

# **Advanced Nanoengineered- Customized Point-of-Care Tools for Prostate-Specific Antigen**

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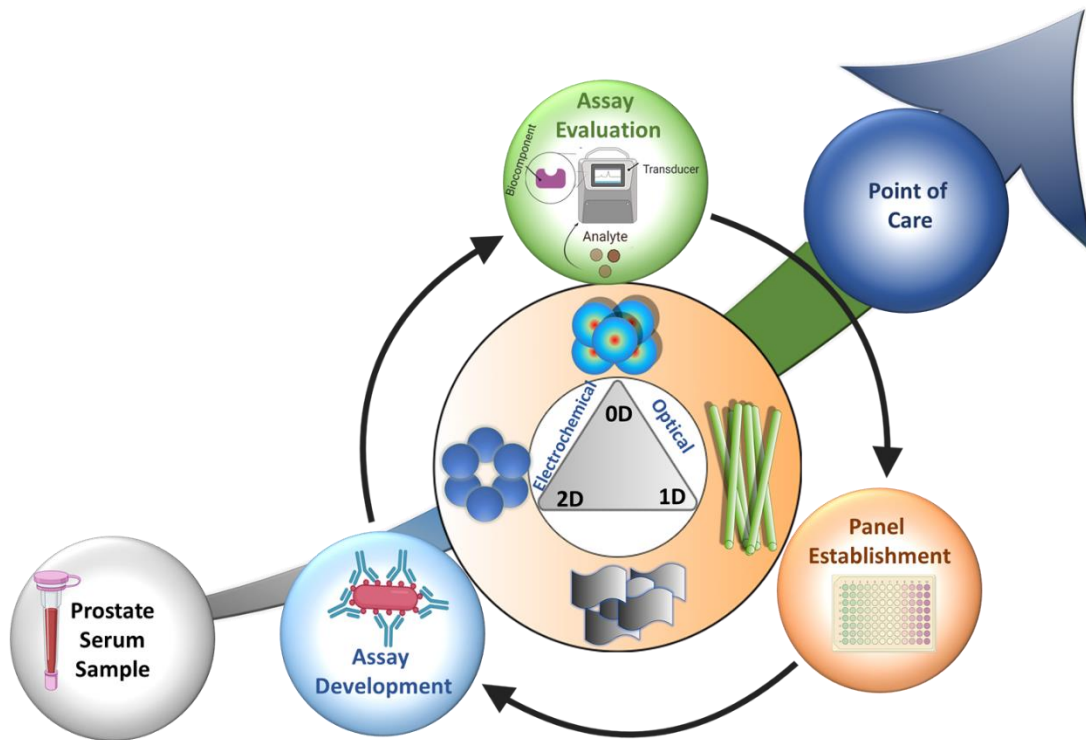
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# Graphical



## **Abstract**

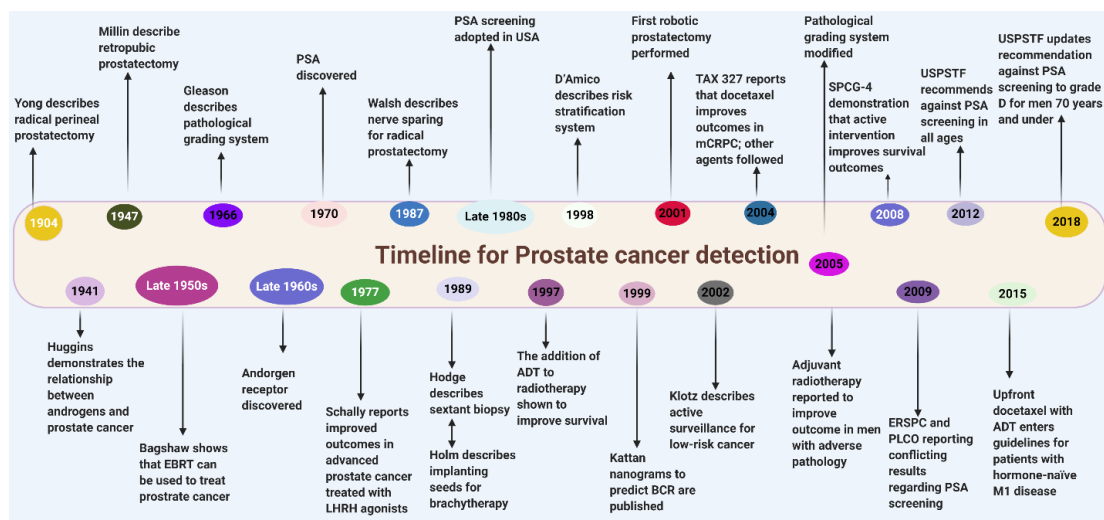
Change in the level of human prostate-specific antigen (PSA) is a major element in the development and progression of prostate cancer (PCa). Most of the methodologies are currently restricted to their application in routine clinical screening due to the scarcity of adequate screening tools, false reading, longer assay time, and cost. Innovative techniques and the integration of knowledge from a variety of domains, such as materials science and engineering, are needed to provide long-term solutions. The convergence of precision point-of-care (POC) diagnostic techniques, which allow patients to respond in real time to changes in PSA levels, provide promising possibilities for quantitative and quantitative detection of PSA. This solution could be interesting and relevant for use in PCa diagnosis at the POC. The approaches enable low-cost real-time detection and are simple to integrate into user-friendly sensor devices. This Review focuses on the investigations, prospects, and challenges associated with integrating engineering sciences with cancer biology to develop nanotechnology-based tools for PCa diagnosis. This article is intended to encourage the development of new nanomaterials to construct high-performance POC devices for PCa detection. Finally, the Review concludes with closing remarks and a perspective forecast.

**Keywords:** Prostate cancer, Prostate-specific antigen (PSA), Screening, Nanomaterials, Point-of-care devices

# 1. Introduction

## 1.1. Prostate cancer

Prostate cancer (PCa) is the second most frequently diagnosed cancer in man, with approximately 1.2 million new cases and over 350,000 deaths yearly. Statistically, 1 in 7 men will be diagnosed with PCa at some point in their lives [1]. PCa is a biologically and clinically heterogeneous disease, with several known histotypes and molecular subtypes with distinct etiologies, risk factor profiles, treatment responses, and prognoses [2]. In contrast, PCa has a very good overall survival rate (98 %) in man diagnosed with PCa will live at least five years following their preliminary diagnosis, and 65–90 % will live for at least ten years [3]. Extensive work in PCa has significant outcomes that have shaped our understanding of and managing this disorder, as shown in **Figure 1**.



**Figure 1:** Major milestones in the development of diagnosis and prevention of prostate cancer.

Despite an increase in the prevalence of PCa, death rates have gradually declined due to earlier detection and better treatment. PCa incidence has decreased in parallel with the development of extensive cancer routine screening, the increased use of diagnostic testing, and the widespread use of improved imaging technology [2]. The most relied diagnostic methods are based on scanning of anatomic [4], functional and molecular imaging information [5]. The

different detection techniques include Magnetic Resonance Techniques (MRI) [6], Computed Tomography (CT) [7], Transrectal Ultrasonography (TRUS) [8, 9], Positron Emission Tomography (PET) [10], Radionuclide Imaging [11] and Positron Emission Tomography/Computed Tomography (PET/CT) [12]. However, these diagnoses demand a significant number of professional expertise at a high cost.

None of these procedures are capable of differentiating between aggressive and indolent PCa. However, such procedures, are time-consuming, subjective, and may not be accurate at disease initiation stage. In 1986, the US FDA approved the screening of PCa in the male by high serum level of prostate-specific antigen (PSA). PSA is an important component of PCa for constant monitoring and identification of disease situations, especially in its forms (complex or free) or the combination with other biomarkers [13]. Since the approval of PSA by US FDA, PSA is getting more controversial as a PCa biomarker. PSA is not specific, hence 75% of men get unnecessary biopsies due to raised PSA level. Multiple needles are used in biopsies, although small tumours may be undetected. These limitations lead to false positives and negatives result, along with contradictory clinical trial findings [14]. Other limitation of PSA as PCa biomarker is its inability to give a clear difference between benign and malignant cancer [15]. PSA has limited sensitivity for detecting PCa. It is mainly when the total PSA (tPSA) level is less than 10 ng/mL. The possibility of PCa in males with tPSA lies between 4.1- 9.9 ng/mL, and negative DRE is about 20%, with the probability of 85% [16]. It also leads other complications like hematuria, rectal bleeding, and urinary tract infection [17].

