Biomedicine & Pharmacotherapy 155 (2022) 113649

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Advances in nanomaterial-based immunosensors for prostate cancer screening

Rabia Khan [a], Fareeha Arshad [b], Israr U. Hassan [c], Gowhar A. Naikoo [b,*], Mona Z. Pedram [d,e,**], Mohammed Saeedi Zedegan [d,e], Hamed Pourfarzad [f], Alaa A.A. Aljabali [g], Ángel Serrano-Aroca [h], Yusuf Haggag [i], Vijay Mishra [j], Yachana Mishra [j], Martin Birkett [k], Murtaza M. Tambuwala [l,*]

[a] Neurology Laboratory, National University of Science and Technology, Islamabad PC 051, Pakistan
[b] Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Salalah PC 211, Oman
[c] College of Engineering, Dhofar University, Salalah PC 211, Oman
[d] Faculty of Mechanical Engineering-Energy Division, K.N. Toosi University of Technology, Tehran, Iran
[e] Department of Analytical Chemistry, University of Kashan, Kashan, Iran
[f] Center of excellence in electrochemistry, faculty of chemistry, University of Tehran, Tehran, Iran
[g] Faculty of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Yarmouk University, P. O. BOX 566, Irbid 21163, Jordan
[h] Biomaterials and Bioengineering Lab., Centro de Investigación Traslacional San Alberto Magno, Universidad Católica de Valencia San Vicente Mártir, 46001 Valencia, Spain
[i] Department of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University, Tanta 31512, Egypt
[j] School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144411, India
[k] Department of Mechanical and Construction Engineering, Northumbria University, Newcastle upon Tyne NE1 8ST, UK
[l] School of Pharmacy and Pharmaceutical Science, Ulster University, Coleraine County Londonderry BT52 1SA, Northern Ireland, UK

ARTICLE INFO

Keywords:
Prostate cancer
Immunosensors
Nanomaterials
Prostate-specific antigen
Metallic nanomaterials

ABSTRACT

Prostate cancer is one of the most common health hazards for men worldwide, specifically in Western countries. Rapid prostate cancer screening by analyzing the prostate-specific antigen present in male serum has brought about a sharp decline in the mortality index of this disease. Immunoassay technology quantifies the target analyte in the sample using the antigen-antibody reaction. Immunoassays are now pivotal in disease diagnostics, drug monitoring, and pharmacokinetics. Recently, immunosensors have gained momentum in delivering better results with high specificity and lower limit of detection (LOD). Nanomaterials like gold, silver, and copper exhibit numerous exceptional features and their use in developing immunosensors have garnered excellent results in the diagnostic field. This review highlights the recent and different immunoassay techniques used to detect prostate-specific antigens and discusses the advances in nanomaterial-based immunosensors to detect prostate cancer efficiently. The review also explores the importance of specific biomarkers and nanomaterials-based biosensors with good selectivity and sensitivity to prostate cancer.

1. Introduction

Prostate cancer (PCa) falls under the category of heterogeneous diseases and, after lung cancer, is the second most frequent cancer in males worldwide [1]. Prostate cancer can be localized or metastatic, with 80% of cases falling under localized prostate cancer. Localized prostate cancer mortality rates are meagre, but it is the opposite when discussing metastasized cancer. Usually, early diagnosis tends to be asymptomatic. Patients majorly complain of urinary discomfort, either facing trouble in getting started, feeling fullness post urinating or a urinary hesitancy, incomplete emptying, or a weak flow [2]. One other common complaint is the increased frequency of urination. These symptoms are collectively categorized under Lower Urinary Tract Symptoms (LUTS) [2]. Patients also tend to experience back pain and urinary retention as the disease progresses because the axial skeleton is the actual site of bony metastatic disease [2]. Fig. 1 gives a general overview of prostate cancer, i.e., the difference between a normal prostate versus prostate cancer (Fig. 1(a)), symptoms of prostate cancer

* Corresponding authors.
** Corresponding author at: Faculty of Mechanical Engineering-Energy Division, K.N. Toosi University of Technology, Tehran, Iran.
E-mail addresses: gahmed@du.edu.om (G.A. Naikoo), mona.zpedram@gmail.com (M.Z. Pedram), m.tambuwala@ulster.ac.uk (M.M. Tambuwala).

https://doi.org/10.1016/j.biopha.2022.113649
Received 26 June 2022; Received in revised form 22 August 2022; Accepted 1 September 2022
Available online 13 September 2022

(Fig. 1(b)), and the risk factors associated with this form of cancer (Fig. 1 (c)).

The prostate tissue expresses a glycoprotein known as prostate-specific antigen, a serine protease present in the epithelial cells of the prostate gland [3,4]. To determine the presence of cancer, prostate-specific antigen (PSA) plasma levels are evaluated. The usual range of PSA should be > 4 ng/mL. Along with evaluating PSA levels, many countries recommend following the digital rectal exam (DRE) protocol for a clear diagnostic picture [3]. However, the PSA levels can sometimes be misleading and give false positive or negative results [5]. Thus, studies have been conducted to find other biomarkers to identify prostate cancer. According to a study published by Schumacher et al. [6], around 100 single nucleotide polymorphisms have been located concerning prostate cancer that targeted during cancer diagnosis. Recently, another favourable technique that gained momentum in the diagnostic field is multiparametric magnetic resonance imaging (mpMRI) [7,8], which is used both for diagnosis and surveillance of disease progression in patients. The general overview of the prostate cancer progression and the different treatment strategies is shown in Fig. 2.

The treatment options for PCa vary according to cancer classification, the oldest being androgen deprivation therapy [9]. If the cancer cells are castration-resistant, patients undergo chemotherapy and radiation. Recent developments have made remarkable progress in improving the standard of drugs used in chemotherapy. Some promising drugs are Abiraterone, docetaxel, and apalutamide [9,10]. Treatment strategies have seen tremendous leaps in effectively managing prostate cancer. However, radiation therapy posed a huge side-effect and was reportedly responsible for erectile dysfunction in patients. Moreover, there was an increased risk of urinary incontinence due to radiation prostatectomy [11]. Therefore, efforts must be directed towards developing better, easily accessible and safer diagnostic methods to detect prostate cancer rapidly.

Biosensors thus offer great alternatives for rapidly detecting prostate cancers [12,13]. Amongst these, the rapidly emerging nanomaterials-based sensors are promising candidates for prostate cancer screening and early diagnosis of tumours by detecting prostate cancer biomarkers, including the PSA. This review thus aims to provide a comprehensive overview of the recent advances in developing nanomaterial-based immunosensors for early prostate cancer screening. First, we provided a quick overview of prostate cancer occurrence worldwide, followed by an in-depth discussion on the various methods for prostate cancer detection. We then highlighted the different types of immunosensors developed in recent years for prostate cancer screening. We also briefly discussed the advantages and disadvantages of nanomaterial-based immunosensors for the detection of prostate tumours. We finally concluded the review by presenting the conclusion and future perspectives of this ever-evolving and promising field.

2. Impact of prostate cancer on males in the world

According to Chu et al. [14,15], it has been enumerated that men of African-American origin are more likely to be diagnosed with prostate cancer than white men, with double the mortality rate as their latter counterparts. An estimate of 1,276,106 new cases was recorded across the globe, thereby making the percentage of cancers in men mount to 7.1% [16]. Reports also suggest that frequent testing augments prostate cancer detection and reduces the mortality rate in Europe [3,17]. The incidence rates in Japan, Korea, and Singapore have been higher than in West Asian countries [4,18,19]. This can be attributed to increased PSA testing and better cancer registries in these countries. The incidence rates are increasing in Pakistan, India, Kuwait, Indonesia, Turkey, Thailand, Iran, Philippines, Vietnam, Sri Lanka, United Arab Emirates, and China. In 2018, a total of 358,989 deaths were due to prostate cancer [16], and the average age of a patient at diagnosis has been calculated to be 66 years [16]. The probability of prostate cancer diagnosis by 79 years is one in 47 countries where the sociodemographic index ranges from low to middle. Therefore, with the ever-increasing

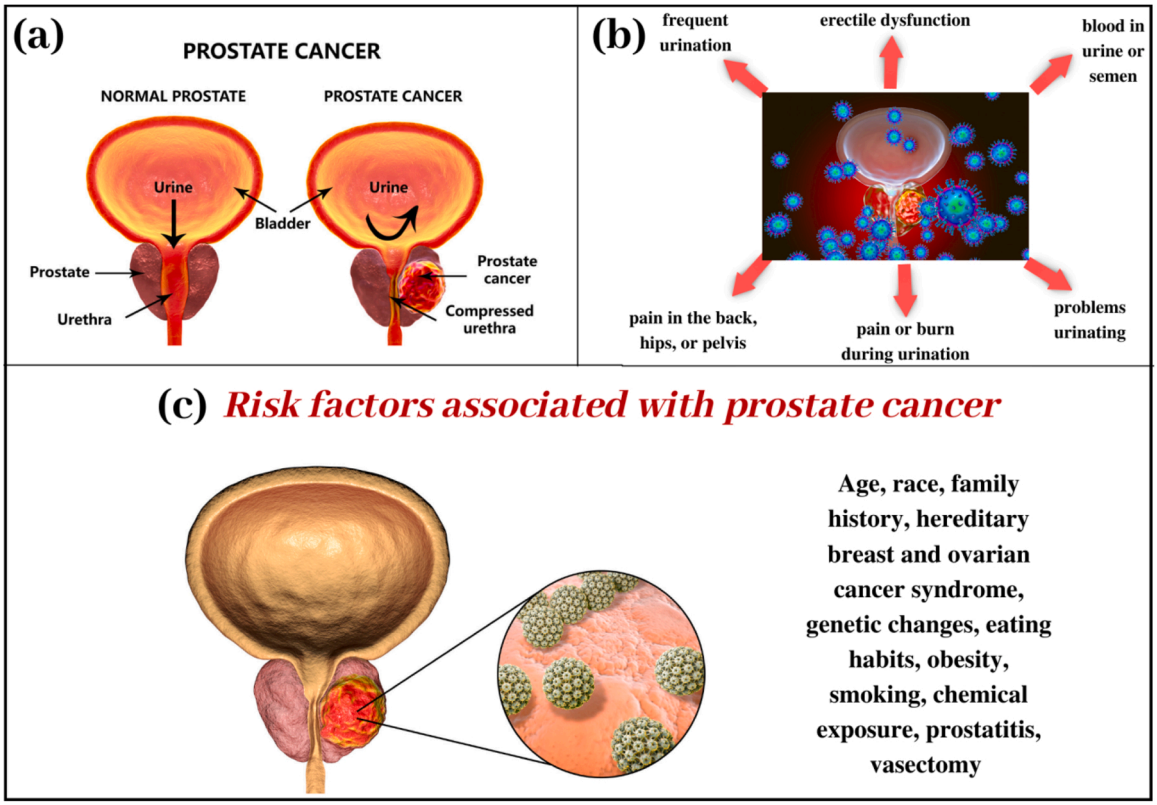

Fig. 1. Prostate cancer (a) Normal prostate versus prostate cancer (b) Symptoms of prostate cancer (c) Risk factors associated with prostate cancer.

