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Recent advancement in sensitive detection of carcinoembryonic antigen using nanomaterials based immunosensors

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Abstract

Carcinoembryonic antigen (CEA) is a prominent cancer biomarker that allows for early diagnosis of various cancers. Present immunoassays techniques help quantify such target molecules in test samples via anti-antibody reaction. Despite their rapid usage, conventional immunoassay techniques demonstrate several limitations that can be easily overcome by employing nanomaterials in sensing assays. Thus, nanomaterial-based immunosensors have gained steady attention from the scientific community owing to their high specificity and low detection limit. Various nanomaterials like platinum, gold, silver and carbon exhibit exceptional properties have allowed promising results in the detection and diagnostics of CEA. Thus, the present review aims to explore the significance and the recent developments of nanomaterial-based biosensors for detecting CEA biomarkers with high sensitivity, selectivity, and specificity. After a brief introduction, we discussed the fundamentals of immunosensors immobilization strategies and common nanomaterials. In the next section, we highlighted the recent advances in the common immunosensors detection approaches for CEA alone and simultaneous detection of CEA with other biomarkers detection. Finally, we concluded the review by discussing the future perspectives of this promising field of biomarkers detection.

Keywords: Carcinoembryonic antigen; Noble nanomaterials; Carbon nanomaterials; Biosensors; Immunosensor; Detection techniques

1. Introduction

Cancer is the second leading cause of death globally [1–3]; therefore, the early diagnosis of tumour markers is crucial for effectively treating cancers. However, due to the complexity of cancer cells, their small size and their diverse nature, they are notorious for being difficult to detect [4]. Also, different concentrations of various biomarkers give vital information on cancer progression and help distinguish cancerous cells from normal cells [5]. Thus, the early detection of crucial biomarkers such as alpha-fetoprotein (AFP), cancer antigen 125 (CA 125), human chorionic gonadotropin (hCG) and carcinoembryonic antigen (CEA) markers is essential to control the rapidly dividing metastatic cancer cells and cancer-related mortality and morbidity [2,6–9]. In recent years, research in "cancer detection" has tremendously increased. This can be observed from the number of publications from the past decade (from 2009 to 2021), as shown in **Fig. 1A**. In addition, different strategies have been employed for multiple cancer biomarkers detection in the last decade, as illustrated in **Fig. 1B**. Among them, electrochemical (EC) methods have gained the most interest by the scientists and researchers.

CEA is one of the first tumour-associated biomarkers to be identified and has consequently been well characterised [10]. CEA is a cell surface glycoprotein involved with cell adhesion [11], is associated with various types of cancers, and is vital in monitoring the effectiveness of remediation [8,12]. Further, this biomarker has a high molecular weight and is extensively used in the clinical diagnosis of colon, breast and ovarian tumours [13–16]. It is typically present at elevated levels in the foetal colon and is typically low in the adult human colonic epithelium. Under normal conditions, the CEA concentration is significantly lower in the colon tissue of adults. However, abnormally elevated CEA levels can also be

observed in several benign diseases and malignant tumours [17]. CEA concentration elevates during inflammation or in the presence of tumours in any endodermal tissue, including gastrointestinal and respiratory tract, pancreas and breast. In healthy individuals, the concentration of CEA is usually below 5 ng/mL [18,19], while in cancer-affected individuals, the CEA concentration in the blood reaches up to 20 ng/mL [3]. These cut-off values are essential to evaluate the patients' prognosis and risk of cancer recurrence [20].

Further, besides being an important biomarker in tumour detection, CEA is important in non-malignant disorders, namely pancreatitis, hepatitis, renal failure, and bowel disease [17]. Thus, there is a high significance for detecting CEA in diagnosing various diseases in healthcare and the medical field. Therefore, quantitative detection of low concentrations of tumour markers such as CEA is vital for the early diagnosis of cancers [7]. **Fig. 1C** illustrates the increase in publications over the last decade with the keyword "carcinoembryonic antigen", which signifies the importance of this cancer biomarker.