Frequent detection can be expensive, time consuming, and expose patients to unnecessary biopsies. So, there is an urgent need to resolve these constraints and establish a real win-win scenario in which better sensitivity and selectivity for PSA can be accomplished synergistically at reduced costs. Contrary to the conventional time-consuming and costly methods, POC can be a good alternative. The ideal POC sensor would require small sample volumes, be

inexpensive per test, have a quick turnaround time, be easily used in all necessary locations, and require no training to operate.

In this context, the development of electrochemical and optical immunosensors based on nanomaterials are the best alternative analytical technologies for accurate detection of PSA [18]. Not surprisingly, the use of nanomaterials is one of the most significant ways to boost the selectivity and sensitivity of sensor for PSA. This Review, however, concentrates on nanomaterials with excellent optical and electrochemical properties in sensing PSA. The strengths and pitfalls of each approach are discussed and criticized. We also discuss challenges to translating these technologies from the laboratory to the POC. Future perspective with relation to the state-of-art patient-friendly PSA-based diagnosis is presented and discussed.

## **2. Point-of-Care (POC) based detection modalities: A trend toward better management of PCa**

Early diagnosis of PCa is a significant clinical concern due to its poor prognosis and late-stage diagnosis. When a disease is in its early stages, only trace amounts of biomarkers are available; therefore, the reliability and sensitivity of screening analyses are critical. Before a novel detection technology to be seriously considered for commercialization, it must be at least three orders of magnitude more reliable than the currently available state of the art [19].

The identification of a single biomarker demands a high level of sensitivity and specificity due to its complexity. Existing detection approaches are based on rigorous experimental steps. With the advancement of clinical diagnostics, current biosensing eventually facilitates "self-use" or usage by a general practitioner. Accordingly, a highly sensitive and quantitative biosensor with POC features is required for use in the diagnosis and treatment of PCa. The testing modality, known as a POC diagnostic device, should ideally be cost-effective, fast, functional without unnecessary sample pre-processing, highly sensitive to identify cancer at an early stage, and specific to prevent over-diagnosis, misdiagnosis, or missed-diagnosis [20]. As a result, the POC would allow for fast clinical decision-making in the diagnosis of PCa, which would

significantly improve patient outcomes by allowing for early treatment and medical intervention.

Recently, optical and electrochemical have shown emerging potential for POC diagnostics. Optical sensors have been studied for their ease of use, high sensitivity, and rapid detection of PSA. Different optical detection methods are used for PSA like colourimetric, electrochemiluminescence, fluorescence, localized surface plasmon resonances (LSPRs), Photoelectrochemical, Surface-enhanced Raman spectroscopy (SERS) methods have usually been used for recognising PSA [21].

Electrochemical detection of clinically required biomolecules is the most often used technology, among others. It is usually inexpensive, simple to apply, and provides high sensitivity, a significant linear range, and low LOD [22]. In the initial stage of PCa analysis, several voltammetry techniques such as differential pulse voltammetry (DPV), cyclic voltammetry (CV), electrical impedance spectroscopy (EIS), and square wave voltammetry (SWV) have been reported to detect PSA (Numan et al., 2020, 2017; Singh et al., 2020b).

This paper presents recent examples of optical and electrochemical biosensors, along with their advantages and limitations.

### **2.1. Electrochemical biosensor detection strategies**

Electrochemical detection has considerable potential for clinical applications due to its sensitivity, speed, simplicity, and low cost. Electrochemical transduction is based on electrochemical reactions that occur during biorecognition process. In this procedure, changes in an electrical signal are based on electrochemical reactions that occur on the surface of an electrode. The changes are generally carried on by imposed potential, current, or frequency. Electrochemical biosensing systems offer user-friendly platforms for monitoring biological processes. It comparatively requires a small sample and is easy to miniaturize [22].

## **Voltammetry/amperometry**

CV and DPV are the most versatile and widely used voltammetry techniques for identifying PSA. In several fields of chemistry and biochemistry, CV is becoming an essential tool. It is generally employed in the research of redox processes as well as the understanding of reaction intermediates. This approach monitors current (limited by analyte diffusion at the electrode surface) while altering the potential over a fixed range in a forward and backward direction [27].

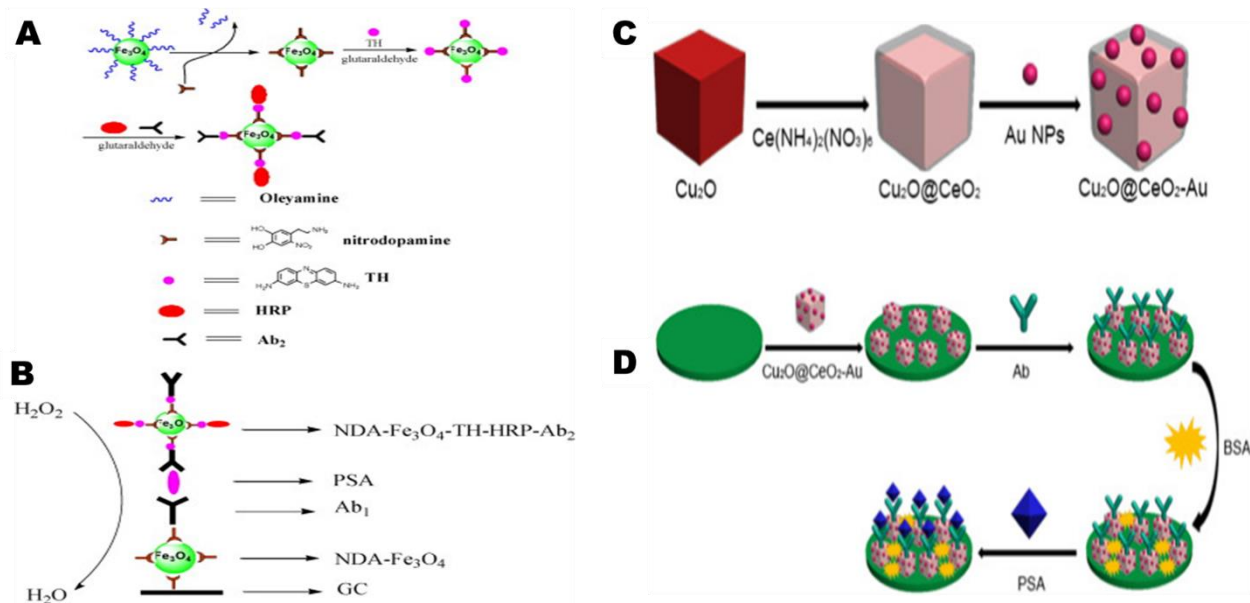
The voltametric and amperometry technologies are distinguished by applying the working electrode (or indicator) potential over the reference electrode and measuring the current. The induction of current is done through electrolysis via electrochemical reduction or oxidation on the working electrode. The rate of molecular mass transport constrains the current in electrolysis to the electrode [28]. Analytical sensitivity is increased by eliminating the capacitive background signal in voltammetric procedures.

The most widely used voltammetry techniques include linear voltammetry, DPV, stripping voltammetry, polarography, AC and square-wave voltammetry. All these approaches have a broad dynamic range and are sensitive to low-level quantitation [29]. When it comes to electrochemical sensing, CV is mostly preferred for detection. In this context, electrochemical immunoassay based on nitrodopamine (NDA) functionalized iron oxide nanoparticles (NDA-Fe<sub>3</sub>O<sub>4</sub>) was developed by Li et al. [30]. It immobilised both the primary anti-PSA antibody (Ab1) and the secondary anti-PSA antibody (Ab2) label. Mediator thionine (TH) was initially conjugated to NDA - Fe<sub>3</sub>O<sub>4</sub> based on NDA amino groups to detect PSA. Next, the amino group TH was used to immobilize Ab2 and horseradish peroxidase (HRP) as shown in **Figure 2** (A & B). Since a large NDA volume was attached to the surface of Fe<sub>3</sub>O<sub>4</sub>, the antibodies and mediator and enzyme loading to the NDA-Fe<sub>3</sub>O<sub>4</sub> were significantly increased. This resulted in



an increased sensitivity of immunosensor quantified by CV. Linearity was reported in the range of 0.005-50 ng/mL with LOD 4 pg/mL. A molecularly imprinted polymer was reported by Yazdani et al. [31], for detection of PSA. It was developed by electrochemical polymerization of the pyrrole on a screen-printed gold electrode in the presence of PSA. PSA served as a molecular template for the polymer. DPV was used to measure the fabricated nano-biosensor, and  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  was used as an electrochemical marker. The nano-biosensor displayed a rapid rebinding rate and excellent PSA recognition ability with a DL of 2.0 pg mL<sup>-1</sup>.