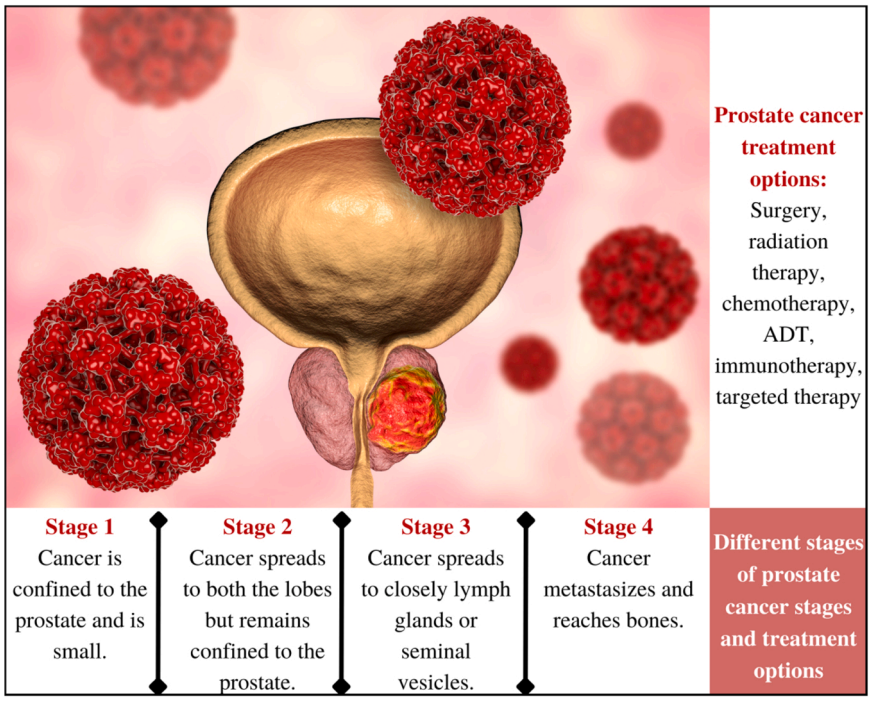

Fig. 2. Progression of prostate cancer and recommended treatment strategies.

rate of prostate cancer, it is essential to develop strategies and methods to allow for the rapid detection of tumour biomarkers. Fig. 3 and Fig. 4 present an estimated number of PCa cases and deaths in 2021, as the American Cancer Society reported.

3. Current methods used to detect Prostate Specific Antigen for prostate cancer diagnosis

Prostate-Specific Antigen (PSA) is a serine protease in the prostate tissue. It comprises a glycoprotein with 240 amino acids and is found in normal and malignant prostate tissue. Usually, it is secreted in the semen for dissolving the semen coagulum. In addition, PSA can also be found in cystic breast fluid, amniotic fluid, periurethral gland, and liver, kidney,

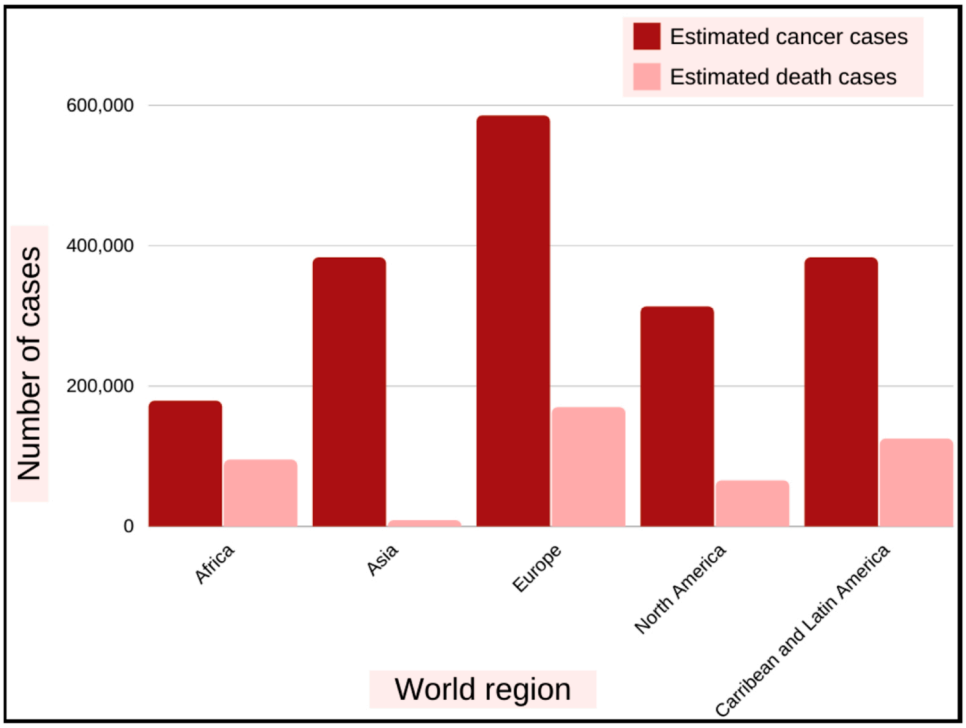

Fig. 3. Number of patients affected by prostate cancer. **(Dark Red)** Estimated cases of Prostate Cancer by 2040. **(Pink)** Estimated deaths of Prostate Cancer by 2040. Data collected by American Cancer Society 2021.

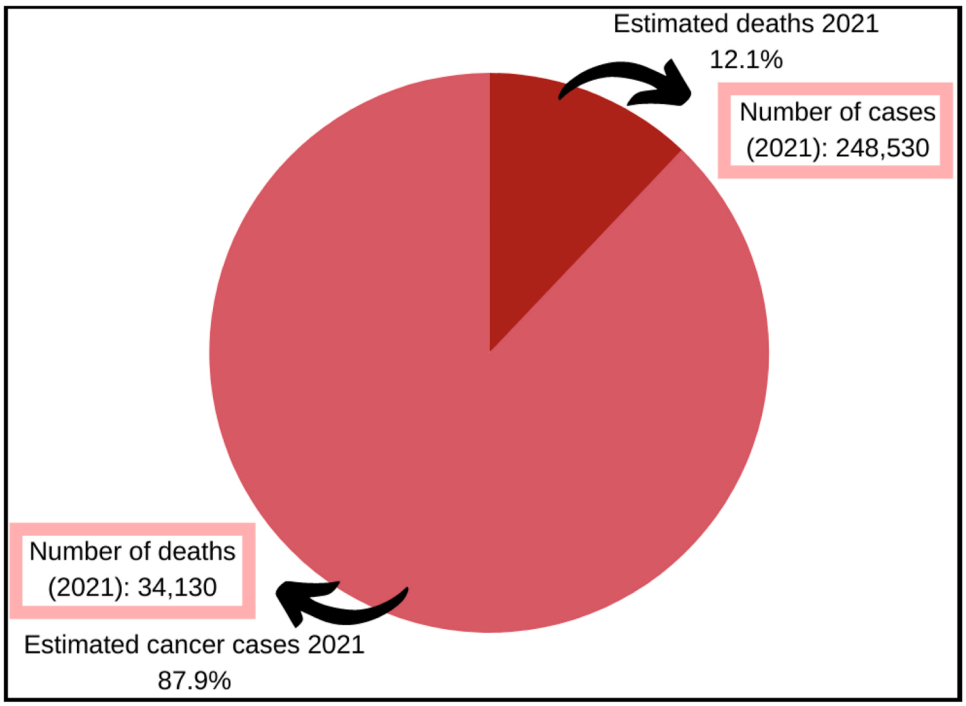

Fig. 4. Estimated global prostate cancer cases and deaths in 2021. Data collected by American Cancer Society 2021.

and lung tumours [20,21]. Prostate cancer tissue exhibits high PSA levels as compared to normal prostate tissue. This is because the gland's epithelial cells secrete the excess protein into the circulatory system, leading to elevated serum PSA concentrations. PSA in the serum has an affinity to bind to serine protease inhibitors, namely macroglobulin and antitrypsin, making it possible to exist in serum as free PSA and bound PSA [21]. Reports suggest that approximately 5–30% of PSA exists in the free form. The reference range for normal prostate tissue shows PSA levels to be $>$ 4.0 ng/mL.

Laboratory technicians measure PSA levels in serum using various immunoassays. As a result, multiple methods are used today to evaluate PSA levels to determine the presence of prostate cancer. Immunoassays are the techniques used to study the specific immunoreaction between an antibody(Ab) and an antigen (Ag). Immunoassays enable the evaluation of trace amounts of compounds similar in molecular or chemical structure [22,23]. Therefore, immunoassays are the best technique for analyzing analytes in complex solutions. The methods used in conventional immunoassays involve immobilizing the Ab-Ag on the surface of plastic tubes, glass fibres, or microtiter plates. The immobilized agent is in contact with the analyte-containing sample (Ab or Ag). The bound and free forms of Ab and Ag separate, leading to quantification, as shown in Fig. 5. The enzyme's activity to conjugate with either Ab or Ag is measured to get quantitation [24]. This is widely used in enzyme-linked immunosorbent assay (ELISA) and in radioactive immunoassay, where the radioactivity is measured. On the other hand, conventional immunoassays come with their share of problems. Since they are primarily operated manually, issues such as poor reducibility, lengthy processes, and slow reactions are frequently encountered.

3.1. *Electrophoretic immunoassay*

One of the most widely used immunoassays in the current decade is the Capillary Electrophoretic (CE) Immunoassay. Antibodies are known for their high specificity and solid binding affinity, making them favourable for detecting and evaluating analytes in plasma, serum, and blood [25]. In order to enable excellent detection of analytes, different CE immunoassays use chemical labels. Usually, fluorescent tags or enzymes are the basis of these chemical labels. CE immunoassays pose

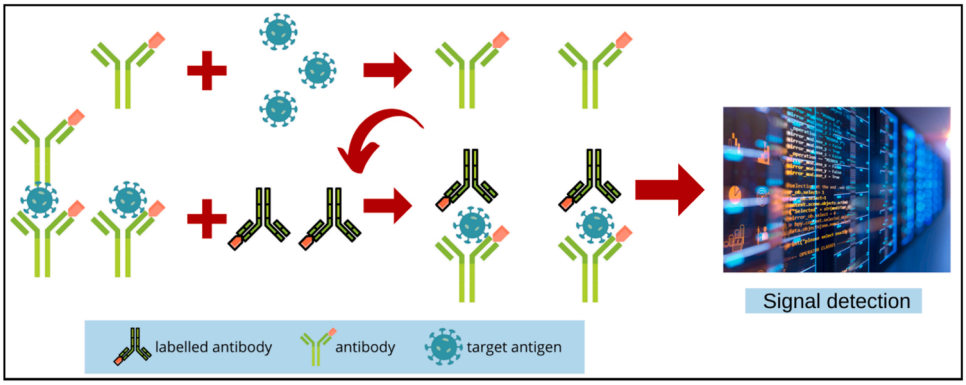

Fig. 5. A schematic illustration of the basic working principle of an immunoassay. The target antigen binds with the antibody, after which the second labelled antibody is added to the substrate. After washing and removing unbound antibodies, the detected signal is deciphered to analyze the presence of the target antigen in the sample.

favourable advantages for easy automation and rigorous high-speed antibodies/analyte/antibody-analyte separation processes. Furthermore, they can intake a minimal quantity of reagents and samples, making it possible to detect minuscule traces of analyte. As a result of this attribute, CE immunoassays are highly desired when analytes need to be detected from single cells. However, the main drawback of CE immunoassays is their tendency to deliver poor concentration-based detection limits compared to ELISA.