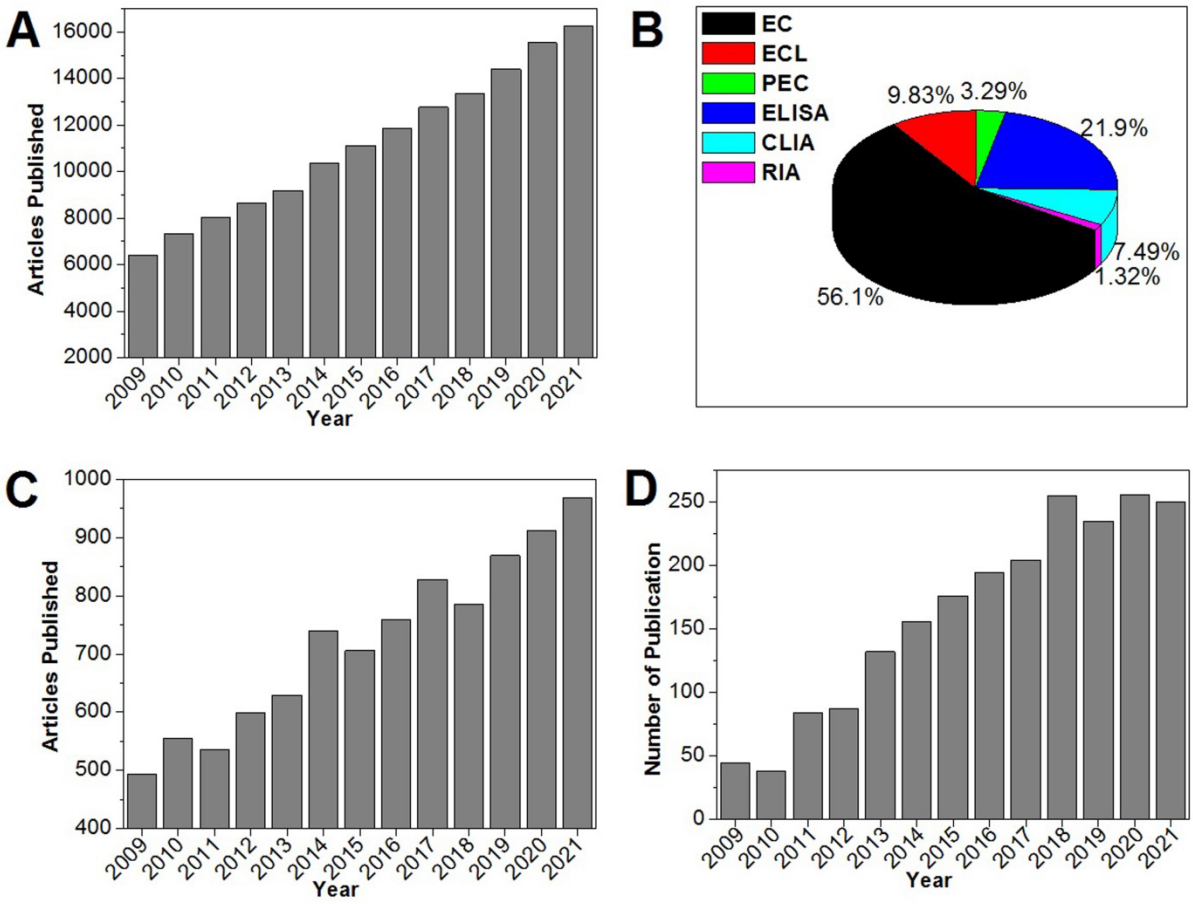

Fig. 1 (A) Articles published from the year 2009-2021 with the keyword “cancer detection” obtained and compiled from the Web of Science™ database on the 07th of April 2022; **(B)** The pie chart of the article published in the years from 2009 to 2021 of the different types of immunoassays and immunosensors developed for the detection of CEA. Data was compiled and obtained from the Web of Science™ database on the 07th of April 2022; **(C)** Articles published from the year 2009-2021 with the keyword "carcinoembryonic antigen" obtained and compiled from the Web of Science™ database on 07th of April 2022; and **(D)** Articles published from the year 2009-2021 obtained and compiled from the Web of Science™ database on the 07th of April 2022 with keywords “cancer immunosensor”. [Keywords: “electrochemical cancer immunosensor” (EC); “electrochemiluminescence cancer immunosensor” (ECL); “photoelectrochemical cancer immunosensor” (PEC); “chemiluminescence immunoassay-based cancer detection” (CLIA); “radioimmunoassay based cancer detection” (RIA); and “enzyme-linked immunosorbent assay-based cancer detection” (ELISA)].

Interestingly, immunosensors have shown promising potential for detecting several biomarkers, including CEA [21–26]. Therefore, in recent years, there has been an increase in the development of immunosensor for the detection of cancer biomarkers, as shown in **Fig. 1D**. However, despite their innumerable advantages for developing point-of-care devices, immunosensors have demonstrated various challenges, especially with real sample analysis. This is because actual samples contain numerous biomolecules, including protein molecules that interfere with the detection, especially within blood samples under neutral or acidic pH conditions [27]. Apart from the interference from other substances [28,29], reproducibility and stability are significant concerns for any sensing devices, including immunosensors; this thus limits their practical applications as a point of care devices [30,31]. Another issue is the variations in clinical biomarker threshold values in the samples that disallows accurate, simultaneous detection of more than four biomarkers [32,33]. Further, antibody-based methods have limitations like high cost, low stability, prolonged preparation time, and low stability [34]. In addition, researchers are trying to overcome the main challenge in immunosensors is to reach an extremely low detection limit. Thus, the primary approach to overcome these limitations is redesigning immunosensors, particularly EC immunosensors, using nanomaterials and nanocomposites [31,35–37].