In amperometry, alterations in the current produced by electrochemical oxidation or reduction are controlled directly across time. Because of its lower detection limit and simplicity, amperometry measurement is widely used with biocatalytic and affinity sensors. To this, core-shell nanocomposites of the amino-functionalized cuprous oxide@ceric dioxide ( $Cu_2O@CeO_2-NH_2$ ) were developed to bond gold nanoparticles (AuNPs) by establishing stable Au-N bonds between Au NPs and  $-NH_2$ . Since the synergistic effect was present in the core-shell  $Cu_2O@CeO_2$  filled with Au NPs ( $Cu_2O@CeO_2-Au$ ). It demonstrated a more robust electrocatalytic behaviour towards hydrogen peroxide reduction ( $H_2O_2$ ) than single  $Cu_2O$ , Au NPs, and  $Cu_2O@CeO_2$ . The developed immunosensor showed a wide linear range of 0.1 pg mL<sup>-1</sup> to 100 ng mL<sup>-1</sup> with a low DL of 0.03 pg mL<sup>-1</sup> under ideal conditions **Figure 2** (C & D) [32].



**Figure 2.** Schematic representation of (A) the preparation of the NDA-Fe<sub>3</sub>O<sub>4</sub>-TH-HRP-Ab<sub>2</sub> (B) immunosensor (C) Method of preparation of Cu<sub>2</sub>O@CeO<sub>2</sub>-Au; (D) The schematic description of the label-free amperometric immunosensor. Reprinted with permission from [32].

### Electrical impedance spectroscopy (EIS)

EIS measures the dielectric properties as a frequency function. According to Bhansali and colleagues, the successful fabrication of an impedance-based miniaturized biosensor was developed by utilizing photolithographic techniques. In this, the human PSA monoclonal antibodies were used as capturing primary antibodies. The newly developed biosensor detects PSA in predicted and actual human plasma with varying PSA concentrations in an ultrasensitive manner. EIS was used for the identification. The sensor had a LOD of 1 pg mL<sup>-1</sup> for PSA with limited non-specific binding (NSB) [33].

Following the development of the Ab-Ag immunocomplex, the electrochemical response of the immunosensor to the target is based on the interaction of the electroactive nanomaterial with the target surface. Numerous non-labelled@label-free electrochemical immunosensors

have been described in the past few decades to detect PCa biomarkers. Binding a nanoparticle to a biomarker for cancer will improve current, ohmic response, and potential. Such electrical or electrochemical modifications make it possible to identify and measure biomarkers. Owing to specific nanoparticles' electrical properties, their target fixation is accompanied by a chemical reaction (i.e., oxidation-reduction), which can be quantified by the signal induced [34].

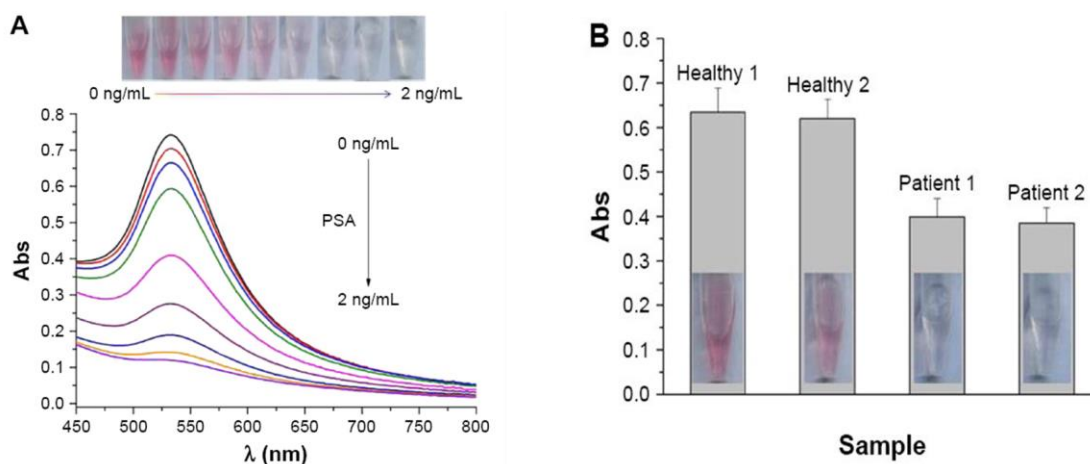
## **2.2. Optical techniques-based PSA detection**

PSA detection has been reported using a range of different approaches in addition to electrochemical techniques. Optical biosensing is used to detect PSA. It has many advantages, including precise and fast quantification, high specificity, easy downsizing, and real-time monitoring of bispecific interaction [35]. Over the past few decades, the optical biosensing approaches, including fluorescent, colorimetric, electrochemiluminescence, fluorescence, localized surface plasmon resonances (LSPRs), Photoelectrochemical, Surface-enhanced Raman spectroscopy (SERS) methods have usually been used for recognising PSA. Optical biosensing systems have features like real-time and label-free measurement, high specificity or sensitivity, small size, minute reactants, and cost-effectiveness have been considered one of the most conventional techniques. These biosensing platforms can be showed significant developments such as fast, sensitive and selective determination.

### **Colorimetric Sensing**

Among various methods for biomolecule detection, colorimetric detection is desirable for its visible radiation, easy operation, and rapid reading. Nanomaterial-based colorimetric immunoassays are commonly based on a change in the optical properties of the nanomaterial due to aggregation or morphology transition or a colour change produced by enzyme-assisted nanoparticle amplification. Among these methods, gold nanoparticles (AuNPs)-based colorimetric assays have been in demand for detection of PSA in the last 20 years. It is because

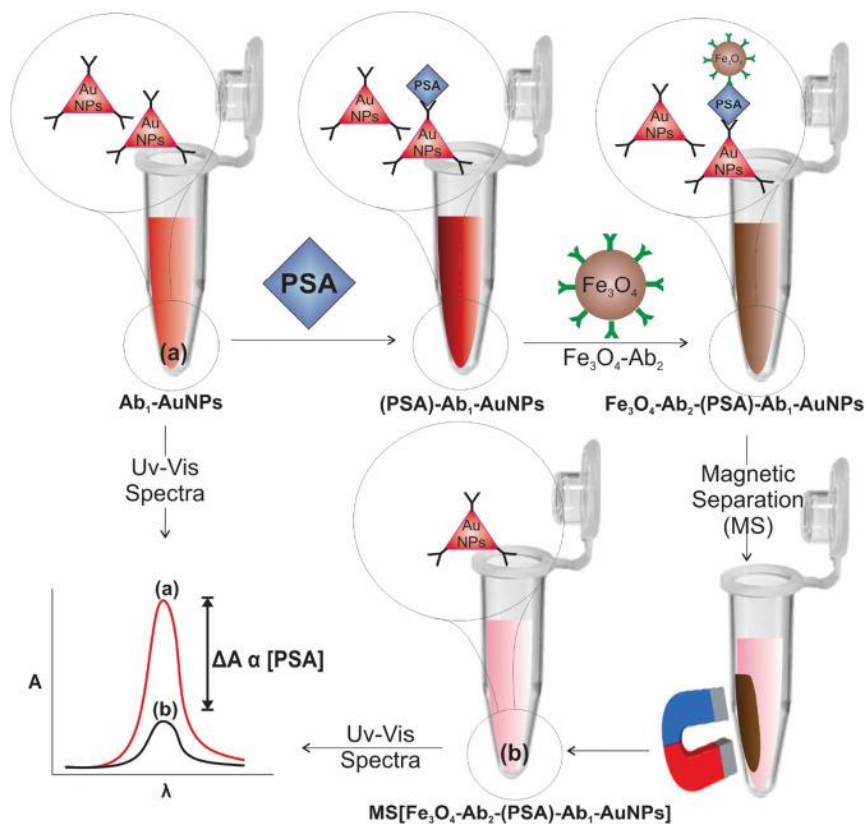
AuNPs show a high extinction coefficient. The colour difference can be easily recognised with the naked eye or spectrometry for quantitative examination [36]. Using AuNPs, Xia et al. established a colorimetric method for clinical PSA detection. The procedure is based on the in-situ synthesis of AuNPs and Cu<sup>2+</sup>-catalysed oxidation of AA. They showed that the PSA substrate peptide (DAHSSKLQLAPP) containing an ATCUN motif of DAH could sequester Cu<sup>2+</sup> by forming an ATCUN–Cu<sup>2+</sup> complex, thereby inhibiting the Cu<sup>2+</sup> catalyzed oxidation of AA. The separation of cleavage step and analysis step by peptide-functionalized MMBs promotes the selective detection of PSA in serum samples. As depicted in **Figure 3 (A)**, the red colour solution eventually became colourless as the PSA content progressed. Quantifications of PSA in serum samples from two healthy donors and two prostate cancers were performed to demonstrate the method's viability for clinical assays. The fluid colour is red for healthy controls, whereas it is colourless for patients (**Figure 3 (B)**). By identifying the most negligible PSA concentration at which the response is clearly distinguished from the background, the detection limit was estimated to be 0.02 ng/mL. Thus, the colourimetric method is promising for determining PSA in a clinical specimen for preoperative diagnosis and screening for prostate cancer. The value obtained is lower than that obtained by measuring the PSA released by a healthy prostate (4 ng/mL) [37].