3.2. Enzyme-linked immunosorbent assay (ELISA)

The principle of ELISA revolves around the antibody-antigen reactions, displaying the chemical interaction between antibodies and antigens. ELISA uses the reaction between antigens and antibodies and paves the way for high selectivity and sensitivity qualitative/quantitative analysis of different proteins, nucleic acids, hormones, plant metabolites, and peptides [25–27]. ELISA immunoassays possess simple procedures, high specificity, high sensitivity, high efficiency, and are safe and eco-friendly; reagents are cost-effective and do not require complicated procedures. However, a significant drawback of ELISA is that there is a high probability of generating false positives. Moreover, problems such as antibody instability, expensive culture media, costly and intensive labour for antibody preparation, low sensitivity, and enzymes losing activity after conjugation is also encountered [28,32,35,37,38], leading researchers to develop improved performance techniques like chemiluminescence immunoassay and fluoroimmunoassay [28]. Nevertheless, despite these setbacks, ELISA is still one of the most commonly used immunoassays in medicine, pharmaceuticals, biotechnology, and plant toxicology [28].

3.3. Chemiluminescence immunoassay

The chemiluminescent immunoassay (CLIA) technique uses a luminescent molecule as a label. This label is also referred to as the accurate indicator of the reaction. The concept of luminescence surrounds the emission generated on the transition of an electron from an excitatory state to a resting state [25]. The frequency of this emitted radiation lies between 300 and 800 nm. The energy left behind in the atom is then exonerated as light. The chemiluminescent immunoassays present indispensable advantages: high specificity, a vast dynamic range, high signal intensity, accelerated signal retrieval, tremendous attributes of stability of reagents, random access, and excellent assay protocol compatibility. Along with numerous promising advantages, the assay has a few limitations, mainly limited test panels, reduced antigen detection, soaring costs, and closed analytical systems [29–31]. New technology has been developed known as the flow-injection chemiluminescent immunoassay (FI-CLIA) [29], which involves rapidly injecting micro-bubbles to ensure increased temperature, a better reagent mixture, and a reduced incubation period. This technology has demonstrated a reduction in analysis time and is one of the most used immunoassays in PSA detection.

3.4. Fluorescence immunoassay

The fluorescent-based immunoassay makes use of a detection reagent or a fluorophore. This reagent is a fluorescent compound that can absorb light at one wavelength and then emit it at another wavelength [25]. The FIA can be homogenous or heterogeneous and can be competitive or non-competitive. The homogenous FIA exempts the need to separate the antibody-bound analyte from the free analyte before measurement. When the antibody binds, the labelled analyte tends to exhibit polarization, making it feasible to monitor the concentration of the analyte directly as the increase in polarization is directly related to the analyte concentration. The advantages of FIA include high sensitivity analytic detection, precision, abridged reagents, easy assay designs, and high speed. Fluorophores also pose good photostability and reactivity properties, along with excellent water solubility [25]. However, the fluorophore tends to alter the binding affinity of the compounds [25].

3.5. Radioimmunoassay

The first-ever immunoassay developed was the Radioimmunoassay (RIA), and it is known as the predecessor of all the latest immunoassays techniques [32]. The working principle of this immunoassay is based on the antigen-antibody reaction in which a radioisotope is used (radioactive antigen), where it is labelled with gamma-radioactive isotopes. Using separation techniques, the bound antigens are separated, and the radioactivity of the remainder is evaluated [23,32,33]. RIA poses good sensitivity, good reaction time, and extreme precision. However, a study by Banks demonstrated the possibility of the immunoreactive particle entering the blood-brain barrier [34]. Over the years, there has been a gradual decline in using the RIA technique as there have been raised regarding its safety due to radioactivity and the threat it poses as a severe health hazard.

4. Nanomaterial based immunosensors used in the detection of PSA for prostate cancer diagnosis

A biosensor usually incorporates a bioreceptor responsible for the recognition of the analyte followed by binding to form an immunocomplex (Fig. 6). To measure the immunocomplex, the antigen-antibody reaction is coupled to a transducer. Transducers process the information between the antibody-antigen complexes and convert it into an electrical signal. This signal is measured and depicted in graphs or numbers [35,36]. An ideal immunosensor comprises specifications such as quick identification of target antigen, generating immunocomplexes without additional reagents, producing high reproducible results, and rapid target identification in the sample. To identify the target, immunosensors can directly determine the target analyte or use indirect methods like using a label. These labels can be enzymes or nanoparticles directed to study the binding reaction.

There have been numerous successful research studies done that demonstrate the efficient use of nanomaterial based immunosensors, and they are gradually taking over the conventional analytical methods used in scientific research giving rise to important outcomes [35–38]. Nanomaterials, owing to their high surface area and unmatched properties have shown to increase the sensitivity and selectivity of the sensors and thus demonstrate high applicability in the field of sensing. Therefore, nanomaterial based immunosensors are ideal towards the detection of PSA for early diagnosis of prostate cancers. The upcoming sections will highlight different types of nanomaterial based immunosensors developed for PSA detection.

4.1. Gold nanoparticles decorated on graphene oxide-based immunosensor for efficient detection of PSA

Graphene exhibits exceptional electrical, mechanical, and optical properties, making it a pioneer in biomedical, optoelectronic, and electronic domains from the nanoscale to macroscale applications. The derivatives of graphene, such as graphene oxide (GO) and reduced GO (rGO), have demonstrated outstanding potential due to their electrochemical and mechanical properties [39]. They present exceptional thermal conductivity, excellent electron mobility at room temperature, high mechanical properties, and a vast surface area [40]. They can form nanocomposites with a metal (MNP), quantum dots, polymers, and metal oxides. These nanocomposites are being extensively used in immunosensing technologies [41]. The optical transparency of a graphene sheet has also been helpful in the electronics industry. Even though graphene has a large surface area and is helpful in many applications, it still faces a few challenges. A significant challenge of graphene is its property of agglomeration and restacking to regenerate

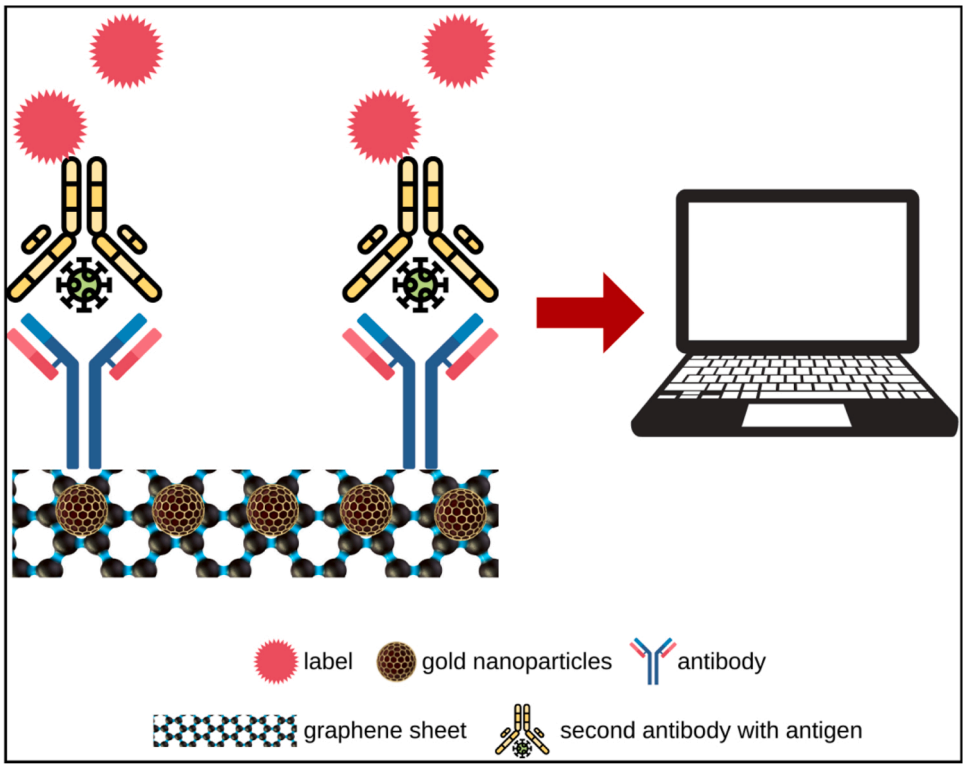

Fig. 6. A schematic illustration of the basic working principle of an immunosensor with graphene sheets and nanomaterial induction on the electrode surface. The reaction is then analyzed based on the signal output generated.

structures [40]. However, different studies show that introducing metal nanoparticles on graphene or its derivatives helps keep the restacking of graphene in check, thereby yielding a way to produce advanced nanocomposites for various applications [42,43]. Graphene-nanoparticle composites used as biosensors enhance the sensitivity, control non-specific adsorption, improve reproducibility, and limit detection [44].

Gold (Ag) metal nanoparticles offer extremely favourable chemical and physical properties, mainly optical, electronic, magnetic, and catalytic [40,41]. Their reduced size and ability to assist the electron transfer process make them attractive in developing electrochemical sensors [45]. They are highly compatible with different biomolecules and provide effective biomolecule immobilization. Gold nanoparticles (AuNPs) exhibit good conductivity, stability, and adsorption properties, equipping them with biosensing quality to easily detect enzymes [40, 46]. Furthermore, they have proven a favourable option in DNA hybridization for calorimetric identification [47]. AuNPs have also been used in photothermal therapy systems as they have flexible optical properties and good biocompatibility [40,48].