In the recent decade, researchers have focused on developing sensitive biosensors that depend on the antigen-antibody interaction as its specific affinity-based recognition approach and detect tumour biomarkers with high sensitivity [38–40]. For instance, Yang *et al*. designed a label-free chemiluminescent (CL) immunosensor using CuS nanoparticles for the inexpensive, rapid and sensitive detection of AFP. Proposed label-free CL immunosensor demonstrated a low limit of detection (LOD) of 0.07 ng/mL with a linear detection range from 0.1 to 60 ng/mL [41]. Further, Lan *et al*. developed an excellent EC immunosensor for the susceptible detection of AFP employing streptavidin-functionalized reduced graphene oxide@polystyrene nanospheres. Developed EC immunosensor portrayed detection of AFP from linear concentration range of 0.1 to 100 ng/mL with a LOD of 0.03 ng/mL [42]. In a similar study, the EC immunosensor proposed by Cao *et al*. for detecting AFP using hedgehog-like Bi_2S_3 nanostructure demonstrated a linear range of 0.2-4 ng/mL with a LOD of 0.1 ng/mL [43]. Moreover, recently different nanomaterials-based strategies have been developed for the sensitive detection of CEA using CL and EC methods, such as Li *et al*. fabricated a CL immunosensor for the inexpensive, rapid and sensitive detection of CEA. The dual-functional cupric oxide nanorods based CL immunosensor showed linear detection range of 0.1-60 ng/mL with a LoD of 0.05 ng/mL [44]. Further, Lan *et al*. have recently reported an EC immunosensor using novel platinum-nanoparticle-decorated reduced graphene oxide@polystyrene nanospheres for the highly selective and sensitive detection of CEA. Developed EC immunosensor demonstrated to detect CEA within the linear range of 0.05 to 70 ng/mL and LOD of 0.01 ng/mL. In addition, the EC immunosensor portrayed high stability and good reproducibility with the potential to detect CEA in clinical serum samples having relative errors in-between –8.07 to 9.10 [45]. Thus, these studies conclude that nanomaterial-based biosensors hold promising potential in this ever-developing field of applications of immunosensors for CEA diagnosis [46].