**Figure 3 (A).** The UV–VIS absorption spectra and photographic representations of the AuNPs formed in DAHSSKLQLAPP-functionalized MMBs at different PSA concentrations **(B)**. PSA levels in the serum of healthy persons and prostate cancer patients [37].

### Surface plasmon resonance sensing (SPR)

The study of interactions between biomolecules using SPR sensing can be used to evaluate the interactions between antibodies and antigens, ligand-receptor kinetics, enzyme-substrate reaction, and epitope mapping. Karami and colleagues confirmed the viability of a colorimetric immunoassay for PSA analysis using colloidal AuNPs with the SPR band. As illustrated in **Figure 4**, antibody-conjugated AuNPs were subjected to PSA antigen molecules in the presence of a second antibody-conjugated  $\text{Fe}_3\text{O}_4$  to form a complex immune network. The SPR signals were estimated using residual  $\text{Ab}_1$ -AuNPs (unreacted NPs). PSA detection has a linear range of  $0.01\text{-}20\text{ ng mL}^{-1}$ , with a detection limit of  $0.009\text{ ng mL}^{-1}$  [38]. Finally, we anticipated that the immunosensor is a simple yet reliable and cost-effective platform. It will be valuable in developing future POC sensing tools to detect biomarkers in a drop of blood.



**Figure 4.** The performance of colorimetric immunoassay for PSA. Reprinted with permission from [38].

### Fluorescence sensing

Fluorescence is a highly efficient bioanalytic method. It has given researchers a better understanding of biological components, processes, molecular detection, expression, and relationships. Silica nanospheres (SiO<sub>2</sub> NPs) have been identified as a helpful platform for the covalent binding of several biomolecules for fluorescence biosensing. Kong et al. developed a label-free fluorescent aptasensor based on aggregation-induced emission (AIE) and SiO<sub>2</sub> NPs for the sensitive "turn-on" detection of PSA for the first time. The binding of the aptamer to the target PSA may result in a tight aptamer confirmation, enabling the PA to be released from the surface of SiO<sub>2</sub> NPs. The AIE molecules tetra phenylethylene derivative 3 (TPE3) clumped together on the SiO<sub>2</sub> NPs surface. And as a result, fluorescence was produced. With the advantages of simple design and rapid responses, the suggested aptasensor demonstrated excellent sensitivity and selectivity for PSA with a detection limit of 0.5 ng/mL [39]. **Table 2** summarizes PSA detection's best-chosen biosensor tools with a description of detection systems, signal enhancement protocols, and primary performance characteristics.

**Table 2.** The different electrochemical or optical-based detection for PSA

Nanomaterials modified electrode	LOD (ng/mL)	Linearity range (ng/mL)	Reproducibility (RSD%)	Technique	References
GQDs-CoPc( $\pi\pi$ )-aptamer (sequential)	0.018	0.034-0.057	3.77	DPV	[40]
IDE/APTES/EDC-NHS/anti-PSA aptamer	1.5	2.5-90	3.10	EIS	[41]
AuNPs/CHI/SPE	0.001	1-18	3.6	CV & EIS	[42]

Au-NS electrode	0.023	0.5-100	5.7	DPV	[43]
Ag@MSNs	0.015	0.05-50.0	-	CV	[44]
GNPs	2	0.2-1	-	LSPR	[45]
Gold nanodisk arrays functionalized with specific aptamers	1.49	1.7 -20.4	-	LSPR	[46]
Mn <sup>2+</sup> -enhanced NaYF <sub>4</sub> :Yb, Er upconversion nanorods	0.112	0.1172-18.75		LET	[47]
Poly (acrylic acid) (PAA)-modified gold magnetic nanoparticles (PGMNs)	0.17			Lateral flow immunoassay	[48]
Non-thiolated poly-Adenine aptamer	0.02		Colorimetry	0.1-100	[49]

Detection of PSA at an early stage can significantly reduce the death rate in the PCa. Optical and electrochemical nanosensors nanomaterials have unique properties. Advances in the nanomaterial sciences and their integration with parallelly developed optical and electrochemical nanosensors improve the performance and speed of the sensor. To maximize signal production, materials that rely on electrical and optical signal transfer must have high fluorescence, electrocatalysis, and conductivity.

### 3. Nano-engineered materials for POC based detection of PSA

Nanotechnology utilisation in medicine is not restricted only to the development of drug carriers. The research focused on nanotechnology may be the key for the development of next generation biosensor for PCa. The evolution of nanotechnology-based PSA screening

approaches has high potential due to their susceptible analytical detection properties, clinical effectiveness, and accessibility. Moreover, the coupling of electrochemical or optical sensors with nanomaterials supports the sensitive and selective detection of PSA.

Over the last decade, advances in PCa nano diagnostics have concentrated on detecting PSA in clinical specimens using nanostructured materials. The fundamental justification for utilizing nanometer-scale products or structures is to manipulate the specific physical properties, including structural, optical, magnetic, and electronic, apparent within the nanoscale range [50].

Nanomaterials can be employed in a variety of ways in the detecting system, including capture probes, electrode fabrication, and electrode coatings. Indeed, such approaches are gaining regulatory acceptability for the evaluation, allowing for the development of safe-by-design nanomaterials for applications in development of immunosensors. They can also apply advanced biomolecule research tools, real-time environmental monitoring systems, and POC diagnostic systems [51].

A significantly helpful feature for early monitoring of biochemical recurrence after prostatectomy demands ultralow detection of PSA. The use of various nanomaterials, such as carbon and non-carbon nanoparticles, are highly essential and primarily used to develop electrochemical sensors [52]. Nanoparticles deliver unique detection characteristics such as higher reactivity, catalytic activity, excellent enhanced electrical conductivity, strong biocompatibility, unique magnetic properties, large area-to-volume ratio, and the potential to minimize electrode fouling [53].

During the last decade, the PCa nano diagnosis by applying nanostructured materials was primarily concentrated for detection of PSA as a target in clinical specimens. The attachment of PSA proteins to the antibody on the surface of microcantilever contributed to the



nanomechanical structural deformation. This deformation could be assessed optically to achieve a clinically significant PSA detection in a backdrop of human serum albumin and plasminogen at  $1 \text{ mg mL}^{-1}$  (maximum of  $0.2 \text{ ng mL}^{-1}$ ). This technology was adopted in field-effect sensors for silicon nanowires that integrated nanowires and surface PSA receptors into highly sensitive PSA protein detection arrays. PSA protein binding to antibodies on the nanowire's surface may lead to a real-time electrical signal to  $0.9 \text{ pg mL}^{-1}$  PSA concentrations in undiluted serum samples [54]. However, the clinical application of cutting-edge nanostructured materials has not been accomplished. This is due to the lack of scientific research experience in applying the latest nanomaterials to practical clinical usage and flawed clinical trials' progress attributed to funding problems and lack of diagnostic marketing awareness.