Research has been focused on studying the optical properties of gold nanoparticles using localized surface plasmon resonance (LSPR), SERS, and Rayleigh resonance scattering [40,48]. In addition, studies have also shown the promising potential of plasmonic nanoparticles, primarily based on single particle detection systems, that possess specialized, localized surface plasmon resonance characters that can be used to develop optical biosensors for the biosensing of several biomarkers [49–51]. In their review study, Tian and colleagues demonstrated that with the combination of single nanoparticle imaging methods, we can obtain detailed information regarding the nanomaterials and their activity, thus potentially applying them to biosensing prostate cancer biomarkers [49]. Thus, single particle detection can also be applied towards developing immunosensors to detect PSA and relevant biomarkers in prostate cancers. For instance, in a study by Wang et al., a single particle detection system was used to develop an aptasensor based on luminescence resonance energy transfer between aptamer modified upconversion nanoparticles (UCNPs-aptamer) and gold nanoparticles [52]. This UCNP-aptamer functions as an energy donor, and the gold nanoparticles work as acceptors. In the absence of the target analyte, the gold nanoparticles get adsorbed on the UCNPs-aptamer surface and cause quenching; therefore, no luminescence is observed. On the contrary, in the presence of target molecules, the aptamers show stronger attraction towards the target molecules than the gold nanoparticles and therefore show luminescence. In another similar study, a supraparticle based on MnO_2-modified gold nanoparticle was developed that showed the promising potential of a single particle enzyme activity assay for sensitive detection of disease-related biomarkers [53]. In another closely related study by Qi and colleagues, a highly sensitive colour-coded single particle detection system was developed based on single gold nanoparticles to detect target biomolecules like pyrophosphate [54]. Furthermore, in another study by the same group, a sensitive localized surface plasmon resonance coupled method based on single particle detection coupled with dark field microscopy was used to detect the target analyte [55]. The authors recorded that the developed technique could perform highly sensitive and selective detection of the target molecule. Such single particle detection systems, thus, can also be employed to detect prostate cancer biomarkers, including the PSA.

Thus, graphene oxide- gold NP composites have shown impressive results in various applications due to their synergistic interaction, which aids in enhanced performance. On the one hand, graphene provides good stability and excellent mechanical strength. On the other hand, AuNPs offer good biocompatibility and immobilization of molecules, thereby broadening the scope of these composites, specifically in the sensing technologies for medical diagnostics, as shown in (Fig. 7). Over the last decade, many studies have reported that graphene oxide AgNPs nanocomposites and immunosensors have been successfully developed to aid prostate cancer detection by targeting the prostate-specific antigen. Some of the immunosensors created using AuNPs, and their limit of detection and linear range have been summarized in Table 1.

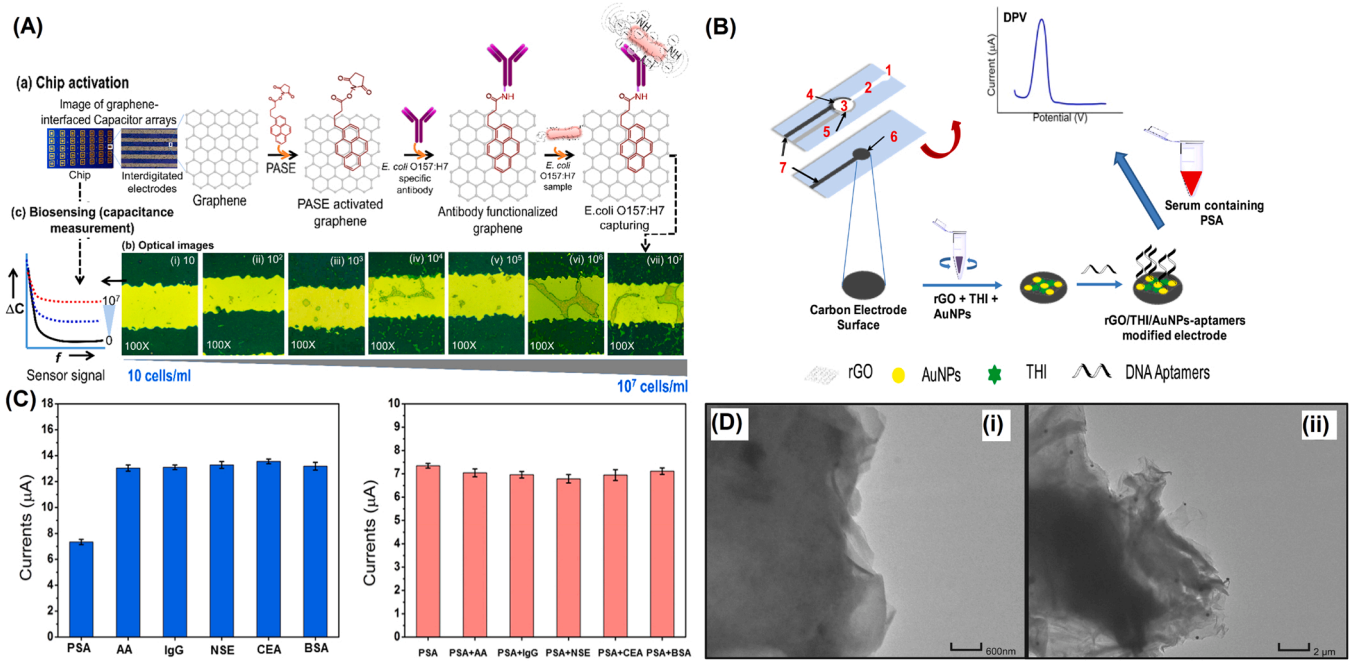

Fig. 7. Gold nanoparticles decorated on graphene oxide-based immunosensor for efficient detection of PSA **(A)** (a) Photographic images of a real graphene-interfaced chip and the process of PASE activation and antibody immobilization. (b) Optical microscopic images of captured target *E. coli* O157:H7 cells on graphene-interfaced chips through specific antibodies covalently attached to chip surfaces. (c) Biosensing of *E. coli* O157:H7 through measuring capacitance change between gold microelectrodes of chips. **(B)** Fabrication and modification process of the microfluidic paper-based aptasensor and the typical response of the detection of analyte (1) injection port; (2) microfluidic channel; (3) reaction site; (4) screen-printed carbon counter electrode; (5) screen-printed reference electrodes; (6) working electrode; (7) screen-printed electrode-lead. **(C)** The evaluation of the selectivity of the proposed aptasensors. DPV responses of the sensor to 1 ng mL^{-1} PSA and 1 ng mL^{-1}AA, IgG, NSE, CEA and BSA, respectively (left); DPV responses of the aptasensor to 1 ng mL^{-1} PSA and 1 ng mL^{-1} PSA mixed with 1 ng mL^{-1}AA, IgG, NSE, CEA and BSA, respectively (right). **(D)** TEM images of (i) rGO/THI and (ii) AuNPs/rGO/THI nanocomposites.
(a) Adapted with permission from ref. [41], copyright@ 2017 (Elsevier). (b) Adapted with permission from ref. [46], copyright@ 2018 (Elsevier).

Table 1
Details of different immunosensors developed for PSA detection and their detection limit and linear range.

No.	**Sensors/Electrodes**	**Detection Limit**	**Linear range**	**Reference**
1.	Graphene-methylene blue/ chitosan nanocomposite	0.013 ng mL^{-1}	0.05–5.0 ng mL^{-1}	[70]
2.	Graphene-silver hybridized mesoporous silica NP	0.002 ng mL^{-1}	0.01 – 10 ng mL^{-1}	[70]
3.	HRP-Ab 2/Au NPs	0.00046 ng mL^{-1}	0.002 ng mL^{-1} to 2 μg mL^{-1}	[71]
4.	AuNPs/rGO/THI	0.01 ng mL^{-1}	0.0005–0.2 ng mL^{-1}	[46]
5.	AuPtAg-ANCs	0.017 ng/mL	0.05–50 ng mL^{-1}	[68]
6.	PdNP@PANI-C_{60}/GCE	1.95 × 10^{-5} ng/mL^{-1}	1.6 × 10^{-4} ng.mL^{-1} to 38 ng.mL^{-1}	[65]
7.	hydroxyl pillar[5]arene@AuNPs@g-C3N4	0.00012 ng/mL^{-1}	0.0005–10.00 ng mL^{-1}	[72]
8.	SWCNT array with Pt microelectrode	0.25 ng/mL	–	[73]
9.	SWCNT with anti-PSA antibody, FET	–	–	[38]
10.	PEDOT/ P3DG/ AuNPs	0.03 ng/mL	–	[74]
11.	PNT/AuNPs/PANI	–	–	[74]
12.	rGO-AuNPs with anti-PSA AB	0.003 ng/ mL		[48]
13.	3D-anti-PSA Graphene-Gold sensor	0.59 ng/ mL		[48]
14	AuNPs/m-PdPtCu	3.3 fg/mL	10fg/mL to 100 fg/mL	[63]
15.	PEDOT:P3DG/AuNPs	0.03 pg/mL^{-1}	0.0001–50 ng mL^{-1}	[74]
16.	AgNPs/rGO nanocomposite	0.01 ng/mL	1.0–1000 ng/mL	[40]
18.	Nanocomposite based on graphene and Au modified electrode	0.59 ng/mL	0–10 ng/mL	[40]
19.	Pt-Cu HNFs	0.003 ng/mL^{-1}	0.01–100 ng/mL^{-1}	[63]
20.	AuNps-GRelectrode	0.59 ng/mL	0–10 ng/mL	[75]
21.	TH/MWCNTs/ IL/GCE	0.02 ng/mL^{-1}	0.2–1.0 ng·mL^{-1}	[56]
22.	MWCNTs/IL/ CS/GCE	0.001 ng/mL^{-1}	0.05–80 ng·mL^{-1}	[56]
23.	Conductive electrode paper based GO/chitosan/AuNPs.	0.001 ng/mL	0.003–20 ng/mL	[48]
24.	AuNPS-GN and CdTe quantum dots coated silica NPs	0.0032 ng/mL	–	[48]
25.	CNT-PtME array	0.25 ng/mL	Upto 1 ng/mL	[73]
26.	SiO2- AgNPs/Ab/BSA/Ag	1 ng mL˙1	0.1–0.001 μg mL	[76]

4.2. Carbon nanotubes modified paper electrode immunosensors

Carbon nanotubes (CNTs) possess excellent properties such as impressive electrocatalytic effect, fast electrode kinetics, solid adsorptive ability, strong electrical conductivity, low cost, and efficient biocompatibility[56]. Due to these attractive synergistic effects, CNTs have become a favourite in designing the latest generation of electrochemical immunosensors. They have improved performance compared to other carbon electrodes when analyzing reaction rates, detection levels, and reversibility. Carbon nanotubes are a byproduct of folded

graphite layers into carbon cylinders and can be considered a unique yet incredibly fresh carbon material.

Paper has become an attractive tool in preparing paper-based immunosensors as it exhibits excellent features such as tool-free, low cost, absorption, and flexible manipulation. This feature makes carbon nanotube-modified paper electrodes an excellent option for detecting PSA and other cancer biomarkers [57]. The conducting paper has proven inefficient for signal conduction in immunosensing technology. Organic and inorganic materials are used to construct CNT. Inorganic materials are not cost-effective, are challenging to process, and can crack on bending even though they have a better electrical performance. Organic materials are preferable because they are cost-effective, flexible, and easier to process[58]. As a result, carbon nanomaterials are suitable protagonists for CP fabrication.

