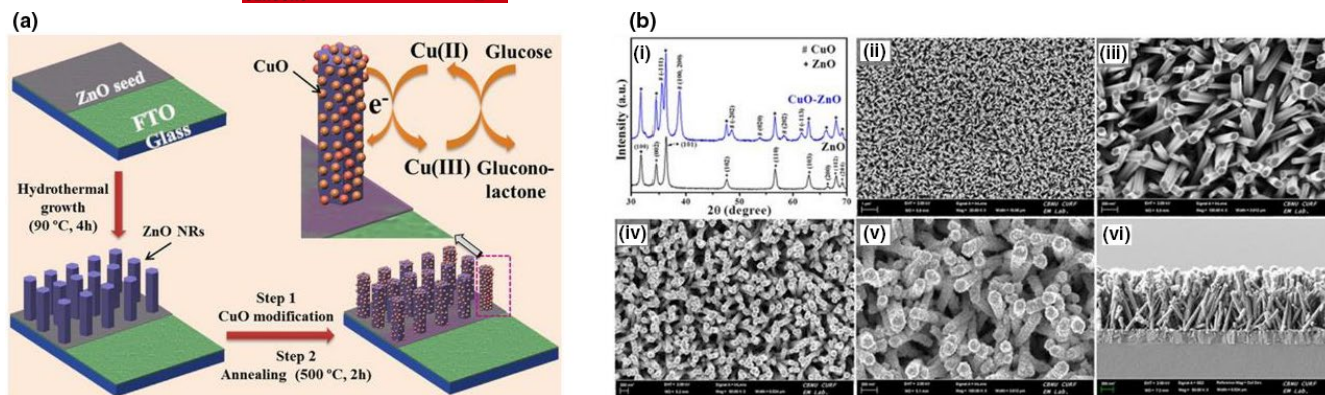


FIGURE 11 Glucose detection by a non-enzymatic glucose sensor. (a) Schematic of NR production and electrochemical detection. (b) NR characterization (i) XRD patterns of as-synthesized ZnO NRs and CuO modified ZnO NRs. (ii) Low- and (iii) high-resolution FESEM images of as-grown ZnO NRs. (iv) Low-, (v) high-resolution, and (vi) cross sectional FESEM view of CuO modified ZnO NRs (Ahmad et al., 2017). Figure adapted with permission from Nature Research

sensing, Zhao et al. (2016) developed a glucose biosensor based on ZnO NR on a chemically reduced graphene film. This system allows the electron transfer between glucose oxidase and the electrode due to the placement of ZnO NR between the redox centre of glucose oxidase and the electrode. With this approach, a biosensor with a sensitivity of 17.64 $\mu\text{A}/\text{mM}$ was achieved (Zhao et al., 2016).

3.1.5 | Quantum dots

Quantum dots are used in luminescence sensors, electrochemical, optical and photoelectrochemical (PEC) biosensors, such as ECL. Carbon and graphene QDs have been utilized in the fabrication of immunosensor. (Li, Jia, et al., 2017; Zhou et al., 2015). Desmin, an intermediate filaments protein type III, is one of the earliest expressed muscle-specific proteins. Its increased expression is also linked to colorectal cancer. Carbon QDs, quasi-spherical graphite NC with photoluminescence at about 455 nm, were used as an antibody substrate for desmin. The linear relation (in the range of 0.7–4.3 ng/ml) between desmin concentration and photoluminescence of carbon QDs makes these particles a potential biosensor for desmin detection (Li, Yan, et al., 2017). In another study, polyarginine-graphene QDs were used to modify glassy carbon working electrode. The QDs addition was transduced in a remarkable increase in surface area and therefore active sites for polyarginine to provide signal amplification. The detection of malondialdehyde, one of common biomarkers of oxidative stress, was achieved at a lower limit of quantification (0.329 nM). This was possible since polyarginine-graphene QDs accelerate the rate of electron transfer of malondialdehyde and have a good electroactivity (Runsewe et al., 2019) for redox reaction of the biomarker, enhancing its detection sensitivity (Hasanzadeh et al., 2017). Carbon QDs were embedded in a ZrHf metal-organic framework (ZrHf-MOF) which shows strong fluorescence and enhanced amino-functionalization. Carbon QDs-ZrHf-MOF was used as a scaffold for securing aptamer strands to detect human epidermal growth factor receptor-2 (HER2) and living HER2-overexpressed MCF-7

cells, and, this way, provide an early cancer detection. The addition of amino-functionalized carbon dots can not only enhance the binding ability of aptamer strands but also boost the electrochemical activity of electrode (Gu et al., 2019).

3.1.6 | Nanocomposites

Some studies combine different nanomaterial to gather in the same biosensing device different properties that contribute for further enhancement of performance. For example, ZnONRs-AuNPs nanohybrids have been produced and used for electrochemical detection of the ovarian cancer antigen CA-125/MUC126. Using cyclic voltammetry measurements, a detection of 2.5 ng/ μl was obtained, which is a 100-fold lower than the detection limit using an immunoblot system (Gasparotto et al., 2017). The Au NPs onto ZnO NR surface provides a favourable platform for efficient loading of anti-CA-125 antibody via binding with cystamine and glutaraldehyde. Researchers (Gasparotto et al., 2017) have tested that biomolecules immobilization on AuNPs increases the their stability and supports the maintenance of biomolecules activity. Besides, by using AuNPs the direct electron transfer between redox species and bulk electrode materials is facilitated, due to their conductive nature, which and improves the electrochemical sensing. ZnO nanostructures are suitable to immobilize and modify proteins due to its biocompatibility and biomimicry, as well as large specific area, high chemical stability and high isoelectric point (IEP ~ 9.5). Their semiconductor properties also offer an excellent channel for effective carrier transport during the redox process. The higher value of sensitivity in this biosensor is a result of high adsorption, effective antibody loading and possible fast electron communication characteristics of both ZnO nanostructures and AuNPs (Gasparotto et al., 2017). Another example of nanocomposites used in biosensors is a glassy carbon electrode modified with 3D graphene and hybrid Au nanocages (AuNCs)/amino-functionalized multiwalled CNTs (MWCNTs-NH₂). This composite device was used for label-free and

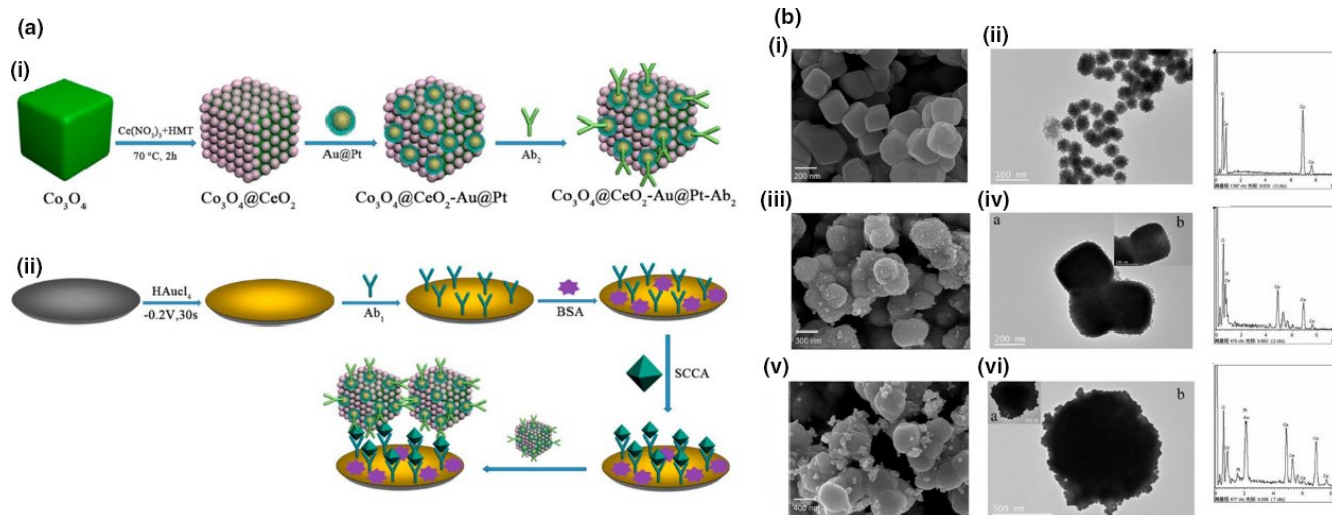


FIGURE 12 (a) Schematic of squamous cell carcinoma antigen detection using electrochemical detection. (i) Fabrication procedure of $\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt-Ab}_2$ label; (ii) The schematic of the sandwich-type electrochemical immunosensor; (b) Characterization of nanocomplex: SEM images of cubic Co_3O_4 NPs (i); $\text{Co}_3\text{O}_4@\text{CeO}_2$ NPs (iv); $\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt}$ NPs (vii); TEM images of Au@Pt NPs (ii); $\text{Co}_3\text{O}_4@\text{CeO}_2$ NPs (v); $\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt}$ NPs (viii); EDX spectra of Co_3O_4 NPs (iii); $\text{Co}_3\text{O}_4@\text{CeO}_2$ NPs (vi); $\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt}$ NPs (ix) (Li, Zhang, et al., 2017). Figure adapted with permission from Elsevier. Nanomaterial for multiplex detection

selective detection of MCF-7 breast cancer cells via differential pulse voltammetry. The fabricated device showed high specificity and sensitivity (in the range of 1.0×10^2 to 1.0×10^6 cells/ml, with LOD 80 cells/ml) for MCF-7 cells detection due to DNA-labelled antibodies and nanomaterial-based signal amplification. This approach combines analyte capture capacity with signal amplification in sandwich-type biomimetic interface. The use of amine-functionalized MWCNTs (MWCNTs-NH_2) as a support is attractive because these modified CNTs have useful properties, such as high electronic conductivity, chemical stability, high dispersibility in water and the ability to facilitate electron transfer to amplify the current signal and improve the stability of the biosensor. Furthermore, hollow AuNCs deliver larger specific surface areas and more exposed active sites, with simultaneous electrocatalytic activity (Yang, Fu, et al., 2018).