### **3.1. Graphene, graphene oxide and carbon nanotube nanomaterial-based electrochemical detection**

Carbonaceous materials, including graphene and carbon nanotubes, have quickly been paid significant attention by the biosensor science communities. Graphitic material is not new, despite its extensive usage as pencils, lubricants, and electrical conductors [55]. However, after the Nobel Prize-winning research on single- or bi-layer graphene, this star substance and its numerous variants such as graphene oxide (GO) and carbon nanotubes (CNTs) have been re-focused with significant interest. Graphene has been an increasingly common nanomaterial since the 2010 Nobel prize in physics on this material [56]. The use of various nanomaterials is also a way to improve the performance of electrochemical biosensors. Alongside the intact graphene that has been successfully synthesized via several methods, graphene oxide attracts more interest in biosensors. The functional groups of epoxy, hydroxyl, and carboxyl allow more flexible surface functionality and lead to excellent aqueous solubility and biocompatibility. The sensitivity of graphene-based sensor devices has grown exponentially,

particularly in the field of biosensors, where it can be utilized to improve conductivity and stability [57].

Increasing interest has been raised towards the utilization of nanomaterials for biosensor development, and carbon nanotubes (CNTs) are the subject of specific attention. CNT is an advanced, well-ordered hollow graphitic nanomaterial consisting of  $sp^2$ -hybridized carbon cylinders. Such materials are known as single-walled nanotubes (SWNTs), which are single sheets of graphene that are twirled into tubes or multi-walled nanotubes (MWNTs), each comprising of multiple concentrated tubes with a similar longitudinal axis [58]. It provides an opportunity for greater efficiency, biocompatibility, portability, and, most significantly, the ease of label-free sensing. Nanotubes and nanowires have very high volume-to-surface ratios and thus are very sensitive. Recent literature on biosensing has documented carbon nanotubes or nanowires as active sensors for various biological analytes [59]. The electrocatalytic behaviour of the CNTs was linked to "topological defects." Pentagonal domains characterize their unique structure at the hemispheric ends or as defects along with the graphite cylinder. This generates regions with a higher charge density than in the regular hexagonal network, resulting in enhanced electroactivity of CNTs [60]. They were commonly used as electrode materials for these purposes, and many electrochemical biosensors were introduced using CNTs as a platform for biomolecule immobilization and electrochemical transduction.

In these PCa sensing, graphene, graphene oxide, and carbon nanotube-based nanomaterial may be helpful in signal amplification to achieve a lower DL for PSA detection in immunosensors.

**Construction of antibody-graphene biosensor interface** - Graphene is also utilized to improve the conductivity and durability of carbon immunosensors, which integrate screen-printed electrodes with great content vegetable parchment. Lu et al., 2012 [61] discussed the fabrication of a new, reversible, and extremely sensitive electro-analytical immunosensor utilizing graphene nanosheets (GS) and horseradish peroxidase (HRP) signal antibody

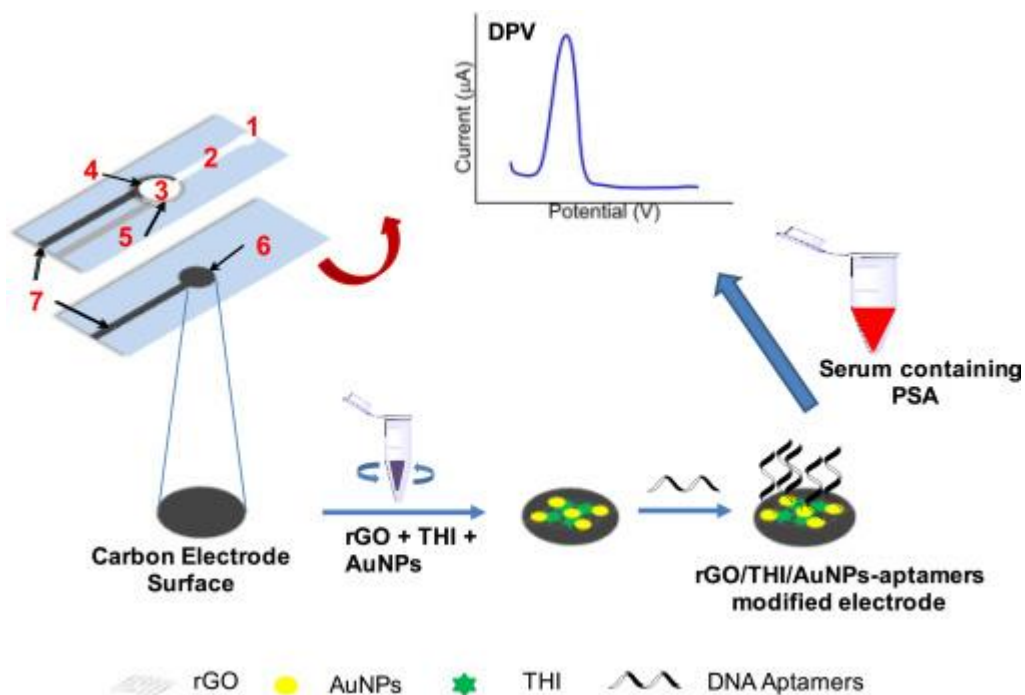
functionalized with gold nanoparticles (HRP-Ab2/Au NPs). Using PSA as a model analyte, this immunosensor displayed a broad linear spectrum of 6 orders of magnitude with a value of less than  $2 \text{ pg mL}^{-1}$ . In clinical applications, this offered exciting ultrasensitive potential- Screen printed electrode-based PSA for ultralow detection sensors up to  $2 \text{ pg mL}^{-1}$  with a broad linear spectrum of 6 magnitude orders [62]. Mao et al. [63] developed a novel electrochemical immunosensor for the detection of PSA. It was based on a nanocomposite film of graphene sheets-methylene blue-chitosan (GS-MB-CS) as an electrode substrate. They used chitosan as a dispersant to create an immune interface on a glass carbon electrode to achieve stronger-oriented antibody assembly. It offered a lot of amino groups for PSA antibody binding. The modification process was controlled by CV. A low DL ( $13 \text{ pg mL}^{-1}$ ) and strong selectivity were achieved. In both previously mentioned references, the PSA recovery was in the range of 99-107% and 100-102% from human serum samples, respectively.

In 2013, Kim and colleagues [64] developed a reduced graphene oxide field-effect transistor (rGO-FET) biosensor for the label-free ultrasensitive detection of a biomarker for prostate cancer, a complex of PSA/ $\alpha$ 1-antichymotrypsin (PSA-ACT). The ultrasensitive with the LD as low as fM range and the broad dynamic range is probably due to dense immobilization of receptor biomolecules and limited non-specific binding. A novel 3D graphene-Au composite was developed by Jang et al to increase the accessible surface area for an antibody combination compared to the 2D graphene layer. A wide linear range of  $0\text{-}10 \text{ ng mL}^{-1}$  with a low DL of  $0.59 \text{ ng mL}^{-1}$  was reported; however, significantly increased electron transfer and high PSA sensitivity [65].

Many aminated graphene quantum dots and carbon graphene quantum dots on the electrode were mixed onto the sheet, as adjusted with Au/Ag -rGO, established by Wu et al., 2016. The DL achieved in concentration range of  $1\text{-}10 \text{ ng mL}^{-1}$  was  $0.29 \text{ pg/mL}$  [66]. More recently, Assari et al. [67] modified the GCE, first with gold and then decorated with gold-

nanoparticulated graphene oxide. AuNPs on reduced graphene oxide has a good surface for the attachment of antibodies. EIS for estimation of PSA was also performed. EIS exists within a concentration of 0.0018 to 41 ng mL<sup>-1</sup> and has a DL of 60 pg mL<sup>-1</sup>. These biosensors are potentially suitable for analyzing many different analytes based on the biosensor application's binding antibody. This is a common feature of different types of affinity biosensors.