2. Biosensor

Sensors are devices that collect data from chemical, physical, or biological changes and convert them into readable signals. While, compact analytical devices that generate output signals in response to specific analytes using biological materials over transducers are called biosensors [47,48]. These integrated receptor-transducer devices allow quantitative or semi-quantitative analytical information about actual biological samples [28,47]. They are generally divided into bio-catalytic sensors [37,49] and bio-affinity sensors [50,51]. Moreover, immunosensors fall under the bio-affinity category [52]. However, in developing new biosensors, certain factors such as sensitivity, selectivity and cost affectivity are considered [53,54]. Typically, biosensors are made up of a recognition element, a transducer and an output interface, as shown in **Fig. 2A**. Further, **Fig. 2A** illustrates examples of the typical biosensors for direct and indirect detection.

2.1. Immunoassay

Immunoassay is an analytical technique that records the specificity and avidity of the immunoreaction between antigen and antibody. The technique has gained remarkable recognition in recent years [55,56] and was first proposed in 1959 by Berson and Yellow. Since its discovery, various immunoassays have been customised and investigated for various applications [57]. Further, with technological advancements, many immunoassay methods have been explored for the precise and sensitive quantification of tumour markers [30,58]. The standard immunoassay includes ELISA [3,5,8], which involves the use of an enzyme to detect the enzyme-antibody conjugate actively. Besides, chemiluminescence immunoassay (CLIA) [3,8,59] is another method that involves light emission due to the excitation of a photon from a chemical reaction. In addition, electrochemiluminescence based immunoassay known as electrochemiluminescence immunoassay (ECLIA), measure the change in electrochemiluminescence (ECL) signal, while electrochemical based immunoassays known

as electrochemical immunoassays (ECIA) [3,5,7] is a technique based on the measurement of electrode potential, interfacial capacitance and impedance of the redox current [57,60–63].