Other studies combined nanomaterial to act as labels, achieving superior levels of performance in electrochemical sensors. For example, Li, Zhang, et al. (2017) reported amino-functionalized cobalt oxide @ ceric dioxide nanocubes with core-shell morphology combined with sea-urchin like gold @ platinum NPs ($\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt}$) conjugate with secondary antibodies to act as labels for signal amplification (Figure 12). These nanomaterials showed outstanding electrochemical properties and improved auxiliary catalytic activity of $\text{Co}_3\text{O}_4@\text{CeO}_2\text{-Au@Pt}$. High electrocatalytic current responses led to the reduction of hydrogen peroxide (H_2O_2) and proved that this nanomaterial composite mimics enzymatic catalyses. In addition to the nanolabels, electrodeposited gold NPs (D-Au NPs) on glassy carbon electrodes were used as antibodies carriers in sensing platforms. This combination allowed a broad linear range of 100 fg ml^{-1} to 80 ng ml^{-1} with a low detection limit of 33 fg ml^{-1} for detection of squamous cell carcinoma antigen, a tumour biomarker of several cancers (Figure 12).

Recently, a surface modified with Au/Ag-rGO , and aminated GQDs (graphene QDs) and carboxyl QDs were combined to form an electrode. In this study, Au and Ag NPs were used for the adsorption of PSA antibody, and meanwhile, GQDs served for ECL signal amplification (Farid et al., 2015; Wu et al., 2016). Additionally, CQDs-based composites have been reported for the detection of microRNA (Hu et al., 2010; Liu, Ma, et al., 2017). GQDs in combination with AuNPs were employed to develop a FRET biosensor for detecting a gene sequence (Shi et al., 2015). An impedimetric immunosensor consisting of a mercaptopropionic acid-modified gold electrode functionalized with silica-coated gold NPs, CdSe QDs and the anti-CA-125 monoclonal antibody was used for the detection of the CA-125 serum biomarker in patients with ovarian cancer (Johari-Ahar et al., 2015).

QDs were also used to detect Leishmaniasis, a parasitic disease. The most common forms include cutaneous leishmaniasis. Surface antigens leishmania-specific were detected based on a method that combined magnetic beads and CdSe QDs with a specificity of 100% and a low LOD of $3,125 \text{ ng/}\mu\text{l}$ for Leishmania DNA (Andreadou et al., 2016).

The combination of nanomaterial arrangements has been employed, not only in electrochemical biosensors, but also in optical and electroluminescent sensors. For example, a novel surface plasmon resonance-based ECL sensor with DNA tetrahedral scaffolds as platform and multiple luminol modified AuNPs as enhancer was developed for the sensitive differentiation of cancer cells via telomerase activity (Feng, Zhou, et al., 2017). Proximal metallic NPs could quench the ECL emission of semiconductor QDs due to Förster energy transfer, but at a certain distance, the coupling of light emission with SPR result in enhanced ECL. Thus, the modification strategies and distances control between QDs and metallic NPs are critical for the ECL intensity of the QDs. The telomerase activity detection limit

was two orders of magnitude lower than commercial ELISA kits. The

a result of the introduction of PTCA into the luminol/ H_2O_2 ECL

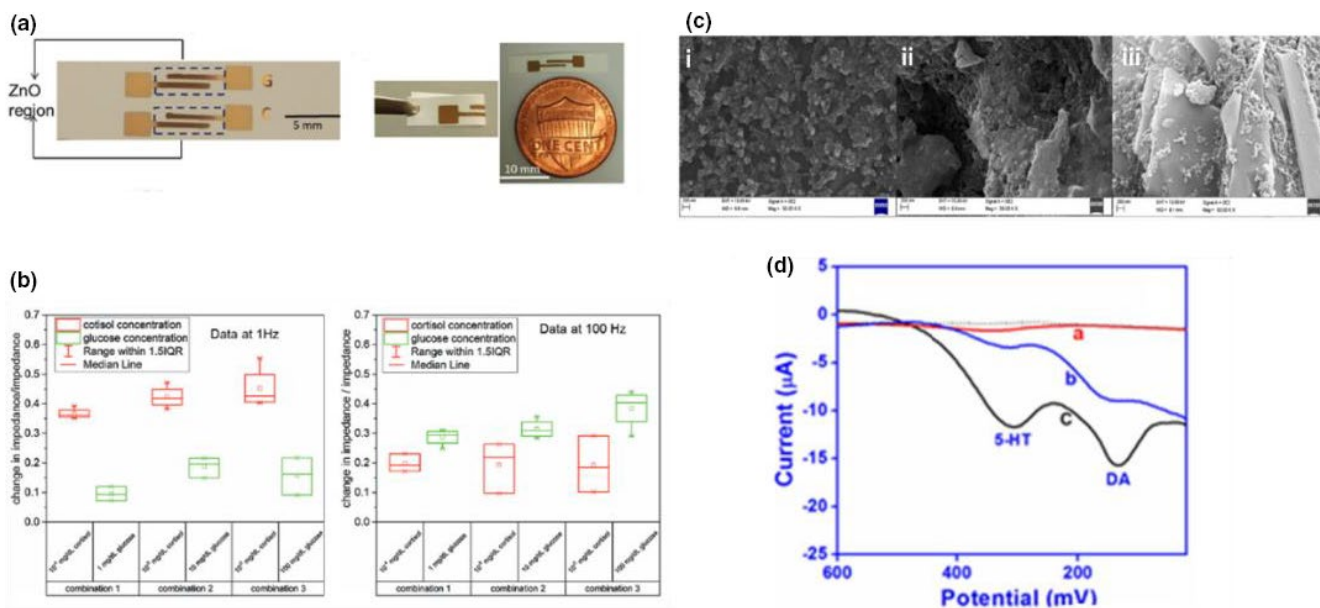


FIGURE 13 (a) Image of a sensor array deposited on polyamide substrate and its comparison with a cent; (b) combined detection of glucose and cortisol molecule with impedance measurements at 1 Hz and 100 Hz frequencies (collectively spiked glucose and cortisol concentrations as displayed on x-axis) (Munje et al., 2017); (c) SEM images observed for (i) unmodified SPCs (ii) graphene/SPCs and (iii) graphene/p-AHNSA/SPCs; (d) a comparison of square wave voltammetry observed for 50 μ M DA and 5-HT at unmodified SPCs (curve a), graphene/SPCs (curve b) and graphene/p-AHNSA/SPCs (curve c) at pH 7.2 (Raj et al., 2017). Figures adapted with permission from Elsevier

performance of the sensors was the result of tetrahedral scaffolds of DNA, modified with three thiol groups, anchored on the surface of cadmium sulphide QDs-GCE, building a uniform telomerase coverage on the electrode surface. The rigid pyramidal structure of DNA tetrahedron also caused the precise regulation of distance separation between AuNPs and QD. In addition, multiple luminol AuNPs assembled on DNA tetrahedral scaffolds caused a greater SPR-ECL signal which contributed to improvement sensors performance (Feng, Zhou, et al., 2017).

Other authors (Heidari et al., 2019) used a similar strategy for detection of p53 protein, which can be used in early detection of cancer, monitoring both the cancer progress and clinical prognosis and achieving a dynamic range of 20 and 1000 fg/ml with a calculated LOD of 4 fg/ml. In this approach, cadmium sulphide NC were immobilized on the glassy carbon electrode and anti-p53 antibody were conjugated to AuNPs and introduced to the process through formation of a sandwich-type immunocomplex. The ECL of cadmium sulphide NC evoked the surface plasmon resonance of AuNPs which in return amplified the CdS NCs ECL intensity (Heidari et al., 2019). Another approach for performance enhancement in ECL sensors relied on PdCu nanocubes supported single-walled carbon nanohorns (PdCu@SWCNHs) immobilized with 3,4,9,10-perylene-tetracarboxylic acid (PTCA) conjugated luminol, forming a novel self-catalysed luminescence emitter. This immunosensor exhibited a wide linear detection range of 0.1 to 25 ng/ml with a relatively low detection limit of 0.05 pg/ml, in clinical serum samples. This performance is

system, responsible for the increment of the luminescence signal, stability and hydrophilicity of pristine SWCNHs. Moreover, PdCu@SWCNHs nanohybrid showed superior catalytic activity towards H_2O_2 that could further amplify the ECL signal of luminol/ H_2O_2 system. Additionally, the good biocompatibility and high specific surface area of PdCu nanocubes allowed successful immobilization of detection antibody (Liu, Wang, et al., 2017).

Electrochemiluminescence/Electrogenerated chemiluminescence immunosensors based on modified transducers embedding metal and silica NPs, (Valenti et al., 2016) magnetic NPs (Gandhi et al., 2016) and NW (Ma et al., 2016), were shown for instance to reach extremely low detection limits. A fluorogenic sensing assay for L-Cysteine detection in MKN-45 and Colo-205 cancer live cells with LOD of 0.51 nM was employed based on a gelatin stabilized gold nanoparticle decorated with reduced graphene oxide (rGO/Au) nanohybrid for the development of an optical sensor. The sensing mechanism is based on the fluorescence recovery due to the stronger interaction between Au NPs and L-Cys, and consequently, the prevention of energy transfer between rGO and Au NPs (Thirumalraj et al., 2018).