**Construction of aptamer-graphene biosensor interface** – Aptamers is ligand-binding nucleic acids that interfere with antibody affinities and selectivity. They are adapted as antibodies and as unique reagents in their own right for analytical applications [68]. The DNA capturing sensor plays a crucial role in identifying and catching the target molecules for interface-based PC biosensors. DNA aptamer, a unique, single-strand DNA (ssDNA) isolating from a random sequence of DNA/RNA libraries, utilizing the *in vitro* collection method known as the systematic evolution of ligands by exponential enrichment (SELEX) [69]. In several published experiments, nanocomposites based on graphene were first prepared, consisting of graphene and different combining elements. For example, Fang and colleagues suggested an amplified assay using DNase I for sensitively detection of PSA based on the PSA aptamer/graphene oxide (QD-aptamer/GO) label of CdSe/ZnS quantum dot. Under optimum controlled conditions, fluorescence strength increased linearly with PSA concentration between 0.1- 3 fg mL<sup>-1</sup> with a quantification limit of 0.05 fg mL<sup>-1</sup>. This has three lower orders of magnitude than those of DNase I [70]. Wei et al. synthesized nanocomposites of gold nanoparticles (AuNPs)/reduced graphene oxide (rGO)/thionine (THI), which were coated on working electrodes to immobilize the DNA aptamer sample, as shown in **Figure 5**. They stated the excellent conductivity of AuNPs and rGO also play an essential role in the transfer of electrons. This results in sensitive detection for PSA, capable of detecting PSA as low as 10 pg mL<sup>-1</sup>, with a linear range of 0.05 to 200 ng mL<sup>-1</sup>[71].



**Figure 5.** Fabrication and modification process of the microfluidic paper-based aptasensor Reprinted with permission from [71].

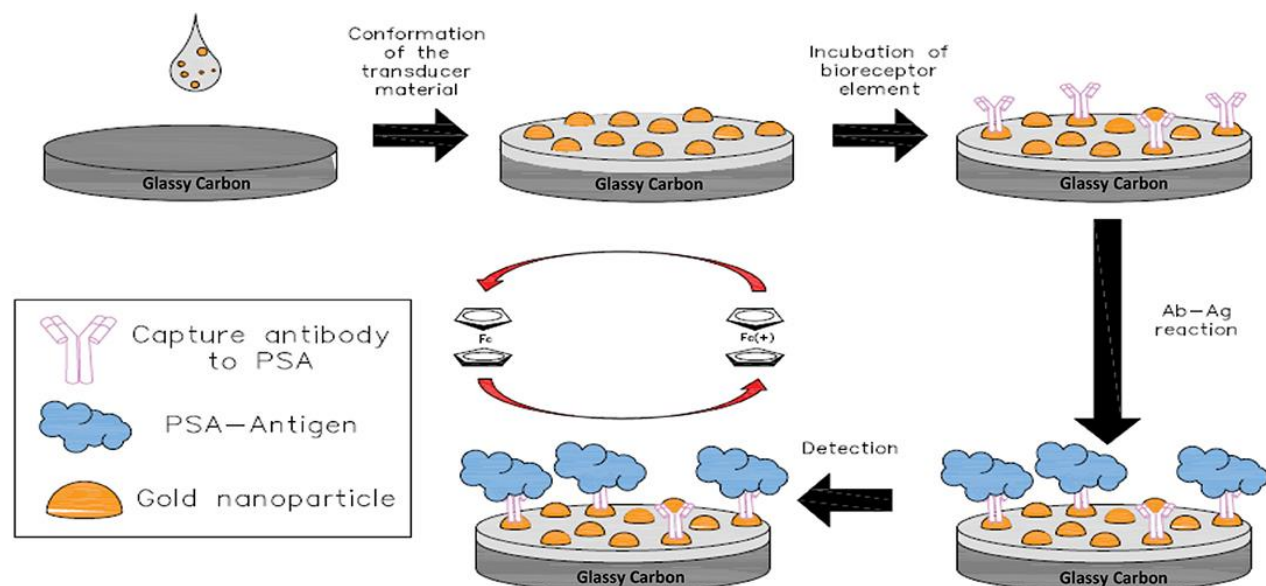
**Antibody-carbon nanotubes/nanowires biosensor interface** – Impressive advancements in material technology, primarily related to device engineering, enabled the use of a wide range of materials with multiple applications. Maehashi et al. [72] reported electrochemical biosensors based on carbon nanotube array (CNTs)-modified electrodes. Electrochemical biosensors based on CNTs play a significant role in CNTs-related biosensors based on their inherent advantages such as higher sensitivity, fast response, ease of processing and favourable portability. Depending on the recognition process, CNT-based electrochemical biosensors may be classified into biocatalytic and bio-affinity sensors [143-144]. CNTs contain carbon allotropes organized in sheets that have been formed into extremely conductive, hollow tubes of different nanometre sizes [75]. Yu et al. [76] developed serum PSA immunosensors utilizing multi-labelled CNT-HRP-Ab<sub>2</sub> particles using this method given DL, 3 times the average noise level above zero PSA power, of 4 pg mL<sup>-1</sup> (150 fM).

Further, an electrochemical immunosensor focused on CNTs was first used to detect PSA in actual biochemical serum and tissue lysates for prostate cancer diagnosis. The PSA detection efficiency was compared with an immunosensor focused on the CNTs and the ELISA techniques. The CNT-based immunosensor had a LOD of  $4 \text{ pg mL}^{-1}$  ( $100 \text{ mol mL}^{-1}$ ) for PSA in 10 mL of undiluted calf serum, which was extremely reactive relative to the ELISA system of 5% precision for human serum samples [77]. The SWCNT forests provided 5 to 10 times greater sensitivity than nanotubes immunosensors due to a significant increase in Ab1 density relative to that on a flat immunosensor, which was recently verified. Authors reported that the increase in sensitivity could be due to the compact packaging of carboxylated SWCNT forest tips, along with the high content of conductive SWCNT forests, translating into a large surface concentration of capture antibodies [78]. Similarly, various other approaches for the development of a PSA biosensor dependent on CNTs have been investigated.

Multi-wall carbon nanotubes (MWCNTs) have been optimized to carry thousands of alkaline phosphatase enzyme molecules per CNT and secondary antibodies to reach an fM protein DL in buffers. In 2013, Salimi and colleagues developed immunosensors focused on the immobilization of PSA-anticorps (anti-PSA) on a robust nanocomposite that incorporates MWCNTs ionic liquid (IL)1-butyl-methylpyrolydinium bis (trifluoromethyl sulfonyl) imide [C<sub>4</sub>mpyr][NTf<sub>2</sub>]. Under an ideal condition, the immunosensor's DPV peak current increased linearly in two concentration ranges, 0.2–1.0 and 1–40 ng mL<sup>-1</sup>, with DL of 20 pg mL<sup>-1</sup> [79].

Later in 2014, Kavosi et al. developed a sensitive electrochemical immunosensor for the detection of PSA. It was based on the covalent immobilization of anti-PSA and redox mediator (thionine) on polyamidoamine dendrimer (AuNPs-PAMAM) embedded in gold nanoparticles and multi-walled carbon nanotubes/ionic liquid/chitosan nanocomposite (MWCNTs/IL/Chit) as substrate. They reported the PSA analysis with a 0.5 ng mL<sup>-1</sup> DL concentration level up to 25 ng mL<sup>-1</sup> [80]. Another group has recently reported various functional multi-wall carbon

nanotubes, with gold nanoparticles as biosensor electrons (fMWCNT-AuNPs-Ab). FMWCNT-AuNPs-50-Ab of a study with the highest performance with a linear range of 0-6 ng mL<sup>-1</sup> and an excellent 4.74 mA g<sup>-1</sup>/ng mL<sup>-1</sup> sensitivity in the human sample as shown in **Figure 6** [81].



**Figure 6.** Illustration of the stepwise process for PSA immunosensor electrode fabrication and detection of the cancer biomarker Reprinted with permission from [81].