Moreover, graphene gold nanocomposites provide a conductive material for fabricating conducive paper electrodes (CP). This GN-Au-CP electrode allows point-of-care detection and has unmatched characteristics like flexibility, low cost, biocompatibility, high throughput production, and modified efficiency [58]. For instance, a study by Ji et al. in 2018 aimed at developing an immunosensor that detected PSA levels. The study used bioactivated MWCNT.s and a micro-pore filter paper. They activated MWCNTs with PSA antibody using N-(3-dimethyl aminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (NHSS). They reported that the developed sensor was comparatively cheaper and faster than the ELISA immunoassay [57]. Furthermore, Salimi et al. fabricated a highly specific electrical immunosensor based on MWCNT to detect PSA. This immunosensor reported excellent results [59]. Another immunosensor was fabricated by Aminur Rahman et al., in which they used MWCNTs/AuNPs to detect PSA. The immunosensor exhibited an

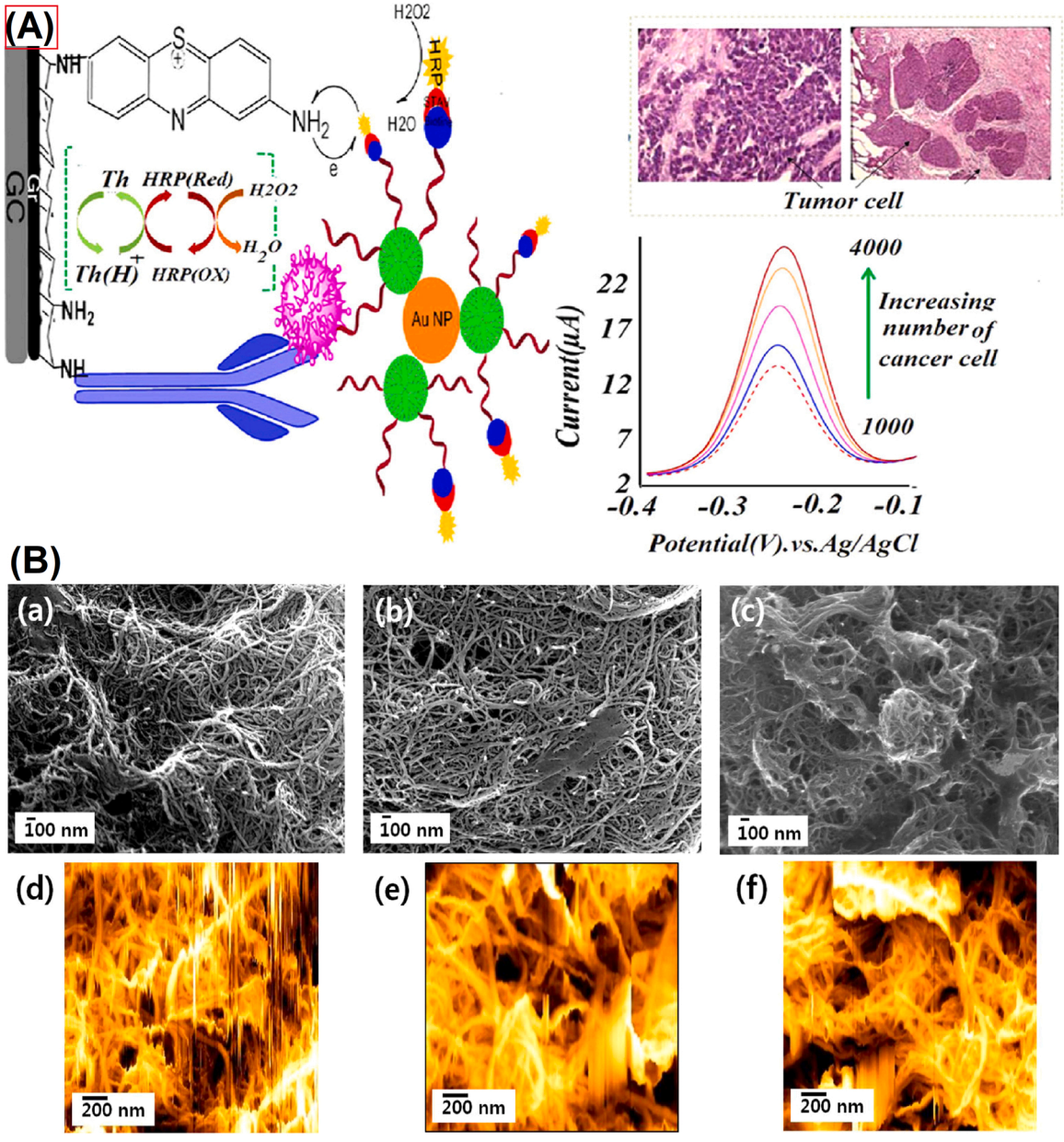

Fig. 8. Carbon nanotubesmodified paper electrode immunosensors for PSA detection (A) Ultrasensitiveelectrochemical immunosensor for PSA biomarker detection in prostate cancercells using gold nanoparticles/PAMAM dendrimer loaded with enzyme linked aptameras integrated triple signal amplification strategy. (B) SEM and AFM images of MWCNTs based sensorelement, (a) and (d) are SEM and AFM images of carboxylated MWCNTs, (b) and (e)are SEM and AFM images of Ab-MWCNTs, (c) and (f) are SEM and AFM images ofPSA-Ab-MWCNTs.
Adapted with permissionfrom ref. [59], copyright@2015 (Elsevier). Adapted withpermission from ref. [57], copyright@2018 (Elsevier).

excellent linear range and detection limit (Fig. 8). CNTs provide an excellent electrocatalytic activity, making it feasible to use them in electrochemical immunosensors based on dehydrogenase/oxidase-enzyme. Moreover, they also enhance the electrochemical signals making their applications in electroanalytical immunosensing a bonus [60]. Table 1 summarizes some immunosensors developed using carbon nanotubes/ nanowires for PSA detection.

4.3. Three-dimensional platinum-copper nanoparticles based immunosensor for amplified detection of PSA

Platinum nanoparticles (PtNPs) are among the most promising nanoparticles in developing electrochemical immunosensors. Their excellent catalytic properties have made them a conducive option over the years [61]. They allow oxygen reduction reaction, hydrogen evolution reduction, along H_2O_2 electrocatalytic reduction. Additionally, interconnected hollow channels and mesoporous PtNPs manifest great electrocatalytic activity for H_2O_2 reduction [62]. Hollow PtNPs and solid Pt nanospheres exhibit discrete characteristics due to their different morphologies [61]. Compared to monometallic nanomaterials, Pt-based bimetallic nanomaterials display advanced activity and stability owing to their synergistic effects [63]. 3D structures, for example, nano coils, nanocones, and nanoframes provide a high surface area and abundant adsorption sites. As a result, an extra quantity of antibodies can be loaded, leading to whisked-up electron transfer [64]. Thus, they rapidly gain momentum as a reliable option for developing immunosensors.

Among the various metal nanoparticles in use, copper nanoparticles (CuNPs) are outstanding as they have a large surface area, small diameter, rapid transfer of electrons, and are cost-effective [65]. As a result, CuNPs appear to have a bright future in electrode fabrication. Attention has been focused on metallic alloy nanostructures based on Pt and Pd as they exhibit good catalytic activity [66]. This was seen in a recent study when a new type of ultrasensitive electrochemical immunosensor for PSA detection was fabricated by the boosted H_2O_2 reduction catalyzed by PtCu HNFs. The immunosensor displayed a more comprehensive linear range of 0.01–100.0 ng mL^{-1} with a lower detection limit of 0.003 ng mL^{-1} (S/N = 3), good reproducibility, outstanding selectivity, and favourable selectivity stability towards PSA detection, indicating its future applications in clinical diagnosis [63]. Thus, such immunosensors hold good potential for detecting PSA biomarkers for early prostate cancer detection.

4.4. Au-Pt-Ag alloyed nanocrystals and fabricated immunosensor for highly sensitive electrochemical detection of PSA

Like Au and Pt nanoparticles, silver nanoparticles (AgNPs) have also proven to be an excellent candidate for developing highly sensitive and selective immunosensors. This was seen in the work where a novel immunosensor was fabricated using Ag nanoparticles-doped Pb (II) metal-organic framework (Ag-MOF) to detect PSA efficiently. Silver nanoparticles did not need any reducing agent and were used with Pb(II) to modify glass electrodes. This fabricated facile immunosensor exhibited a linear range of 0.001–50 ng mL^{-1} with a detection limit of 0.34 pg mL^{-1} [67]. In recent years, trimetallic heterogeneous catalysis has also shown promising results, owing to its excellence in biocompatibility, chemical stability, and active surface area. In addition, their defined and precise hollow and dendritic structures allow for multiple active sites for binding and signal amplification [68].

For instance, Pradeep et al. fabricated a novel immunosensor based on palladium nanoparticles (PdNPs), polyaniline (PANI), and fullerene-C60 nanocomposite film for PSA detection. It exhibited a linear range of s 1.6×10^{-4} ng.mL^{-1} to 38 ng.mL^{-1} and detection limit of 1.95×10^{-5} ng.mL^{-1} [65]. Jiang et al. developed an immunosensor based on Gold-platinum bimetallic functionalized tin oxide graphene (GS-SnO_2-Au@Pt) and (Cu^{2+}@Ag-Au) nanospheres. The bimetallic graphene traps antibody one onto the surface due to its large surface area and good biocompatibility. The nanospheres were used to label the second antibody (Ab_2). The proposed immunosensor exhibited excellent performance and demonstrated a linear range of (10 pg mL^{-1} to 100 ng mL^{-1}) and detection limit (3.84 pg mL^{-1}) [69]. In another study by Shi et al., a novel immunosensor was developed for PSA detection based on the magnified catalytic activity of $K_3[Fe(CN)_6]$. The electrode materials used are the AuPtAg ANCs. The proposed immunosensor performed exceptionally with a detection limit of 0.017 ng/mL and linear range of 0.05 ~ 50 ng/mL, along with excellent reproducibility, stability, and selectivity [68]. This study thus proved to be a positive approach for developing new catalysts for better and promising medical research and diagnosis applications. (Fig. 9).

5. Advantages and disadvantages of nanomaterial-based immunosensors for prostate cancer screening

Nanomaterial-based immunosensors have been demonstrated to possess more detection sensitivity than conventional sensors based on enzymes, allowing rapid and accurate biosensing even for minute concentrations of the sample. With nanotechnology-derived sensors for prostate cancer screening, many of the shortcomings of the conventional screening methods can be addressed: for instance, the requirement for high sample volume, the use of invasive methods like drawing blood samples via syringes, and the need for skilled professionals to handle the samples. These shortcomings hint that the conventional methods are not very user-friendly, nor do they empower the patients to carry out their testing. On the contrary, nanomaterial-based biosensors, like immunosensors, hold promising potential for developing miniaturized, user-friendly devices that can use non-invasive methods and allow patients to carry out cancer screening independently. Furthermore, the high specificity, sensitivity, and selectivity of nanomaterial-based immunosensors can help reduce the chances of false positive or false negative results during prostate cancer screening. In addition, such sensors also help monitor cancer recurrence and even complete recovery [77]. Therefore, nanomaterials like GO and AuNPs, owing to their unique properties like the large surface area, enhanced conductivity, and distinct shapes, can be successfully employed in developing detection platforms like nanoimmunosensors to detect prostate cancer biomarkers. As discussed in the previous sections, nanomaterials can be used to develop cheap, disposable, environmentally friendly sensors with high sensitivity and selectivity for the target analytes.