High-throughput analysis systems are fundamental for current healthcare needs, since more information in shorter time is necessary for an accurate diagnosis and early treatment. To address this challenge, biosensors with the ability of detect, simultaneously, multiple and different analytes, and with high sensitivity, in the same sample had been developed (KK & Chong, 2011). This capability is

extremely important in the diagnosis of diseases that required the screening of several biomarkers, whose marker levels can have a very low concentration. As these target molecules exist at the nanoscale, biosensors with similar dimensions may allow a better integration between nanotechnology and biology, leading for faster diagnostics and more target therapeutics (Romeo et al., 2016; Wang et al., 2006). The ability to perform simultaneous multiple detection of biomarkers is desired and developed by all the diagnostic technologies such as POC, wearable and implantable.

Munje et al. (2017) developed a nanoporous flexible polyamide substrate with a region with zinc oxide thin films (Figure 13a), as the active region, for the detection of cortisol and glucose, since these two molecules have physiological interconnection and are directly correlated with type II diabetes. The use of the nanoporous polyamide substrate allows for nanoconfinement of biomolecules, which affect the electron transfer kinetics inside the nanopore. Thus, the mass transportation within the nanopores is impacted and the diffusion time is reduced, leading to a high surface-to-volume ratio, enhancing the detection. Nanoporous surfaces also increase selectivity, through the size-based exclusion, which reduces the noise of complex environment and stability of biomolecules captured inside a nanopore. The results of electrochemical detection of cortisol and glucose in synthetic sweat are demonstrated in Figure 13b, at 1 and 100 Hz.

Using a different approach, Raj et al. (2017) developed a biosensor for dopamine (DA) and serotonin (5-hydroxytryptamine) (5-HT). As previously mentioned, dysregulation of dopaminergic transmission

has been associated with neurological disorders such as Parkinson's disease, Schizophrenia and Tourette's syndrome, among others. On the other hand, serotonin is involved in several gastrointestinal disorders like secretion, irritable bowel syndrome, food hypersensitivity and inflammatory bowel disease. It was demonstrated that the respective release of DA and 5-HT influence each other. For the DA and 5-HT simultaneous detection, graphene and a conducting polymer of 4-amino-3-hydroxy-1-naphthalenesulfonic acid (p-AHNSA) composite modified screen-printed carbon sensor (SPCs) was developed. The application of conducting polymer for modification of the surface of the electrode has been found to be as a superior strategy to increase electrical conductivity and chemical stability. The microscopic analysis (Figure 13c) of the graphene-polymer nanocomposite film showed that the p-AHNSA was accumulated on the SPCs surface with nanorod shaped particles, which interacted with graphene and formed a porous surface. This nanocomposite network is uniformly constructed through the entire surface and provides a larger electroactive surface area in comparison to the unmodified sensor, promoting an easy and efficient electron transfer across the film.

For simultaneous determination of DA and 5-HT, a mixture of both components in a pH 7.2 phosphate buffer was used and unmodified SPCs, graphene/SPCs and graphene/p-AHNSA/SPCs was electrochemically tested. As observed in Figure 13d, in unmodified SPCs, the oxidation peak current of DA and 5-HT are overlapping, making accurate detection difficult. Instead for p-AHNSA or graphene-modified SPCs, two peaks for DA and 5-HT were detected.

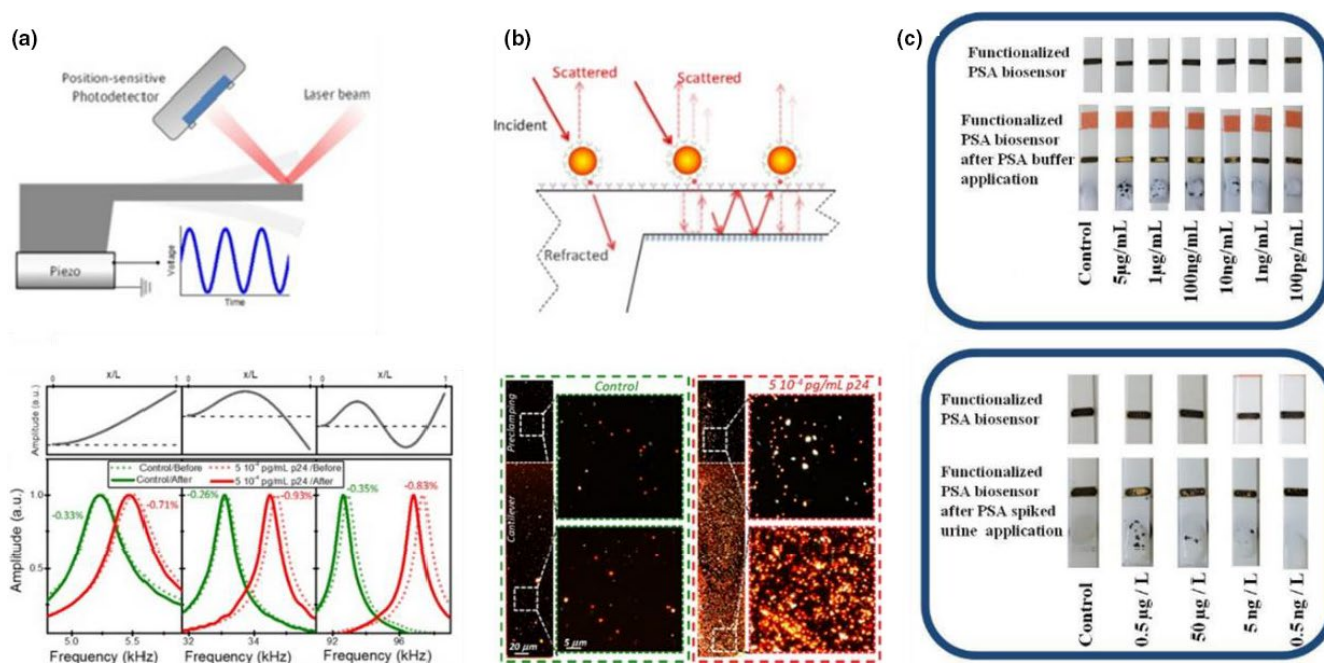


FIGURE 14 (a) Schematics of the optical beam deflection method for measuring the microcantilever vibration and the resonance frequency peaks (graph below) of a silicon cantilever. The dotted lines represent the signal before and the solid lines the signal after the immunoreaction assay for the control (human serum) and for 5×10^{-4} pg/ml of p24 in human serum; (b) Schematics of the multiple pathways for light scattering by the gold NPs bound to the microcantilever and optical images of the microcantilevers after the immunoassays (Kosaka et al., 2017) and (c) results of the biosensing of different concentrations of PSA in buffer (top) and in urine (bottom) (Suaifan & Zourob, 2017). Images reproduced with permission from Public Library of Science and Elsevier

The LODs and dynamic ranges were 2 and 3 nM, 0.05–100 μ M and 0.05–150 μ M for DA and 5-HT in graphene/p-AHNSA/SPCs respectively (Raj et al., 2017). Although some preliminary studies in human plasma samples were made, this biosensor needs more work until a simultaneous DA and 5-HT can be detected in human samples.

By functionalizing carbon dots with three different receptors, Zheng et al. (2019) showed that multiple bacteria can bind to them with different affinity. Using a mathematical statistical method 'liner discriminant analysis (LDA)', they were able to discriminate six kinds of bacteria through their response pattern produced from the variable fluorescence decrease of the carbon dots due to the binding ability differences of the bacteria.

Three independent SiNW biosensors with three different antibodies (PSA, carcinoembryonic antigen (CEA) and mucin-1) immobilized on them were developed (Stern et al., 2007). The conductance-versus-time measurements offered simultaneous monitoring of PSA, CEA, and mucin-1, that were loaded sequentially to the SiNW FET arrays. The multiplex device revealed specific since the addition of serum containing PSA only resulted in a concentration-dependent conductance increase for NW1, containing immobilized antibody for PSA, and not in the other NWs (Stern et al., 2010).

Wang et al. (2020) demonstrated that in the presence of target miRNAs, duplex-specific nuclease (DSN)-assisted target recycling can be triggered, resulting in the release of QD and recycling of target miRNAs. MNs are linked with dual-colour QD through single-stranded DNAs (ssDNA) that are complementary with miR296 and miR-16, respectively. In the presence of target miRNAs, the forming miRNA/ssDNA hetero-duplexes will be simultaneously cleaved by DSN, causing the release of target miRNAs and QD into the suspension. With the subsequent target recycling, simultaneous quantification of miR-296 and miR-16 can be realized by merely recording fluorescence of QD in the suspension under single excitation light. The whole simultaneous detection can be realized by merely mixing the multifunctional nanocomposites with the samples. Then, the simultaneous quantification was realized by recording the corresponding amplified fluorescence signal of QD in the suspension. Femtomolar sensitivity and improved specificity was obtained with the reported method in the simultaneous detection of hsa-miRNA-296-5p and hsa-miRNA-16-5p.

Multiplex detection of biomarkers is essential not only needed for precise diagnostics and further treatment, but also for high-throughput diagnostic platforms. Therefore, quantifying simultaneously different biomarkers in several samples is a current challenge that nanomaterial-enhanced sensors are trying to solve, either for POC, wearable or implantable technologies.