**Carbon nanotubes and graphene-** Chemical functionalization CNTs and graphene must be performed to allow a biocompatible surface further conjugated with other molecules to employ CNTs graphene in biosensors. The process of functionalization has a significant impact on the performance of the sensor system. Several researchers have reported progress in fabricating sensitive PSA biosensors using carbon nanotubes and graphene. Lu et al. [61] reported that Au nanoparticles (AuNPs) dotted carbon nanotubes (MWCNTs)-graphene composite for high-performance electrochemical immunosensor, which were immobilized on the working electrode. The strategy provided an efficient linear response of 0.005 to 500 mIU mL<sup>-1</sup> with a low DL of 0.0026 mIU mL<sup>-1</sup>.

Recently, the sensing interface has implemented a strategic framework for PSA electrochemical immune-sensing of MWCNT/thionine-NH<sub>2</sub>-rGO-COOH-antibody. To do this, a functionalized reduced graphene oxide (His-rGO) multi-walled carbon nanotube

(MWCNT)/L-histidine has been demonstrated as a bifunctional nanoplatforms for covalently attaching thionine redox indicators and anti-PSA antibodies (Ab). The immunosensor displayed a linear concentration range of 10 fg mL<sup>-1</sup> to 20 ng mL<sup>-1</sup>, with a low detection limit of 2.8 fg mL<sup>-1</sup> for PSA [82].

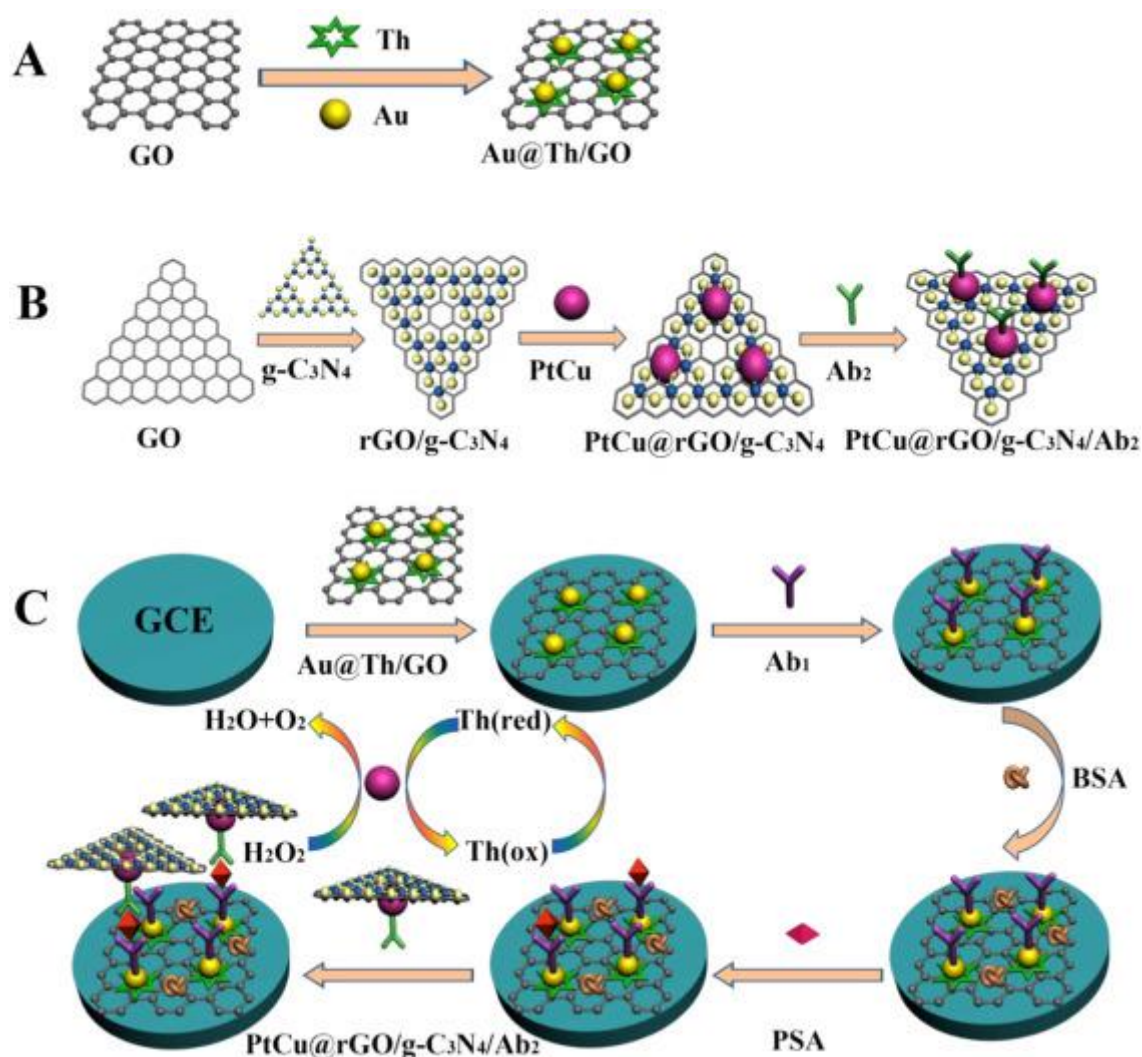
### 3.2. Non-carbon nanomaterial-based electrochemical detection of PSA

To boost the electrochemical properties of biosensors, non-carbon nanomaterials have been recently used as an alternative to support the element of the electrode. We presented different forms of nanomaterials in this section and divided them into two groups, non-metallic and metallic, which were used as an additional component or nanostructured electrode [83].

**Metallic nanoparticles** – The properties of metallic nanoparticles (MNPs) have a wide surface area to increase the catalytic action, immobilization capacity of biomolecules and demonstrate unusual capacities for electron transfer and excellent biocompatibility [84]. In the immunosensor area, AuNPs, being the most commonly used nanocarriers, become particularly attractive. AuNPs have many unique features, such as ideal biocompatibility, good electrical conductivity and large surface-to-volume ratios [85]. PSA and free-PSA (f-PSA) SED immunosensor was fabricated by Han et al. [86]. AuNPs modified Prussian blue nanoparticles, and AuNPs modified nickel hexacyanoferrates nanoparticles decorated with mesoporous graphene tubes, identical to onions. The DL for fPSA and PSA is 6.7 pg mL<sup>-1</sup> and 3.4 pg mL<sup>-1</sup>. Later, Au loaded with thionine functionalized graphene oxide (Au@Th/GO) electrochemical immunosensor (**Figure 7**) for PSA detection was reported by Feng et al. [87]. The developed immunosensor had a linear concentration range from 50 fg mL<sup>-1</sup> to 40 ng mL<sup>-1</sup>, with a low DL of 16.6 fg mL<sup>-1</sup> (S/N = 3) for PSA. Suresh et al. [42] developed a nanocomposite chitosan (CHI) film coated on a screen-printed electrode (SPE) and gold nanoparticles (AuNPs) for PSA



detection. Under controlled conditions, a linear increase in steady-state current with PSA concentration across 1-18 ng mL<sup>-1</sup> range with 0.001 ng mL<sup>-1</sup> maximum detection.



**Figure 7.** (A) Fabrication procedure for Au@Th/GO; (B) PtCu@rGO/g-C<sub>3</sub>N<sub>4</sub>/Ab<sub>2</sub>; (C) Sandwich-type electrochemical immunosensor for PSA detection. Reprinted with permission from [87].

Among the numerous noble metal nanoparticles (e.g. silver, copper, platinum, etc.), the Silver nanoparticles (AgNPs) are subject to modern innovative techniques resulting in extremely novel morphologies and characteristics. These nanoparticles hold numerous strengths, enabling the easier transfer of electrons and accommodating more active sites on their surface [88]. The use of metal nanoparticles alone is not enough for high sensitivity identification.

Hence, it requires more comprehensive approaches. Cho et al. [89] demonstrated the repairing structural deficiencies of reduced graphene oxide and silver nanoparticle incorporation, which significantly enhanced the electrical conductivity of graphene oxide. Thunkhamrak et al. [90] have successfully established an important voltammetric immunosensing framework for sensitive PSA detection. They utilized GO modified screen-printed carbon electrode (SPCE), which was hybridized with ex-situ prepared silver nanoparticles (AgNPs) as a probe and signal transducer. The fabricated immunosensor demonstrated a robust PSA response with a DL of  $0.27 \text{ ng mL}^{-1}$  and a complex configuration range of  $0.75\text{--}100.0 \text{ ng mL}^{-1}$ .