However, this technology has shortcomings that still need to be addressed. For instance, as cancer progresses, dynamic changes related to the biomarkers occur. Owing to these changes, neither monitoring PSA levels alone may not give accurate results nor will its detection help identify the treatment options to be used for the patient for that particular stage. Therefore, further research needs to be done to identify more prostate cancer biomarkers like prostate-associated proteins, exosomes, and circulating tumour cells that are unique to a given stage of tumour progression so that proper treatment conditions are provided to the patient. Also, different nanomaterials can be combined to improve their sensitivity and selectivity and confer unique properties so that multiple analytes can be detected using a single nanoimmunosensor.

Furthermore, developing biocompatible nanomaterials with lower toxicity is essential to improve their eco-friendly properties. Further miniaturization of nanoimmunosensors will widen the doors towards their clinical application with the development of user-friendly point-of-care devices. Machine learning-based programs can also help quick signal processing during prostate cancer detection using nanoimmunosensors, thereby helping obtain rapid and accurate results. Therefore, further theoretical and experimental knowledge enhancement is necessary to advance this promising field.

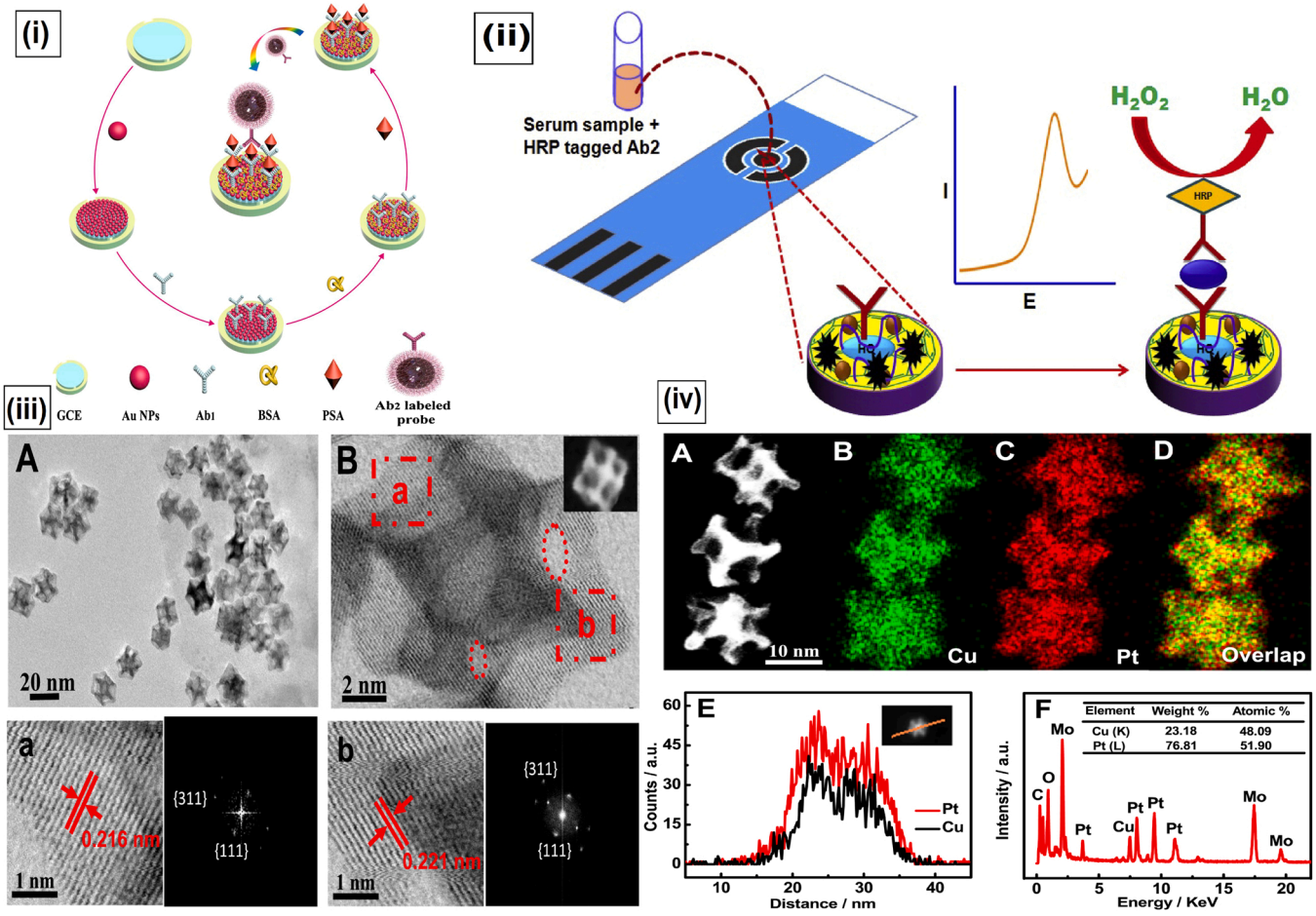

Fig. 9. Au-Pt-Ag alloyed nanocrystals and fabricated immunosensor for sensitive electrochemical detection of PSA. **(i)** The schematic illustration of the designed sandwich-type electrochemical immunosensor. **(ii)** Illustration of PSA sensing in serum sample via paper-based immunosensor as a proof of concept. **(iii)** Medium-magnification TEM images (A) and high-resolution TEM images (B and a–b) of PtCu HNFs. Insets in B show the HAADF-STEM image. Red dotted boxes indicate the disordered areas. **(iv)** HAADF-STEM image (A), the elemental mapping images (B-D), line scanning profiles (E), and EDS spectrum (F) of PtCu HNFs. Insets in E and F show the HAADF-STEM image, and the weight and atomic ratios of Cu and Pt, respectively.
(a) Adapted with permission from ref. [66], copyright@ 2018 (Elsevier). (b) Adapted with permission from ref. [65], copyright@ 2020 (Elsevier). (c) Adapted with permission from ref. [63], copyright@ 2019 (Elsevier).

6. Conclusion

The increasing rates of prostate cancer incidence and mortality among men have become a global concern. The few decades have seen the development of newer cancer screening methods to monitor cancer progression and improve the life quality of the patient. Traditional detection methods like ELISA that rely on antigen-antibody reaction are efficient and can quantify a variety of biomolecules. However, immunoassays tend to present a few unfavourable disadvantages like low selectivity in some cases and low specificity, yielding undesirable results. Therefore, alternative detection methods like immunosensors play a prominent role in the quick and easy diagnosis of prostate cancer based on PSA analysis. In addition, as discussed in the previous sections, the sensitivity, selectivity, and specificity of the immunosensors for target analytes increase with the incorporation of nanomaterials. Hence, nanomaterial-based immunosensors are promising candidates for detecting prostate cancer biomarkers.

Such immunosensors have been shown to demonstrate lower detection limits, simplified protocols, and reduced tools. Various noble metals like gold, silver, and platinum are increasingly used in combination with carbon nanotubes and graphene sheets to meet the highest standards of effectiveness and specificity in detecting PSA in probable patients. Studies have also shown the promising activities of trimetallic compositions of noble metals like Pd-Ct-Cu and Au-Ag-Pt nanocrystals with commendable results. Thus, more efficient NPs can be developed with controllable properties like increased sensitivity and accessibility with further technological advancements.

However, despite the rapid progress in this field, especially at the laboratory level, their clinical applications in natural settings have been poorly reported. Owing to their positive advantages like rapid results, economically feasible, real-time diagnosis, and portable system, their prototype must be practically tested to allow their practical applications in clinical settings. Also, the stability of the nanomaterial-based immunosensors in single or reusable electrodes must be further explored to increase their shelf life. Nanomaterials like GO and AuNPs being biocompatible can be used for in vivo cancer screening. More work needs to be done to understand better the mechanism behind the interaction between the nanomaterials and the target analyte to get more accurate results and decrease the chances of false positives or negative results. With further research, the short-term and long-term effects of nanosensing technologies can be easily mitigated. Thus, efforts should be taken seriously to overcome the difficulties of developing highly sensitive nanocomposites to detect prostate cancer biomarkers. Nanosensing has a pivotal role in shaping the future structure of the diagnostic, pharmaceutical, and healthcare industries.

Funding

No funding is available for this work.

Authors contributions

Equal contribution from author 1 and 2 - Rabia Khan1 and Fareeha Arshad2. All other co-authors listed have made a substantial, direct, and intellectual contribution to the drafting of this manuscript.

All authors have read and approved final version for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

G. A. Naikoo, I. U. Hassan acknowledge the support provided by the Dhofar University, Salalah, Oman.

References

[1] A. Khoo, L.Y. Liu, J.O. Nyalwidhe, O.J. Semmes, D. Vesprini, M.R. Downes, P. C. Boutros, S.K. Liu, T. Kislinger, Proteomic discovery of non-invasive biomarkers of localized prostate cancer using mass spectrometry, Nat. Rev. Urol. (2021), https://doi.org/10.1038/s41585-021-00500-1.

[2] S.W.D. Merriel, G. Funston, W. Hamilton, Prostate cancer in primary care, Adv. Ther. (2018), https://doi.org/10.1007/s12325-018-0766-1.

[3] S. Sandhu, C.M. Moore, E. Chiong, H. Beltran, R.G. Bristow, S.G. Williams, Prostate cancer, Lancet (2021), https://doi.org/10.1016/S0140-6736(21)00950-8.

[4] M. Daniyal, Z.A. Siddiqui, M. Akram, H.M. Asif, S. Sultana, A. Khan, Epidemiology, etiology, diagnosis and treatment of prostate cancer, Asian Pac. J. Cancer Prev. (2014), https://doi.org/10.7314/APJCP.2014.15.22.9575.

[5] H. Barlow, S. Mao, M. Khushi, Predicting high-risk prostate cancer using machine learning methods, Data (2019), https://doi.org/10.3390/data4030129.