3.2 | Nanomaterial for point-of-care diagnostics devices

A better healthcare management can be achieved with quick diagnostics and appropriate data analysis, making point-of-care (POC) devices imperative in the development of smart diagnostic systems

for personalized health care. POC devices perform fast and accurate diagnostics. These devices should be user-friendly, low-cost, sensitive, selective and robust. Another important feature of POC devices is the usage of mass production methods for device manufacturing (Barbosa & Reis, 2017). There are several methodologies to produce a signal in POC devices, and they often depend on the transducer used. Thus, optical and electrochemical are the most common POC devices, examples of which are pregnancy tests and glucose metre, respectively. POC devices were integrated with nanomaterial in different sensing platforms, improving their performance and offering innovative detection systems. Various POC devices have been developed in recent years, paving the way to next-generation of POC testing (Noah & Ndangili, 2019; Quesada-González & Merkoçi, 2018).

In order to achieve an early detection of HIV, it was developed a sandwich immunoassay based on nanomechanical and optomechanical transduction for the detection of p24 antigen (Kosaka et al., 2017). The immunoreactions occurred in the surface of a single crystal silicon microcantilever where AuNPs were used as mechanical and plasmonic labels. The use of AuNPs presented some advantages, like excellent electrical conductivity, ease of surface modification and synthesis, and good biocompatibility. The changes in the AuNPs were detected by the microcantilever, which acts as a mechanical resonator, weighing the mass of NPs and as an optical cavity, for the plasmonic signal created by NPs. With this approach, signal detection can be achieved mechanically or optically. In the first method, the microcantilever vibration is measured by the scanning optical laser beam deflection technique. In the second method, when NPs are bounded to the microcantilever, the optical cavity modes and the localized surface plasmon modes couple each other at particular frequencies, forming a hybrid plasmonic supermode, which enhances the scattering of the NPs (Figure 14a, b). With this work, authors (Kosaka et al., 2017) achieved a detection limit of 10^{-17} g/ml, which is two orders of magnitude below that detected by standard methods (nucleic acid amplification). Additionally, this technology claims the possibility of miniaturization with upscale methods for POC applications. However, the detection of antigen p24 was performed in human serum, needing to be validated in blood (Kosaka et al., 2017).

Suaifan and Zourob (2017) also took advantage of NPs and developed an electrochemical and optical biosensor for PSA detection. The biosensor analyte was created by covalently binding PSA-specific peptide substrate, through its N-terminus, with a carboxyle-terminated magnetic -NPs to a gold sensing platform. The link between NPs-peptide and the sensor platform was then eradicated. An external magnetic field was applied, and NPs-peptide were attracted away from the gold sensing platform, changing its properties, as the impedance or the colour. These changes were measured by electrochemical impedimetric spectroscopy and by optical techniques like surface plasmon resonance and colorimetric methods, as observed in Figure 14c. A gradual decrease in the charge transfer resistance was observed with the application of different PSA concentrations. This reduction might be due to the variation of the self-assembled monolayer structure due to NPs-peptide moieties

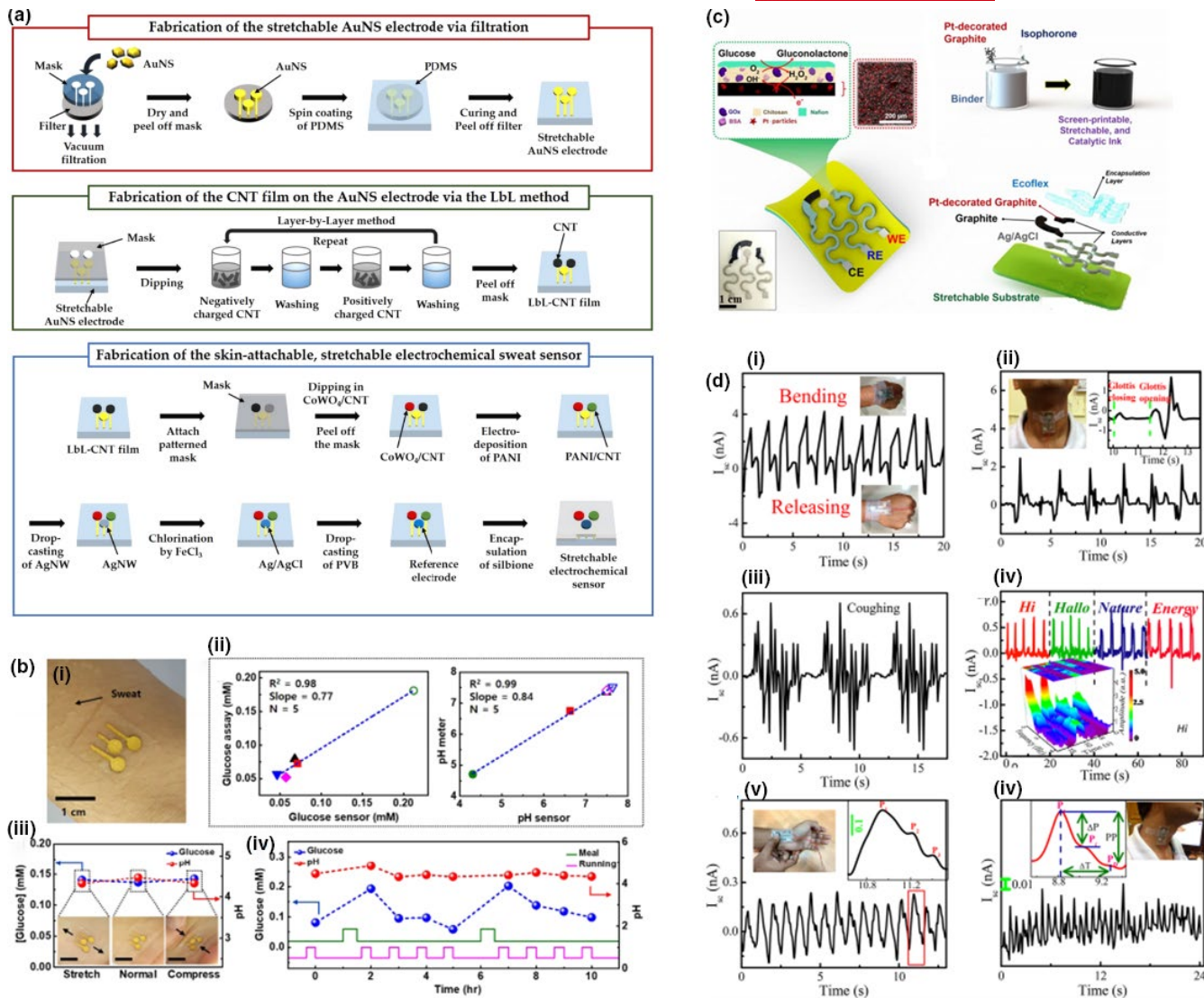


FIGURE 15 (a) Diagram showing the fabrication of the flexible, skin-attachable, electrochemical sweat sensor for glucose and pH detection; (b) (i) Photographic image of the electrochemical sensor attached to the skin wet with sweat, (ii) Correlation of glucose concentration (left) and pH (right) measured by the developed biosensors (x-axis) with those available commercially (y-axis) in human sweat, (iii) performance of the biosensor attached to the skin in human sweat under mechanical deformation and (iv) change of glucose concentration and pH measured by the developed sensor attached onto skin along with different activities of meal injection and running for 10 h; (Oh et al., 2018) (c) Schematic of the stretchable sensor design, the enzymatic immobilization process, the catalytic ink and screen-printing process (Abellán-Llobregat et al., 2017); (d) Time-dependent current responses during (i) repeated bending–releasing cycles of the wrist, (ii) swallowing motions, (iii) repeated coughing actions, (iv) speaking of different words, (v) monitoring radial artery and (vi) carotid artery pulses (Ghosh & Mandal, 2017). Figures reproduced with permission from ACS publications and Elsevier

dissociating from the sensing platform, upon PSA application. The elimination of the blocking and washing steps has made the screen-printed electrode and paper-based low-cost POC biosensor a possibility. However, more work is necessary to miniaturize and scale up production methods, for use as a commercial POC device for early diagnosis of prostate cancer.

New technologies based on microfluidic paper-based analytical devices, incorporating MWCNTs were recently proposed for the detection of cancer markers (e.g., cancer antigen 125 (CA125) and carcinoembryonic antigen (CA)) (Wang et al., 2012). The MWCNTs were functionalized using carboxylic groups and coated with chitosan.

This enabled their integration with emerging paper electronics and provides low-cost, and disposable portable devices for POC testing. A linear range for CA125 (0.001–75 U/ml), and for CA (0.05–50 ng/ml) detection, which are much lower than the normal levels in real serum samples of the two cancer markers of 1.7–32 U/ml, and 0–10 ng/ml, were reported (Wang et al., 2012). Chikkaveeraiah et al. (2009) reported a SWCNT-based sensor to measure four prostate cancer biomarkers in cancer patient serum simultaneously using an ECL immunoarray. The device provides adequate sensitivities within the normal clinically relevant levels for each biomarker, in addition to their real-time measurement. This device has the potential for

application as POC diagnostic assays. Recently, Wan et al. (2011) developed an ECL immunosensor for the simultaneous detection of PSA and interleukin-8 (IL-8) with a screen-printed carbon electrode based on MWCNTs as detection platform. They reported a LOD (LOD) of 5 pg/ml for PSA and 8 pg/ml for IL-8, suggesting also a multiplexing capability, making this immunosensor a promising approach for POC testing in clinical diagnostics.