A novel technique was stated by Zhao et al. [91] for triple signal amplification immunoprobe. The labelled antibody was co-mobilized directly on platinum nanoparticles (ptnps), and in Bovine serum, albumin-stabilized copper nanoclusters (BSA-cuNCs) was developed. The first immunosensor development was made by anti-PSA immobilization, which was done exclusively on gold nanoparticles. Then, after PSA became an immune adsorbent, it was electrochemically deposited on the glass carbon electrode. After that, under ideal conditions, a broad linear range from  $0.5 \text{ pg mL}^{-1}$  to  $100 \text{ ng mL}^{-1}$  and an ultralow detection of  $145,69 \text{ fg mL}^{-1}$  was attained by the proposed immunosensor. Lately, an electrochemical immunosensor for highly sensitive PSA detection was published by Liu et al. [92]. The designed immunosensor was focused on combining a polydopamine-modified porous graphene sensing platform and a non-enzymatic metal-organic framework (MOF) conjugated secondary antibody. This method achieved a wide linear response ranging between  $0.1$  to  $10 \text{ ng mL}^{-1}$ , along with a relatively low DL of  $0.025 \text{ ng ML}^{-1}$ .

Electrical imbalance is one of the significant drawbacks of metallic nanoparticles due to their susceptibility to salt concentrations, contributing to aggregation and precipitation. As a result, adequate chemical and biological changes to the surfaces of the nanoparticles are required for

their use. On the other hand, metallic nanoparticulated signals hold inconsistency with signal amplification, leading to reproducibility restrictions [93].

**Inorganic-organic hybrid nanoparticles for the electrochemical detection of PSA** – Hybrid inorganic-organic composites reflect a new class of modern materials with significant potential. Materials are built with strong physical properties of ceramics and with an outstanding option of functional group chemical reactivity consistent with organic chemistry. Fan et al. [94] reported mesoporous silica nanoparticles (MSN-acetal) used to immobilize the electron mediator thionine (Th) for 3,9-Bis(3-aminopropyl)-2,4,8-tetraoxaspiro[5.5]-undecanes. The molecules encapsulation was done by capping of MSN-Acetal pores by modified carboxylic acid Au nanoparticles. Under ideal conditions, a broad linearity range of 0.001–5.0 ng mL<sup>-1</sup> along with a small DL of 0.31 pg mL<sup>-1</sup> was shown by the electrochemical immunosensor. Thus, a developed cargo release system came up as a ground-breaking and productive approach for detecting PSA as there was a correlation response signal with a PSA concentration.

More recently, Argoubi et al. [95] came up with designing a new label-free electrochemical apta-sensing framework for detecting PSA. It was focused on mesoporous silica thin film-coated gold electrodes as a sensing interface. This apta-sensing strategy focuses on preventing the diffusion of [Fe(CN)<sub>6</sub>]<sup>3/4-</sup> redox probe from crossing the nanochannels of the mesoporous film. Owing to the recognition of the target biomarker by its anti-PSA-specific DNA aptamer covalently attached to the outermost layer of the silica nanopores. This framework seems highly sensitive to PSA between 1 to 300 ng mL<sup>-1</sup> and with DL of 280 pg mL<sup>-1</sup>.

Kavosi et al. [96] reported the triple signal amplification approach to developing an ultrasensitive PSA immunosensor. The thionine and PSA antibody form a covalent bond with graphene oxide/chitosan film, which acts as a redox probe. The AuNPs -PAMAM dendrimer/HRP linked aptamer used in sandwich shape as an electrochemical label. In this work, the DL

and linear concentration range are  $10 \text{ fg mL}^{-1}$  and  $0.1 \text{ pg mL}^{-1}$  to  $90 \text{ ng mL}^{-1}$ , respectively, obtained under an optimized condition using DPV as a measurement technique.

To achieve high accuracy and specificity in detecting chemical markers, researchers have made strenuous efforts to invent tailor-made electronic and optical devices/methods.

#### **4. Limitations and disadvantages**

As presented in this review, PSA has been intensively researched as a possible biomarker for PCa in the last decade. However, analysing PSA in biological samples is not simple, and there have been various hurdles in translating PSA-based biomarkers into the clinic. A serious barrier has been its specificity and sensitivity, which range from 20 - 40% and 70 -90%, respectively [98]. In recent years, significant research efforts have been made to improve the sensitivity, specificity, and LOD of PSA detection utilising various approaches for clinical decision making. To address this obstacle, many studies have shown that nanoengineering of materials may be used to efficiently boost sensitivity and selectivity for PSA. The detection of PSA has been made possible by the development of a wide range of optical and electrochemical sensors such as fluorescence, SERS, LSPR/SPR, impedimetric, voltammetry and amperometric sensors. These strategies demonstrated increased selectivity and sensitivity when compared to conventional PSA detection methods, and some of them demonstrated the potential for field POC. Optical nanosensors are capable of detecting PSA through naked eye at micromolar with high selectivity. Electrochemical nanosensors, on the other hand, have the advantages of rapid detection, ease of fabrication, and greater portability. Furthermore, the electrochemical sensors can provide LOD values down to the picomolar level, which is 2-3 orders better than the LOD values obtained from optical nanosensors, which is a significant improvement. Overall, both optical and electrochemical nanosensors have demonstrated promising potential for PSA detection and provide the platform of POC.

Another hurdle that still needs to be overcome is that only a tiny minority of reported POC have been approved for biological/clinical applications due to reproducibility and stability. While significant attempts have been made to introduce these sensors to the market, many have not been wholly commercialized or used for actual applications due to technological or fabrication problems. Thus, the reproducibility and stability of biosensors are expected to be further improved, calling for more advanced nano engineering techniques. It has been suggested that effective POC development requires a sensitive and selective approach, rather than the traditional approach. Due to nano engineering of materials, similar to human body and physiology, sensors are expected to reduce the differences between in vitro and in vivo preclinical studies and act as a clinical test for personalized clinical applications.

## **5. Conclusions and perspectives**

For the early diagnosis of prostate cancer, PSA came up as one of the most significant biomarkers. The past decade has seen the emergence of various new technology or sensors for PSA detection. An accurate diagnosis at earlier stages of cancer is crucial to yield better disease monitoring and successful treatment for PCa. Many conventional PSA detection methods have been developed but these methods are limited due to laborious, non-portable, time-consuming, and expensive. The diagnostic test performance characteristics of PSA are variable. Rapid technical advancements have contributed to the improvement of biomarker-based detection systems for real-time monitoring of activities related to prostate cancer development, progression, and response.

Considering the potential translational value of next-generation detection techniques, cost-effective and easy to implement advanced detection techniques are needed. Nanotechnology carries the ability to reduce the existing constraints of single reference laboratory or costly specialized instruments, allowing the development of a point of treatment for a specific cost-effective diagnosis of PCa. The unifying features of both nano-and micro-scale materials put

together persuasive capabilities for analyte capture and analysis. The combination of micro-devices and materials that allow nanoscale molecular and cell tagging has generated superior performance devices. Significant opportunities that exist at the interface of optical, electrical techniques, nanotechnology, and molecular diagnostics have enabled the development of next-generation on time sensitive and selective detection of PSA.

Having realized the potential role of nano biosensors in numerous disease detection, this Review took a systematic look at the recent development in POC based biosensors for PSA detection. There is no doubt that developments in biosensing technologies will add to the initiative to develop point-of-care and bedside applications in diagnostics and regular monitoring. For POC, nanomaterials provide incredible opportunities to expand new sensing platforms. Extraordinary attempts were made to easily diagnose disease not just for laboratory study but also in practical samples. The development of robust techniques and sensitive capture platforms that use readily accessible body fluids, particularly urine, could offer novel approaches for disease staging and diagnosis.

We hope that these efforts will be focused on key clinical concerns. Thus, analysis can be served as a valuable initiation point leading toward PSA to make sure efforts are focused towards the most relevant clinical decision-making areas in prostate cancer patient treatment. We expect that incorporating sophisticated nanotechnologies into POC systems would result in high efficiency and maximize viability. These developments can contribute to more straightforward and more accurate clinical diagnostic devices. We expect novel openings in drug development, disease modelling and personalized medicine after tumour chip models become generally accepted in academia, industry and healthcare.

## **Declarations**

**Conflict of interest** The authors declare no competing interests.

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