[6] F.R. Schumacher, A.A. Al Olama, S.I. Berndt, S. Benlloch, M. Ahmed, E.J. Saunders, T. Dadaev, D. Leongamornlert, E. Anokian, C. Cieza-Borrella, C. Goh, M.N. Brook, X. Sheng, L. Fachal, J. Dennis, J. Tyrer, K. Muir, A. Lophatananon, V.L. Stevens, S. M. Gapstur, B.D. Carter, C.M. Tangen, P.J. Goodman, I.M. Thompson, J. Batra, S. Chambers, L. Moya, J. Clements, L. Horvath, W. Tilley, G.P. Risbridger, H. Gronberg, M. Aly, T. Nordström, P. Pharoah, N. Pashayan, J. Schleutker, T.L. J. Tammela, C. Sipeky, A. Auvinen, D. Albanes, S. Weinstein, A. Wolk, N. Håkansson, C.M.L. West, A.M. Dunning, N. Burnet, L.A. Mucci, E. Giovannucci, G.L. Andriole, O. Cussenot, G. Cancel-Tassin, S. Koutros, L.E. Beane Freeman, K. D. Sorensen, T.F. Orntoft, M. Borre, L. Maehle, E.M. Grindedal, D.E. Neal, J. L. Donovan, F.C. Hamdy, R.M. Martin, R.C. Travis, T.J. Key, R.J. Hamilton, N. E. Fleshner, A. Finelli, S.A. Ingles, M.C. Stern, B.S. Rosenstein, S.L. Kerns, H. Ostrer, Y.J. Lu, H.W. Zhang, N. Feng, X. Mao, X. Guo, G. Wang, Z. Sun, G.G. Giles, M. C. Southey, R.J. MacInnis, L.M. Fitzgerald, A.S. Kibel, B.F. Drake, A. Vega, A. Gómez-Caamaño, R. Szulkin, M. Eklund, M. Kogevinas, J. Llorca, G. Castaño-Vinyals, K.L. Penney, M. Stampfer, J.Y. Park, T.A. Sellers, H.Y. Lin, J.L. Stanford, C. Cybulski, D. Wokolorczyk, J. Lubinski, E.A. Ostrander, M.S. Geybels, B. G. Nordestgaard, S.F. Nielsen, M. Weischer, R. Bisbjerg, M.A. Røder, P. Iversen, H. Brenner, K. Cuk, B. Holleczek, C. Maier, M. Luedeke, T. Schnoeller, J. Kim, C. J. Logothetis, E.M. John, M.R. Teixeira, P. Paulo, M. Cardoso, S.L. Neuhausen, L. Steele, Y.C. Ding, K. De Ruyck, G. De Meerleer, P. Ost, A. Razack, J. Lim, S. H. Teo, D.W. Lin, L.F. Newcomb, D. Lessel, M. Gamulin, T. Kulis, R. Kaneva, N. Usmani, S. Singhal, C. Slavov, V. Mitev, M. Parliament, F. Claessens, S. Joniau, T. Van Den Broeck, S. Larkin, P.A. Townsend, C. Aukim-Hastie, M.G. Dominguez, J. E. Castelao, M.E. Martinez, M.J. Roobol, G. Jenster, R.H.N. Van Schaik, F. Menegaux, T. Truong, Y.A. Koudou, J. Xu, K.T. Khaw, L. Cannon-Albright, H. Pandha, A. Michael, S.N. Thibodeau, S.K. McDonnell, D.J. Schaid, S. Lindstrom, C. Turman, J. Ma, D.J. Hunter, E. Riboli, A. Siddiq, F. Canzian, L.N. Kolonel, L. Le Marchand, R.N. Hoover, M.J. Machiela, Z. Cui, P. Kraft, C.I. Amos, D.V. Conti, D. F. Easton, F. Wiklund, S.J. Chanock, B.E. Henderson, Z. Kote-Jarai, C.A. Haiman, R. A. Eeles, Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci, Nat. Genet. (2018), https://doi.org/10.1038/s41588-018-0142-8.

[7] J. Kurhanewicz, D. Vigneron, P. Carroll, F. Coakley, Multiparametric magnetic resonance imaging in prostate cancer: Present and future, Curr. Opin. Urol. (2008), https://doi.org/10.1097/MOU.0b013e3282f19d01.

[8] J.J. Fütterer, A. Briganti, P. De Visschere, M. Emberton, G. Giannarini, A. Kirkham, S.S. Taneja, H. Thoeny, G. Villeirs, A. Villers, Can clinically significant prostate cancer be detected with multiparametric magnetic resonance imaging? a systematic review of the literature, Eur. Urol. (2015), https://doi.org/10.1016/j.eururo.2015.01.013.

[9] M.Y. Teo, D.E. Rathkopf, P. Kantoff, Treatment of advanced prostate cancer, Annu. Rev. Med. (2019), https://doi.org/10.1146/annurev-med-051517-011947.

[10] C. Ritch, M. Cookson, Recent trends in the management of advanced prostate cancer, F1000Research (2018), https://doi.org/10.12688/f1000research.15382.1.

[11] S.C. Kamran, A.V. D'Amico, Radiation therapy for prostate cancer, Hematol. Oncol. Clin. North Am. (2020), https://doi.org/10.1016/j.hoc.2019.08.017.

[12] Z. Akbari jonous, J.S. Shayeh, F. Yazdian, A. Yadegari, M. Hashemi, M. Omidi, An electrochemical biosensor for prostate cancer biomarker detection using graphene oxide–gold nanostructures, Eng. Life Sci. (2019), https://doi.org/10.1002/elsc.201800093.

[13] F.S. Tabar, M. Pourmadadi, H. Rashedi, F. Yazdian, Design of Electrochemical Nanobiosensor in the Diagnosis of Prostate Specific Antigen (PSA) Using Nanostructures, in: 27th Natl. 5th Int. Iran. Conf. Biomed. Eng. ICBME 2020, 2020. ⟨https://doi.org/10.1109/ICBME51989.2020.9319418⟩.

[14] L.W. Chu, J. Ritchey, S.S. Devesa, S.M. Quraishi, H. Zhang, A.W. Hsing, Prostate Cancer Incidence Rates in Africa, Prostate Cancer (2011), https://doi.org/10.1155/2011/947870.

[15] P. Kheirandish, F. Chinegwundoh, Ethnic differences in prostate cancer, Br. J. Cancer (2011), https://doi.org/10.1038/bjc.2011.273.

[16] C.H. Pernar, E.M. Ebot, K.M. Wilson, L.A. Mucci, The epidemiology of prostate cancer, Cold Spring Harb. Perspect. Med. (2018), https://doi.org/10.1101/CSHPERSPECT.A030361.

[17] Global cancer observatory, Prostate Cancer fact sheet Globocan 2020, Cancer Today. (2020).

[18] D. Il Kang, J. Il Chung, H.K. Ha, K. Min, J. Yoon, W. Kim, W.I. Seo, P.M. Kang, S. J. Jung, I.Y. Kim, Korean prostate cancer patients have worse disease characteristics than their American Counterparts, Asian Pac. J. Cancer Prev. (2013), https://doi.org/10.7314/APJCP.2013.14.11.6913.

[19] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Piñeros, A. Znaor, F. Bray, Global cancer observatory: cancer tomorrow. Int. J. Cancer, International Agency for Research on Cancer, Lyon, France, 2019. ⟨https://gco.iarc.fr/tomorrow⟩.

[20] S.W. Oh, Y.M. Kim, H.J. Kim, S.J. Kim, J.S. Cho, E.Y. Choi, Point-of-care fluorescence immunoassay for prostate specific antigen, Clin. Chim. Acta (2009), https://doi.org/10.1016/j.cca.2009.04.013.

[21] D.C. Pérez-Ibave, C.H. Burciaga-Flores, M.Á. Elizondo-Riojas, Prostate-specific antigen (PSA) as a possible biomarker in non-prostatic cancer: a review, Cancer Epidemiol. (2018), https://doi.org/10.1016/j.canep.2018.03.009.

[22] J.W.A. Findlay, W.C. Smith, J.W. Lee, G.D. Nordblom, I. Das, B.S. Desilva, M. N. Khan, R.R. Bowsher, Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective, J. Pharm. Biomed. Anal. (2000), https://doi.org/10.1016/S0731-7085(99)00244-7.

[23] I.A. Darwish, Immunoassay methods and their applications in pharmaceutical analysis: basic methodology and recent advances, Int. J. Biomed. Sci. (2006).

[24] J.J. Bao, Capillary electrophoretic immunoassays, J. Chromatogr. B Biomed. Appl. (1997), https://doi.org/10.1016/S0378-4347(97)00177-1.

[25] S. Ahmed, J. Ning, D. Peng, T. Chen, I. Ahmad, A. Ali, Z. Lei, M. Abu bakr Shabbir, G. Cheng, Z. Yuan, Current advances in immunoassays for the detection of antibiotics residues: a review, Food Agric. Immunol. (2020), https://doi.org/10.1080/09540105.2019.1707171.

[26] J.R. Crowther, The ELISA guidebook, Methods Mol. Biol. (2000), https://doi.org/10.1385/1592590497.

[27] Y. Gao, X. Huang, Y. Zhu, Z. Lv, A brief review of monoclonal antibody technology and its representative applications in immunoassays, J. Immunoass. Immunochem. (2018), https://doi.org/10.1080/15321819.2018.1515775.

[28] M.S. Tabatabaei, R. Islam, M. Ahmed, Applications of gold nanoparticles in ELISA, PCR, and immuno-PCR assays: a review, Anal. Chim. Acta (2021), https://doi.org/10.1016/j.aca.2020.08.030.

[29] L. Cinquanta, D.E. Fontana, N. Bizzaro, Chemiluminescent immunoassay technology: what does it change in autoantibody detection? Autoimmun. Highlights (2017) https://doi.org/10.1007/s13317-017-0097-2.

[30] M.A.U. Azim, M. Hasan, I.H. Ansari, F. Nasreen, Chemiluminescence immunoassay: basic mechanism and applications, Bangladesh J. Nucl. Med. (2018), https://doi.org/10.3329/bjnm.v18i2.35240.

[31] D. Wild, Immunoassay for beginners, Immunoass. Handb. (2013), https://doi.org/10.1016/B978-0-08-097037-0.00002-6.

[32] R.S. Yalow, S.A. Berson, Immunoassay of endogenous plasma insulin in man, Obes. Res (1996), https://doi.org/10.1002/j.1550-8528.1996.tb00274.x.

[33] K. Wakabayash, Radioimmunoassay, J. Synth. Org. Chem. Jpn. (1980), https://doi.org/10.5059/yukigoseikyokaishi.38.139.

[34] W.A. Banks, Peptides and the blood-brain barrier, Peptides (2015), https://doi.org/10.1016/j.peptides.2015.03.010.

[35] I.H. Cho, J. Lee, J. Kim, M.S. Kang, J.K. Paik, S. Ku, H.M. Cho, J. Irudayaraj, D. H. Kim, Current technologies of electrochemical immunosensors: Perspective on signal amplification, Sens. (Switz.). ((2018), https://doi.org/10.3390/s18010207.

[36] R. Zumpano, F. Polli, C. D'Agostino, R. Antiochia, G. Favero, F. Mazzei, Nanostructure-based electrochemical immunosensors as diagnostic tools, Electrochem (2021), https://doi.org/10.3390/electrochem2010002.

[37] S. Lara, A. Perez-Potti, Applications of nanomaterials for immunosensing, Biosensors (2018), https://doi.org/10.3390/bios8040104.

[38] J.V. Veetil, K. Ye, Development of immunosensors using carbon nanotubes, Biotechnol. Prog. (2007), https://doi.org/10.1021/bp0602395.

[39] L. Tian, L. Liu, Y. Li, Q. Wei, W. Cao, 3D sandwich-type prostate specific antigen (PSA) immunosensor based on rGO-MWCNT-Pd nanocomposite, N. J. Chem. (2015), https://doi.org/10.1039/c5nj00355e.