3.3 | Nanomaterial for wearable diagnostics devices

Advanced wearable electronic devices, which can be worn or attached onto skin, had gain an increased interest in healthcare management, fitness and biomedicine, and it is expected to be expanded even further in the years ahead (Jayathilaka et al., 2019). Significant advances in wearable biosensors able to monitor the patient's physiological state have been reported (Rodrigues et al., 2020). Since the tendency is to move towards integrated wearables, the conversion of actual devices into integrated devices involves a significant size reduction, while retaining their functional capabilities. Thus, nanomaterial play a huge role in the development of new wearable biosensors (Jayathilaka et al., 2019; Yang et al., 2017).

One of the biggest challenges in the development of wearable biosensors is reliability and un-interrupted monitoring of signals. Human epidermis is constantly exposed to bending and stretching movements during common physical activity. The excessive noise caused by the skin barrier, in addition to skin irritation and discomfort has an adverse effect on these devices. Taking this into account, a stretchable and skin-attachable electrochemical sensor for detecting glucose and pH in sweat was developed (Oh et al., 2018). Stretchable electrodes were fabricated by layer-by-layer deposition of CNTs on top of patterned Au nanosheets (AuNS) prepared by filtration onto stretchable substrate (Figure 15a). The use of nanosheets and NTs allows the detection of small changes, increasing the sensitivity of the final biosensor.

Graphene-based flexible composites with different materials and structures have resulted in excellent pressure sensors (Lv et al., 2017). Graphene has also been used as a component of nanosheets-based stretchable sensors for human motion detecting and monitoring. Human motions and physical activities at different sites of the human body generate a wide range of crucial signals such as movement disorders, respiratory disorders and blood pressure (Trung et al., 2016), which could be captured by highly sensitive pressure, tactile or strain sensors for continuous monitoring. Graphene and its derivatives have found usage in pressure sensors because of their significant piezoresistive (or piezocapacitive) properties.

Hydrothermally synthesized cobalt tungstate (CoWO_4)/CNTs nanocomposite, with high conductivity and large surface area, were used as working electrode on top of CNTs/AuNS, for glucose detection, without use of any enzyme (Oh et al., 2018). Polyaniline (PANI)/CNTs, prepared via electropolymerization, were used for

pH detection. Additionally, silver nanowire was deposited on the AuNS electrode to produce the solid-state stretchable reference electrode. The developed electrochemical sensor was encapsulated in a sticky polymer (silbione) and was conformably attached onto skin. The monitoring results show highly selective sensitivity, with readings of $10.89 \mu\text{A}/\text{mM}\cdot\text{cm}^2$ to glucose and $71.44 \text{mV}/\text{pH}$ with pH in sweat. The results were consistent even under repetitive deformations of stretching by 30%. During glucose and pH sensing, there was no observed interference from other chemical components and ions existing in the human sweat (Figure 15b). Long-term stability of the sensor was performed, without any notable deterioration of the sensitivity of either glucose or pH. This study was only conducted for 10 days. Continuous monitoring of the change in glucose concentration and pH due to meal ingestion and running could be tested with this biosensor, since values could be stably detected in sweat after exercising, and regardless of the skin movements, surpassing the major drawbacks of actual wearable biosensors (Oh et al., 2018).

Abellán-Llobregat et al. (2017) developed two distinct stick-on platinum nanoparticle (PtNPs)-based stretchable electrochemical biosensors with the objective of measure glucose in the sweat. These authors used Pt-decorated graphite as the ink filler (Figure 15c). Additionally, PtNPs have high electroactivity, even with if the amount of Pt on the electrode is low, thus reducing the cost of the biosensor. One of the developed devices was enzyme-free, while the other took advantage of the PtNPs system to detect H_2O_2 at less positive potentials; thus, minimizing, the interference of electroactive species, such as ascorbic acid or uric acid, which are electroactive at more positive potentials and can interfere in glucose detection. Both biosensors were tested with glucose in phosphate buffer. The enzyme-free biosensor detection was carried out by recording the glucose oxidation current by chronoamperometry. The results obtained shown a sensitivity of $0.69 \pm 0.06 \mu\text{A}/\text{mM}\cdot\text{cm}^2$ and a LOD of 6.6 mM.

The enzymatic detection using biosensor was performed after immobilization of glucose oxidase (GOx) on a Pt-decorated graphite electrode. Glucose quantification was detected by measuring the current as a result H_2O_2 reduction also by chronoamperometry. This greatly improved the sensitivity levels obtained with the enzyme-free sensor. Stretchability was also tested to understand the influence of stress-strain on glucose detection. Results shown that the GOx/Pt-graphite biosensor can be stretched up to $75 \pm 1.3\%$ of its original size. Although authors claim a significant correlation between GOx/Pt-graphite-based biosensor in human perspiration and human blood with a commercial glucose metre, the measurements were taken in a glass container and not in the skin. Thus, this biosensor needs to be studied in human skin before it can be elected as a wearable biosensor.

The development of wearable biosensors to be used directly onto the epidermis requires substrates with properties similar to the human skin, like biocompatibility and air permeability, among others. Despite the efforts to produce flexible wearable sensors, most of the reported sensors still present limited air permeability,

reducing the potential for continuous physiological detection, due to inflammation of skin (Khan et al., 2019). Thus, Yang, Li, et al. (2018) developed a breathable pressure sensor based on poly(vinylidene fluoride) nanofibre membranes and silver NW. The porous structure of the nanofibre membrane allows the air permeability and excellent breathability (Gurley value =17.3 s/100 ml, that is, there are necessary 17.3 seconds for 100 ml of air to pass through a specified area of a separator under a specified pressure), making this a potential sensor for continuous monitorization of human physiological signals, such as respiration and heart rate (HR). For that, the sensor could be attached, for instance, in a face mask. Additionally, the sensor's biocompatibility was assessed through its attachment, for 24 h, to the forearm, showing no allergic reaction.

Another challenge in wearable biosensors development is their power supply. The commonly used separated power source has some inherent disadvantages, like joule heating effect. Thus, the development of self-powered wearable biosensors is necessary.

In order to surpass this limitation, Ghosh et al. (2017) developed a self-powered wearable bio-inspired piezoelectric biosensor, capable of monitoring real-time human physiological signals such as arterial pulses, vocal cord vibration and gentle wrist movements. For this fish skins waste were used as it is composed of collagen nanofibrils, which is a biocompatible and biodegradable piezoelectric polymer. The piezoelectricity properties are due to the presence of hydrogen bonding within the polypeptide chains. Due to the rich

hydrogen bonding network of the stable crystalline structure of the collagen nanofibrils, they present superior dielectric property compared to other available biopolymers. The dielectric properties are mainly influenced by trapping of free charges between fibres and air pores, known as Maxwell–Wagner–Sillars interfacial polarization effect (Samet et al., 2015). Taking this into account, authors developed a fish skin-based nanogenerator able to behave as an ultrasensitive, highly durable pressure sensor, which successfully monitors real-time human physiological signals, as observed in Figure 15d. Furthermore, the fish skin-based nanogenerator produces sufficient output power to operate consumer electronics under human perception (Ghosh & Mandal, 2017). The electricity generation capability of this wearable biosensor, harvesting the bio-mechanical energy with longer endurance makes it valuable for a range of applications in continuous healthcare monitoring.

3.4 | Nanomaterial for implantable diagnostic devices

Implantable biosensors can be an extraordinary tool to achieve early diagnostics and to continuously monitoring a disease. The understanding of the physiology and the pathological processes in organisms, especially in humans, can be aided by in vivo sensors. However, implantable biosensors are far away from mass commercialization,

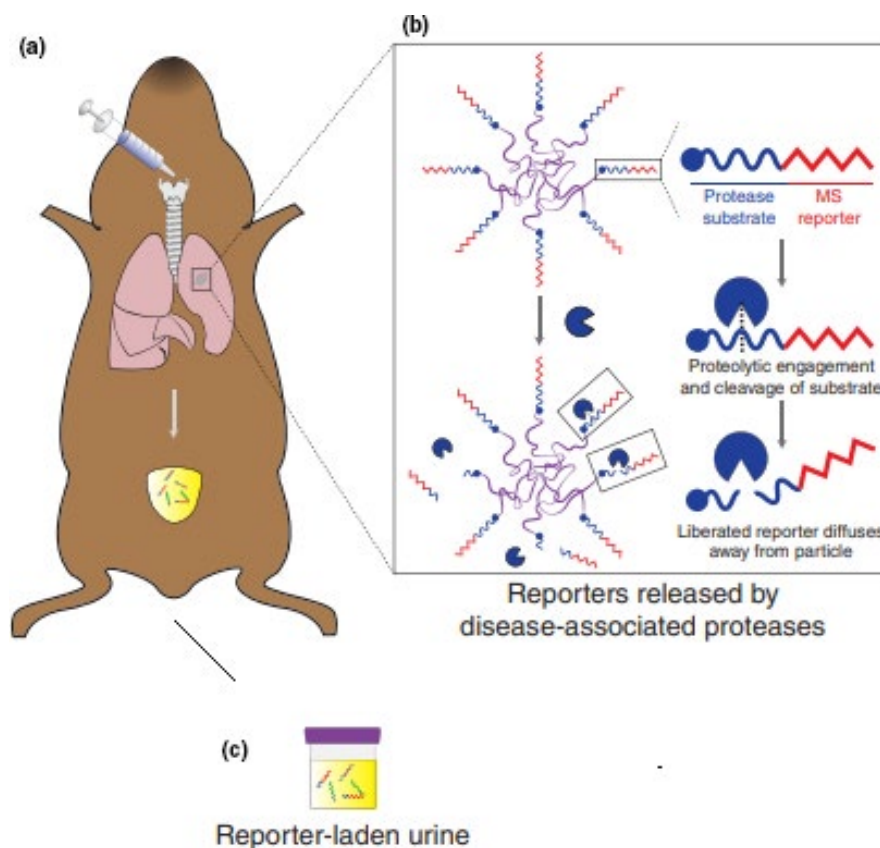


FIGURE 16 Schematic showing urine testing in a mouse flank tumour model: (a) intravenous injection; (b) renal clearance and (c) urine collection (Kirkpatrick et al., 2020). Figure adapted with permission from AAAS

since they still present some challenges that need to be addressed. The biggest challenge corresponds to the foreign body response, but characteristics like porosity, surface chemistry, size, roughness or degradation time are also major limitations.