[40] G. Darabdhara, M.R. Das, S.P. Singh, A.K. Rengan, S. Szunerits, R. Boukherroub, Ag and Au nanoparticles/reduced graphene oxide composite materials: Synthesis and application in diagnostics and therapeutics, Adv. Colloid Interface Sci. (2019), https://doi.org/10.1016/j.cis.2019.101991.
[41] A. Pandey, Y. Gurbuz, V. Ozguz, J.H. Niazi, A. Qureshi, Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic E. coli O157:H7, Biosens. Bioelectron. (2017), https://doi.org/10.1016/j.bios.2016.12.041.
[42] Y. Si, E.T. Samulski, Exfoliated graphene separated by platinum nanoparticles, Chem. Mater. (2008), https://doi.org/10.1021/cm801356a.
[43] R. Pasricha, S. Gupta, A.K. Srivastava, A facile and novel synthesis of Ag-graphene-based nanocomposites, Small (2009), https://doi.org/10.1002/smll.200900726.
[44] F. Laghrib, S. Saqrane, Y. El Bouabi, A. Farahi, M. Bakasse, S. Lahrich, M.A. El Mhammedi, Current progress on COVID-19 related to biosensing technologies: New opportunity for detection and monitoring of viruses, Microchem. J. (2021), https://doi.org/10.1016/j.microc.2020.105606.
[45] J.-N. Chazalviel, P. Allongue, On the origin of the efficient nanoparticle mediated electron transfer across a self-assembled monolayer, J. Am. Chem. Soc. (2011), https://doi.org/10.1021/ja109295x.
[46] B. Wei, K. Mao, N. Liu, M. Zhang, Z. Yang, Graphene nanocomposites modified electrochemical aptamer sensor for rapid and highly sensitive detection of prostate specific antigen, Biosens. Bioelectron. (2018), https://doi.org/10.1016/j.bios.2018.08.067.
[47] J. Zhou, J. Ralston, R. Sedev, D.A. Beattie, Functionalized gold nanoparticles: synthesis, structure and colloid stability, J. Colloid Interface Sci. (2009), https://doi.org/10.1016/j.jcis.2008.12.002.
[48] L.A. Al-Ani, M.A. AlSaadi, F.A. Kadir, N.M. Hashim, N.M. Julkapli, W.A. Yehye, Graphene– gold based nanocomposites applications in cancer diseases; Efficient detection and therapeutic tools, Eur. J. Med. Chem. (2017), https://doi.org/10.1016/j.ejmech.2017.07.036.
[49] M. Tian, Z. Yuan, Y. Liu, C. Lu, Z. Ye, L. Xiao, Recent advances of plasmonic nanoparticle-based optical analysis in homogeneous solution and at the single-nanoparticle level, Analyst (2020), https://doi.org/10.1039/d0an00609b.
[50] Z. Ye, R. Weng, Y. Ma, F. Wang, H. Liu, L. Wei, L. Xiao, Label-free, single-particle, colorimetric detection of permanganate by GNPs@Ag core-shell nanoparticles with dark-field optical microscopy, Anal. Chem. (2018), https://doi.org/10.1021/acs.analchem.8b04024.
[51] D. Chen, Z. Song, M. Lian, Y. Yang, S. Lin, L. Xiao, Single-particle fibrinogen detection using platelet membrane-coated fluorescent polystyrene nanoparticles, Nanoscale (2021), https://doi.org/10.1039/d0nr08492a.
[52] F. Wang, Y. Han, S. Wang, Z. Ye, L. Wei, L. Xiao, Single-particle LRET aptasensor for the sensitive detection of aflatoxin B1 with upconversion nanoparticles, Anal. Chem. (2019), https://doi.org/10.1021/acs.analchem.9b02599.
[53] F. Wang, Y. Li, Y. Han, Z. Ye, L. Wei, H. Bin Luo, L. Xiao, Single-particle enzyme activity assay with spectral-resolved dark-field optical microscopy, Anal. Chem. (2019), https://doi.org/10.1021/acs.analchem.9b01300.
[54] F. Qi, Y. Han, Z. Ye, H. Liu, L. Wei, L. Xiao, Color-coded single-particle pyrophosphate assay with dark-field optical microscopy, Anal. Chem. (2018), https://doi.org/10.1021/acs.analchem.8b03211.
[55] F. Qi, Y. Han, H. Liu, H. Meng, Z. Li, L. Xiao, Localized surface plasmon resonance coupled single-particle galactose assay with dark-field optical microscopy, Sens. Actuators, B Chem. (2020), https://doi.org/10.1016/j.snb.2020.128347.
[56] T. Feng, Y. Wang, X. Qiao, Recent advances of carbon nanotubes-based electrochemical immunosensors for the detection of protein cancer biomarkers, Electroanalysis (2017), https://doi.org/10.1002/elan.201600512.
[57] S. Ji, M. Lee, D. Kim, Detection of early stage prostate cancer by using a simple carbon nanotube@paper biosensor, Biosens. Bioelectron. (2018), https://doi.org/10.1016/j.bios.2017.11.035.
[58] M. Sharifi, M.R. Avadi, F. Attar, F. Dashtestani, H. Ghorchian, S.M. Rezayat, A. A. Saboury, M. Falahati, Cancer diagnosis using nanomaterials based electrochemical nanobiosensors, Biosens. Bioelectron. (2019), https://doi.org/10.1016/j.bios.2018.11.026.
[59] B. Kavosi, A. Salimi, R. Hallaj, F. Moradi, Ultrasensitive electrochemical immunosensor for PSA biomarker detection in prostate cancer cells using gold nanoparticles/PAMAM dendrimer loaded with enzyme linked aptamer as integrated triple signal amplification strategy, Biosens. Bioelectron. (2015), https://doi.org/10.1016/j.bios.2015.07.064.
[60] R. Akter, M.A. Rahman, C.K. Rhee, Amplified electrochemical detection of a cancer biomarker by enhanced precipitation using horseradish peroxidase attached on carbon nanotubes, Anal. Chem. (2012), https://doi.org/10.1021/ac300110n.
[61] D. Pedone, M. Moglianetti, E. De Luca, G. Bardi, P.P. Pompa, Platinum nanoparticles in nanobiomedicine, Chem. Soc. Rev. (2017), https://doi.org/10.1039/c7cs00152e.
[62] A.L. Stepanov, A.N. Golubev, S.I. Nikitin, Y.N. Osin, A review on the fabrication and properties of platinum nanoparticles, Rev. Adv. Mater. Sci. (2014).
[63] Y. Chen, P.X. Yuan, A.J. Wang, X. Luo, Y. Xue, L. Zhang, J.J. Feng, A novel electrochemical immunosensor for highly sensitive detection of prostate-specific antigen using 3D open-structured PtCu nanoframes for signal amplification, Biosens. Bioelectron. (2019), https://doi.org/10.1016/j.bios.2018.10.057.
[64] F. Nosheen, Z.C. Zhang, J. Zhuang, X. Wang, One-pot fabrication of single-crystalline octahedral Pt-Cu nanoframes and their enhanced electrocatalytic activity, Nanoscale (2013), https://doi.org/10.1039/c3nr00833a.
[65] L. Suresh, J.S. Bondili, P.K. Brahman, Development of proof of concept for prostate cancer detection: an electrochemical immunosensor based on fullerene-C60 and copper nanoparticles composite film as diagnostic tool, Mater. Today Chem. (2020), https://doi.org/10.1016/j.mtchem.2020.100257.
[66] M. Li, P. Wang, F. Pei, H. Yu, Y. Dong, Y. Li, Q. Liu, P. Chen, A novel signal amplification system fabricated immunosensor based on Au nanoparticles and mesoporous trimetallic PdPtCu nanospheres for sensitive detection of prostate specific antigen, Sens. Actuators, B Chem. (2018), https://doi.org/10.1016/j.snb.2018.01.136.
[67] H. Ma, X. Li, T. Yan, Y. Li, Y. Zhang, D. Wu, Q. Wei, B. Du, Electrochemiluminescent immunosensing of prostate-specific antigen based on silver nanoparticles-doped Pb (II) metal-organic framework, Biosens. Bioelectron. (2016), https://doi.org/10.1016/j.bios.2015.12.080.
[68] Y.C. Shi, A.J. Wang, P.X. Yuan, L. Zhang, X. Luo, J.J. Feng, Highly sensitive label-free amperometric immunoassay of prostate specific antigen using hollow dendritic AuPtAg alloyed nanocrystals, Biosens. Bioelectron. (2018), https://doi.org/10.1016/j.bios.2018.03.052.
[69] L. Jiang, Y. Li, Z. Gao, P. Wang, D. Li, Y. Dong, Sensitive detection of prostate specific antigen based on copper ions doped ag-au nanospheres labeled immunosensor, J. Electrochem. Soc. (2019), https://doi.org/10.1149/2.1341915jes.
[70] C.I.L. Justino, A.R. Gomes, A.C. Freitas, A.C. Duarte, T.A.P. Rocha-Santos, Graphene based sensors and biosensors, TrAC - Trends Anal. Chem. (2017), https://doi.org/10.1016/j.trac.2017.04.003.
[71] M. Yan, D. Zang, S. Ge, L. Ge, J. Yu, A disposable electrochemical immunosensor based on carbon screen-printed electrodes for the detection of prostate specific antigen, Biosens. Bioelectron. (2012), https://doi.org/10.1016/j.bios.2012.06.019.
[72] X. Zhou, L. Yang, X. Tan, G. Zhao, X. Xie, G. Du, A robust electrochemical immunosensor based on hydroxyl pillar[5]arene@AuNPs@g-C3N4 hybrid nanomaterial for ultrasensitive detection of prostate specific antigen, Biosens. Bioelectron. (2018), https://doi.org/10.1016/j.bios.2018.04.036.
[73] L. Agüí, P. Yáñez-Sedeño, J.M. Pingarrón, Role of carbon nanotubes in electroanalytical chemistry, A Rev., Anal. Chim. Acta (2008), https://doi.org/10.1016/j.aca.2008.05.070.
[74] N. Salahuddin, M. Elfiky, A. Matsuda, Sensors and biosensors nanocomposites based on polymer/inorganic nanostructures, Handb. Polym. Nanocomposites Ind. Appl. (2021), https://doi.org/10.1016/b978-0-12-821497-8.00027-7.
[75] H.D. Jang, S.K. Kim, H. Chang, J.W. Choi, 3D label-free prostate specific antigen (PSA) immunosensor based on graphene-gold composites, Biosens. Bioelectron. (2015), https://doi.org/10.1016/j.bios.2014.08.008.
[76] F. Farshchi, M. Hasanzadeh, E. Solhi, Immunosensing of prostate cancer in human plasma samples using immobilization of antibody on the surface of mesoporous silica-modified silver nanoparticles and its immunocomplex with prostate-specific antigen, Anal. Methods (2019), https://doi.org/10.1039/c9ay02058f.
[77] B.J. Kang, M. Jeun, G.H. Jang, S.H. Song, I.G. Jeong, C.S. Kim, P.C. Searson, K. H. Lee, Diagnosis of prostate cancer via nanotechnological approach, Int. J. Nanomed. (2015), https://doi.org/10.2147/IJN.S91908.