Implantable nanomaterial-enhanced sensors are emerging as a possibility to overcome these limitations, taking advantage of their size and unique properties (Rebello et al., 2019; Shafiee et al., 2019). For example, in spite of the tumour detection tools available, clinical detection of tumours remains a challenge. Currently, tumour sizes of 1 cm diameter are detected using imaging techniques or by analysing the blood markers shed by the tumour and can take up to 10 years to reach this size (Kwon et al., 2017). An activity-based nanosensor using the protease activity in a tumour was able to shed peptide fragments concentrated in urine. (Kwong et al., 2013; Warren et al., 2014). These peptides (tumour-penetrating ligands that engage active tumour trafficking pathways initiated by receptor binding) were conjugated to iron oxide NPs. These NPs were injected into mice whose urine was collected. The size of tumour in the urine was measured. The process is schematically illustrated below (Figure 16). The results indicate that the difference in tumour volumes between detection via application of HE4 blood serum ELISA (blood biomarker) and tumour-penetrating ABNs was 2.4-fold, that is, smaller size tumour can be detected by ABN's (Loynachan et al., 2019).

In vivo sensing explored gold nanoclusters (AuNCLs) intrinsic catalytic activity in a multifunctional protease nanosensor. This device, which responded to disease microenvironments, was designed using biotinylated protease-cleavable peptides to template and

stabilize the growth of catalytic AuNCLs. These were further coupled to neutravidin (NAv), due to its high affinity for biotin.

The AuNCLs-NAv complex was intravenously administered into mice (Loynachan et al., 2019). The complex was specifically dis-assembled by proteases at the site of disease. These proteolytically liberated AuNCLs circulated via the bloodstream and were efficiently filtered into the urine through the kidneys due to their small size (<5 nm). Urine was tested with AuNCLs using a colorimetric assay for indication of disease state. The NPs showed stability in physiological environments as they retained their catalytic activity. These complexes avoided non-specific protein adsorption. These complexes were used in vivo detection in a colorectal cancer mouse model and successfully detected AuNCLs in urine from tumour-bearing mice with a facile colorimetric readout.

Carbon nanotubes are widely used in electrochemical biosensors due to their unique electrical properties, allied with their high sensitivity, fast response, easy operation and favourable portability (Sireesha et al., 2018). Zhang et al. (2017) engineered tunable defects and oxygen-containing species in CNTs, developing an aligned carbon nanotube fibre (CNF) as an electrochemical biosensor for the ratiometric detection of ascorbic acid levels in live rat brains with Alzheimer's disease. At a low potential, the fibre surface facilitates the ascorbic acid oxidation, leading to higher sensitivity and selectivity and avoiding interference from other species that exists in brain. In order to evaluate the in vivo performance of these biosensors, they were implanted in a rat brain with and without Alzheimer's disease. The electrochemical results obtained by the implantable biosensors can be observed in Figure 17a. Two separate oxidation

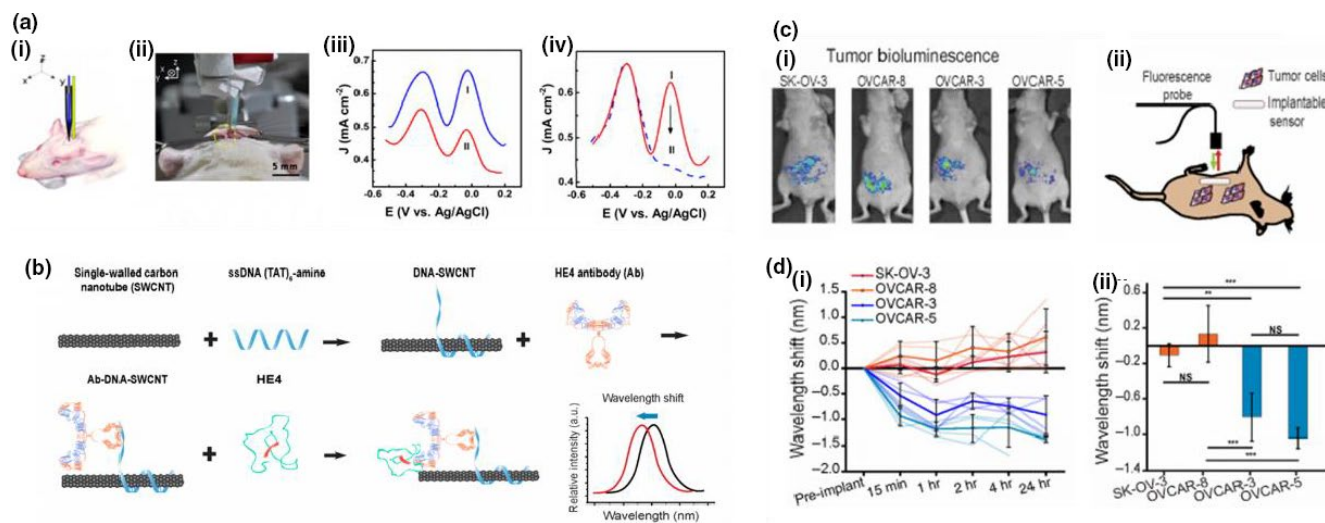


FIGURE 17 (a) (i) Diagram showing the in vivo setup for determining ascorbic acid in rat brain; (ii) optical images before and after the stereotaxic implant into the brain; differential pulse voltammetry recorded at (iii) the normal rat (blue) and rat brain models of Alzheimer's diseases (red) and at (iv) the rat brain model of Alzheimer's diseases before (red) and after (blue) injection of ascorbate oxidase (Zhang et al., 2017); (b) Schematic of CNTs-based biosensor synthesis; (c) (i) representative bioluminescence images denoting tumour burden in the peritoneal cavity of nude mice inoculated with luciferase-expressing cell lines; (ii) method diagram of HE4 measurement in live tumour-bearing mice; (d) (i) Variation in sensor emission centre wavelength after implantation into mice with four different orthotopic intraperitoneal tumour models, where the lighter lines represent traces from the emission within each mouse. (ii) Sensor response from all mice at 60 min after implantation. Cells of four different luciferase-expressing cell lines were used (Williams et al., 2018). Figures reproduced with permission from ACS publications and AAAs

TABLE 3 List of completed and ongoing clinical trials nanosensors for diagnostics applications. Information obtained from: <http://clinicaltrials.gov>

NCT number	Date and status	Study aim	Patients age	Procedure/Samples
NCT04074629	2019–2023 Recruiting	To study the plausibility of skin-based volatile organic compounds (VOCs) as biomarkers for multiple sclerosis diagnosis	18–75 yrs	The skin related VOCs will be collected by 'off-line' method using polydimethylsiloxane patches from different body locations for sensor system
NCT04086329	2019–2021 Not yet Recruiting	To measure the efficacy of an electrochemical oxygen nanosensor to measure in vivo mitochondrial function in human muscle tissue, and its ability to discriminate mitochondrial myopathy patients from healthy volunteers	18–65 yrs	Placement of the sterilized nanosensor involves a small incision for manual placement of the nanosensor in muscle forearm tissue
NCT04119154	2019–2021 Recruiting	Prospective feasibility and validation study of a novel contrast microhalography (CEM) device for diagnosis of malignancy in Botswana	≥18 yrs	Fine needle aspirates evaluated by CEM device
NCT03228095	2017–2025 Enrolling by invitation	To test VOCs detecting technologies as diagnostic and monitoring tools for digestive tract and infectious diseases	≥18 yrs	Plasma/serum samples will be used to obtain information
NCT02332213	2015–2017 Completed	To address the role of confounding factors, including lifestyle factors, diet, smoking as well as addressing the potential role of microbiota in the composition of exhaled volatile markers.	≥18 yrs	Plasma/serum samples will be used to obtain information
NCT01291342	2011–2012 Completed	Early detection of 'Pre-eclampsia' and other pregnancy complications using a simple and inexpensive toll termed NA-NOSE.	18–75 yrs	Volatile biomarkers appearing in exhaled breath and/or blood samples
NCT04022109	2019–2026 Not yet Recruiting	To determine the potential of volatile marker testing for gastric cancer screening	≥18 yrs	Volatile biomarkers appearing in exhaled breath and/or gastric contents.
NCT01420588	2011–2020 Completed	To study the feasibility of a novel method in oncology based on breath analysis with a nanosensors array for identifying gastric diseases	18–75 yrs	Volatile biomarkers appearing in exhaled breath and/or saliva

peaks were clearly observed at approximately -290 and -60 mV at the CNF microelectrode in the normal rat brain, whereas only one peak was obtained at -290 mV in pure artificial cerebrospinal fluid (used as control), suggesting that ascorbic acid was present in the rat brain. Despite authors claim high selectivity and accuracy, long-term stability of the implantable biosensors needs to be studied further in order to increase the potential of this application.

Although CNTs are used in electrochemical biosensors, they also possess characteristics that make them great choices for optical biosensors, like photoluminescence in NIR and strong resonance Raman scattering (Zhu, 2017). The NIR photoluminescence depends on the bandgap energy, which affects the local dielectric environment of the CNTs. The DNA wrapped around the CNTs modulates this local environment when a target analyte binds. The transition from a B to Z confirmation (DNA polymorphism)—because of the presence of divalent metal cations—causes a red shift in the bandgap fluorescence (Barone et al., 2005; Heller et al., 2006). This transition is prevalent in blood, living mammalian cells, and tissues. An optical CNTs-based biosensor for early detection of ovarian cancer, through the sensing of HE4 biomarker, was developed (Williams et al., 2018). This optical biosensor was developed through derivatizing of NIR-emitting CNTs to transduce the binding of HE4 to an immobilized antibody. This antibody-CNTs complex responded specifically to HE4 by modulation of the nanotube emission wavelength (Figure 17b). For *in vivo* validation, the biosensor was implanted into mice and its emissions were measuring an optical probe system for 24 hours (Figure 17c). From those results (Figure 17d), it is possible to observe a shift in biosensor emission wavelength, which correspond to the detection of HE4 in cancerous mice with high express of HE4, when compared to control (with low expression of HE4). While this study promises great potential for early ovarian cancer detection, same limitations need to be overcome before it can pass to the next clinical translation phase. For example, biocompatibility needs to be evaluated and the long-term functionalization and stability requires further investigation (since authors only claim consistent emission *in vivo* for 38 days). In addition, biosensors performance, like sensitivity and detection limit, should be studied to verify its accuracy for this application.

Graphene is another well-known material used in electrochemical sensors. The effect of graphene in *in vivo* applications depends on how the graphene was synthesized and purified (Pinto et al., 2013). Implantable sensor using pristine graphene has found applications as a glucose detection system, (Gu et al., 2012; Lee et al., 2018; Pu et al., 2018) neural stimulator and signal recorder, (Blaschke et al., 2017; Kostarelos et al., 2017), cardiac monitoring (Chen et al., 2013) and biological molecule sensor (Chen et al., 2011, 2012; Kasry et al., 2011). High conductivity, electrochemical stability, flexibility and transparency make graphene an ideal candidate for neural interface design.

The optical transparency of graphene is useful in the study of neural implants (Kuzum et al., 2014; Park et al., 2018). Graphene microelectrode arrays and graphene field-effect transistors have been used for neural stimulation (Thunemann et al., 2018). Wet-spun reduced graphene oxide have been used for free-standing penetrating electrodes (Apollo et al., 2015).

Blaschke et al. (2017) employed flexible arrays of graphene solution-gated FET to record brain activity *in vivo* which had a higher signal-to-noise ratio than traditional Pt electrode of similar dimensions. Nano-enhanced sensors offer great potential to redefine the study and diagnostic of diseases, through their ability to sense a biological or chemical parameter in real time. However, the challenges involved in the designing of implantable nano-enhanced sensors are numerous and this potential will only become a clinical reality when these sensors completely fulfil all necessary requirements for *in vivo* applications.

4 | CURRENT LIMITATIONS OF NANOMATERIAL IN DIAGNOSTIC BIOSENSORS APPLICATIONS AND FUTURE PERSPECTIVES

Nanomaterial has successfully addressed diagnostic biosensors challenges using different approaches as presented in Section 3. However, these approaches have not led to a viable product nor achieved clinical validation. Therefore, current limitations of nanomaterial approaches in diagnostics biosensors and future perspectives for nanomaterial in medical diagnostic biosensors are discussed below.

Some nanomaterial-enhanced sensors have passed clinical validation and are currently commercialized, in particular glucose sensors such as i-STAT and Glucocard, and some are already in clinical trial phases (Table 3). However, most of the developed nanotechnology approaches reported in literature does not reach the final stage of development (Mahato et al., 2018). There are several reasons for this. The most important issue that limits the development and application of nano-enhanced sensors in real life is related to the safety and toxicity of nanomaterial, their composition and particular properties. Second, most developed technologies focus only on analytical sensitivity and detection limit as performance parameters, and neglect parameters such as accuracy, precision and stability. This is the reason why most reported biosensors do not use biological samples in their proof of concept. There is also lack of attention regarding the time of response, and time of operation of newly reported biosensing techniques, which despite being crucial parameters of any biosensor applied in any real situation, are rarely reported. Third, biosensors need to be integrated into a fluidic and automated device, the so-called lab-on-chip (LOC) devices, with integrated detection modes in order to be successfully commercialized. This is extremely complex and demands a multidisciplinary team with access to a diverse set of equipment's to do it. And finally, the nanomaterial production techniques are not always scalable, which hinders the product commercialization.

4.1 | Safety and toxicity of nanomaterial

The safety and toxicity of nanomaterial can compromise the future development of nanomaterial-enhanced sensors, due to added cost of the manufacturing process due to security measures; damages to

the environment and public health when discharged in the case of point-of-care technologies; and the potential harm to patient health in the case of wearable or implantable technologies. These aspects are limiting the potential for commercialization and should be taken into consideration for future biosensors development. The possible toxic effects of nanomaterial are dependent on base material, shape, size, coatings, etc. However, to understand the toxicity associated with nanomaterial it is necessary to conduct control experiments and carefully analyse the data. Different studies of the same nanomaterial, with different cell lines, amounts, shapes, among other parameters, could achieve different results of toxicity, being extremely difficult to determine whether the cytotoxicity observed is physiologically relevant.

Metallic-based nanomaterial is the most widely used nanomaterial. For instance, gold NPs are known for the outstanding biocompatibility. However, the cell type used in toxicity assays can lead to different results. It was concluded that 33 nm citrate-capped gold nanospheres are not toxic to hamster kidney and human hepatocellular liver carcinoma cells (Patra et al. 2017), but can be toxic to human carcinoma lung cell line (Alkilany & Murphy, 2010; Fratoddi et al., 2015; Patra et al., 2007).

The same can be observed in the case for silver NPs toxicity can be achieved by: (a) the release of Ag⁺ ions, (b) the interaction of cells with silver NPs and (c) the formation of reactive oxygen species (ROS). Due to its small particle size and enormous specific surface area, the ions dissolution of nanosilver is much more rapid than in bulk material. Additionally, NPs have the ability to generate ROS, and to interact with, leading to a high toxicity for cells (Rebelo et al., 2016; Reidy et al., 2013).

Regarding carbon-based nanomaterial, the toxicity of several materials was studied and MWCNTs were found to be the less toxic, among the CNTs, carbon NPs and carbon nanofibres tested, showing the influence of shape in toxicity. In general, the toxicity of CNTs increases, significantly, when carbonyl, carboxyl and/or hydroxyl groups are present on their surface (Magrez et al., 2006; Yuan et al., 2019).

With regard to QDs and its individual and unique physicochemical properties, there are discrepancies in the current literature about their toxicity. This happens because not all the QDs synthesized are alike. QD toxicity effect depends on their chemical composition, since they can contain compounds such as Cd and Hg. QDs toxicity depends on several parameters, resultant from individual physicochemical characteristics and environmental conditions, like concentration, size, charge, coating, and oxidative, photolytic and mechanical stability (Bottrill & Green, 2011; Filali et al., 2020). Thus, while some studies show their toxicity (Hoshino et al., 2004; Lovrić et al., 2005), in others that evidence is not demonstrated (Ballou et al., 2004; Voura et al., 2004) and there are studies where the QDs toxicity depends on the dosage used (Li et al., 2020). Toxicity can be minimized by functionalizing the QD surface with biocompatible molecules. Methods for QD surface modification are presented elsewhere (Medintz et al., 2005; Smith et al., 2008; Xing & Rao, 2008).

In conclusion, it is not possible to affirm the toxicity of a nanomaterial, per se, since it depends on a plethora of factors and the

comparison between different literature studies should be carefully made, since, most of the times, the conditions are not the same. This means that nano-enhanced sensors development needs to take in consideration toxicity effects, especially when a wearable or implantable sensors is envisioned.

4.2 | Primary developmental focus on sensitivity and detection limit

Researchers aim to demonstrate engineering excellence of their nanosensing technologies in terms of increased endpoint detection sensitivities and lower detection limits. However, they tend to ignore other analytical performance parameters mentioned above in this review, as well as batch-to-batch variability for a given material and fabrication method. The end result is that proof of concepts are published and the work moves towards a next hypothesis to prove. This has led to the lack of data proving reproducibility and chip-to-chip variability for a given material and fabrication method. Indeed, without this benchmark, many devices will be unsuccessful in the commercial arena, despite incorporating high levels of academic innovation, particularly in the application of critical result endpoints such as in medical diagnostics. Other important aspect is the excessive focus on sensitivity and detection limit itself. The most common performance metric used to estimate sensor sensitivity is the change in signal normalized by the original value of the signal (Cui et al., 2001). A more apt definition would be using the change in signal per unit change in analyte concentration which can be visualized through the calibration curve. Perhaps the most important metrics of sensor performance is the size of the device itself. Decreasing the sensing elements dimensions impacts the two most important performance metrics of biosensors: LOD and response time. The LOD of biosensors and its correlation with sensor dimensions, signal transduction efficiency and reaction-transport kinetics affects sensor performance. Signal transduction efficiency is related to the minimum number of analytes that must be captured at the sensor surface for obtaining an acceptable signal-to-noise ratio, while the reaction-transport kinetics are related to the time required for that capture to occur (Soleymani & Li, 2017). The response time of a sensor is dictated by (a) the mass transport of the analyte to the surface of the sensor where the binding reaction can happen (Sheehan & Whitman, 2005; Squires et al., 2008) and (b) the affinity of that particular receptor-analyte system which becomes a limitation for the detection of trace amounts. Therefore, in many cases the signal-to-noise ratio is improved in miniaturization; however, the target analytes capture on sensor surfaces takes longer due to the increase in mass transport times (Kaisti, 2017).

Most sensors are essentially exposed-channel transistors that suffer from the drawbacks associated with other electronic devices, like electronic noise. A better understanding of the noise sources and will be needed for improved sensitivity and detection limit (Rajan et al., 2013). Also, ionic environment in which most sensing experiments are usually carried out and which does not allow for

ultrasensitive detection in physiological fluids. The ions in the electrolyte solutions accumulate and form a layer around the analyte of interest and screen the charges present on the analyte. A long Debye length ($\lambda_D = 0.3/\sqrt{A}$), where A is the ionic strength, is desirable to ensure less charges are screened. This inhibits the ultrasensitive detection in physiological fluids, which is dependent on the binding affinity given by the equilibrium dissociation constant (K_D). K_D determines the occupancy of the receptor sites at a certain bulk analyte concentration.

Several design changes have been proposed to reduce the response time of these biosensors into acceptable ranges to capture the enhanced signal-to-noise ratio of biosensors with critical dimensions. These changes include employing external forces such as pressure-drive flow (Yanik et al., 2010), electric field (Freedman et al., 2016) and thermal gradients (Wang & Cheng, 2016), or internal forces delivered through self-propelled nano/micromotors (Gu et al., 2012). Diffusion-limited transport can be mitigated by di-electrophoretic manipulation of biomolecules (Lee et al., 2010; Yasukawa et al., 2007) and AC electroosmotic flow (Gong, 2010; Hart et al., 2010) to drive the motion of molecules to the sensor surface.

In addition, the dimensionality of sensors affects their sensitivity. Based on electrostatics considerations, it is well known that two-dimensional cylindrical NW are more sensitive to adsorbed charges compared to one-dimensional planar ion-sensitive field-effect transistor (ISFET) or chemical field-effect transistor (CHEMFET) (Nair & Alam, 2006). These authors showed the detection limit of a typical 2D nanowire sensor for the same response time is three to four orders of magnitude higher compared to planar 1D sensor,

Researchers have applied novel technologies in the development of biosensors. As the devices have gotten smaller enhancing the signal-to-noise ratio, little consideration has been placed to the effect of optimizing the mass transport to the sensor and the chemical reaction that govern the binding kinetics. These affects the ultimate performance of the device. This results in the development of biosensors that do not meet healthcare current needs and therefore are not use in the diagnostic market. This is because not all diagnostic tests need to have low detection limits and high sensitivity to be of clinical use. However, all sensing devices needs to be robust, reproducible, accurate, scalable and cost-effective in order to be considered commercially (Mohammed et al., 2015).

4.3 | Integration of lab-on-chip devices

Biosensor components need to be integrated into a device that allows simultaneously sample and reagent load, and signal detection in an automated form, called lab-on-chip (LOC). LOC is an analytical device which is capable to scale down laboratory functions to a chip format up to a range of a few square centimetres (Panda & Pyarajan, 2014). Usually LOC systems contain microfluidic channels for the transportation of the sample. In addition to the microfluidic system, several functionalities are combined on a LOC system according to the analytical problem. The most important features integrated into

analytical LOC systems are sample preparation, separation and a detection system (Wongkaew et al., 2019).

The integration of biosensors into LOC devices has been reviewed elsewhere (Chen & Shamsi, 2017; Luka et al., 2015; Nikoleli et al., 2018). However, it is important to understand that LOC for diagnostic purposes demand merging nanomaterial, and knowledge in such diverse fields as microfluidics, engineering, chemistry, biology and even electronics. This is extremely challenging in terms of product development, since the whole product needs to be scalable, reproducible and cost-effective, which includes studying of reproducibility and robustness from nanomaterial production, to microfluidic and signal detection integration.

The problem with many lab-on-a-chip devices are that such devices require the use of supplementary equipment such as fluidic pumps, power supplies and signal acquisition devices (microscopes, spectrometers, etc). Most of these devices are significantly larger than the lab-on-a-chip systems themselves, typically taking up a significant volume of space on a laboratory bench, negating many of benefits related to device miniaturization. Also, LOC technologies usually demands skilled users, which introduces complications related to user training and standardization protocols, hampering technology commercialization (Mohammed et al., 2015).

4.4 | Scalable production techniques

Many innovations can present complexity in the fabrication of individual components, and the complete device may require several highly specialized and labour-intensive manufacturing techniques. Fabricating such devices generally draws more on the skill and experience of an individual user, across several interactive process, rather than being something that can be streamlined into a single manufacturing process. Frequently, researchers strive to demonstrate innovative functionalities at the trade-off of increased complexity and, ultimately, a reduced capacity for mass manufacturing (Mohammed et al., 2015). Many traditional fabrication methods used to create micro and nano scale structures do not translate well into large-scale production. Moreover, mass production backend processes and quality control can add up to 80% of total fabrication costs (Becker, 2009). Therefore, high complexity is not desirable for industry settings, because it usually means cost increment.

4.5 | Future Perspectives

Nanomaterial-enhanced biosensors have indeed offered great contributions towards addressing diagnostic challenges. However, if the aim is to have commercial exploitation, researchers should opt for fabrication techniques that are readily up-scalable, using designs which minimize the complexity and process stages required for backend processing. In addition to the design and fabrication, the choice of materials becomes crucial, not only with respect to the cost and desirable intrinsic material properties for the applications in question, but also the toxicity of these nanomaterial.

It is imperative that future studies will investigate more the chip-to-chip variability and repeatability of use, to provide more statistically relevant results for the developed work, but to also aid manufacturers in determining the feasibility of producing a product using a particular design, fabrication technique or material (Mohammed et al., 2015). It is also important that future studies address a real-world need, instead of characterizing the technology based in their analytical sensitivity.

The key to overcome the limitations of nanomaterial-enhanced sensors seems to be based in technology transfer, a process that should be taken into consideration since early stages of technology development (Daniel & Alves, 2019). This process should bring together researchers with their industrial counterparts in order to achieve a delicate balance of functionality, cost, sensitivity and device complexity with how this will translate to the economies of scale for mass manufacturing.

5 | CONCLUSION

Significant effort has been expended towards sensors and devices for health care. In a relatively short time (~two decades), biosensors have made remarkable progress in detecting markers for diseases. However, clinical diagnostics still offer challenges that biosensors are not yet able to fulfil. These challenges are related to diagnostic performance, that demands robust, reproducible, precise, fast, sensitive and specific tests for quantitation of health biomarkers. And to a series of other diagnostic features that would significantly improve early diagnosis and treatment of several health conditions such as continuous monitoring, wearable and implantable capacity, multiplex detection and point-of-care characteristics.

Several different types of nanomaterial-enhanced sensors have been reviewed and discussed under the principles of detection and the shape of nanomaterial, demonstrating in which way they were capable to address current diagnostic sensors challenges. These sensors can detect low concentrations of analyte with short response time. Nanomaterial can come in shapes of NPs, NTs/NW, nanosheets, NR, QDs or some combinations thereof. Detection outputs can be either electrical, optical or mechanical. Optical biosensors exploit the reaction between analyte and ligand that produces luminescence that can be detected using various types of spectroscopy. Mechanical sensors detect adsorbed analyte based on their deflection and change in resonance frequency as a result of analyte binding. The emergence of micro/nanofluidics has enabled the integration of automated systems. In vitro sensors, for integration in LOC, using nanomaterial are expected to make significant breakthrough in the near future given their remarkable sensitivity, real-time long-term monitoring of tissues and organs while also providing assistance with diagnosis and therapeutics. In vivo sensors, the wearable and implantable sensors, need to overcome several hurdles, the chief among them is the issue of toxicity, biofouling which affects the reliability of the implantable systems, and the risk of infection via foreign body reaction.

With continuing research further performance enhancement of existing nanodevices and newer nanomaterial-enhanced sensors, using novel methods of detection can be expected. The uniform quality of diagnostic devices is essential for widespread use in clinical settings. In this regard, wide variety of available nanomaterial and the lack of standardized tests for toxicity evaluation or the absence of regulatory requirement for use in clinical settings is a hindrance to a quick widespread acceptance to these devices. Because of their multifunctionality (therapeutic, diagnostic and barrier avoiding agents), there may be multiple federal agencies whose approval is needed leading to significant time requirement for ascertaining their suitability of clinical use. Nanotechnology will play an important role for both in vivo and ex vivo analysis for imaging and detection of biomolecules for health care.

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ORCID

Ana I. Barbosa  <https://orcid.org/0000-0001-7695-5186>

Rita Rebelo  <https://orcid.org/0000-0002-2709-4516>

Rui L. Reis  <https://orcid.org/0000-0002-4295-6129>

Vitor M. Correlo  <https://orcid.org/0000-0002-5516-7583>

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