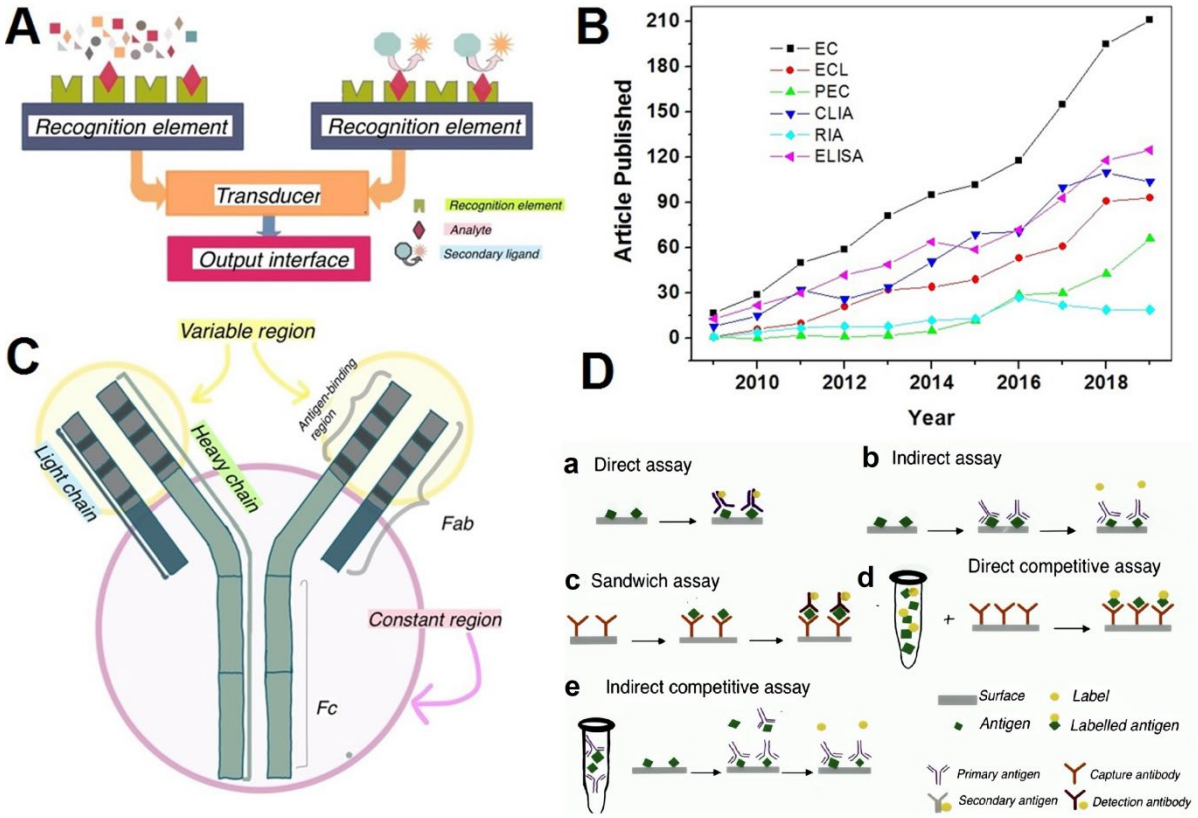

Fig. 2 (A) Illustration of typical biosensors: left illustration shows a typical biosensor based on direct/label-free detection (non-label), and the right illustration shows a typical biosensor based on labelled/indirect detection; **(B)** Articles published from 2009 to 2021, of the different types of immunosensor developed for the detection of CEA. Data of articles published each year was obtained from the Web of Science™ database on the 07th of April 2022 [Keywords: "electrochemical cancer immunosensor" (EC); "electrochemiluminescence cancer immunosensor" (ECL); "photoelectrochemical cancer immunosensor" (PEC); "chemiluminescence immunoassay based cancer detection" (CLIA); "radioimmunoassay based cancer detection" (RIA); and "enzyme-linked immunosorbent assay based cancer detection" (ELISA)]; **(C)** An illustration of an IgG molecule of an antibody structure which shows the essential chains, domains and sites. The Fab fragments are the site of binding between the antibody and antigen. The two strands are held together *via* disulfide bonds; and **(D)** Illustration of the different types of sensing format: (a) direct assay format; (b) indirect assay format; (c) sandwich assay format; (d) direct competitive assay format and (e) indirect competitive assay format.

2.2. Immunosensors

Immunosensors are biosensors based on the antigen-antibody interactions, which form a stable immunocomplex over the transducers [20,27,64,65]. Immunosensors are compact tools that provide rapid and sensitive detection and are applied to detect specific antigens or antibodies [66,67]. Moreover, immunosensors are particularly important in clinical analyses of cancer biomarkers and other diseases, including autoimmune and cardiac diseases [13]. These sensors comprise three essential components: (1) a recognition element which is usually an antigen or an antibody that has been immobilised onto a surface of a solid substrate, (2) a transducer which is a physical component that translates the signal from antigen-antibody interaction into a readable physical signal and (3) a detector which reads and amplifies the collected signal [68]. Thus, an immunosensor is based on a transducer's coupling with a biorecognition element [69,70]. Based on their transduction mode, immunosensors can be classified into optical immunosensors for luminescence, fluorescence ECL and refractive index measurements, photoelectrochemical which measure photoelectric current and EC immunosensors, which include amperometric, potentiometric, impedance, conductometric and piezoelectric devices [13]. As evident from **Fig. 2B**, there has been a tremendous increase in the development of different types of immunosensors in the past decades, especially EC immunosensors.

2.3. Significance of antibodies in immunosensor

Antibodies are Y-shaped immunoglobulin (Ig) (**Fig. 2C**), which are synthesised by plasma cells to respond to a foreign substance entering the body. Generally, there are five different classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE) which differ in the constant and variable regions of the heavy chains, which give them different effector functions. Among the five types, IgG is frequently used in immunoassays as it is available in

large amounts and can be found in blood and extracellular fluid [70]. Antibodies are widely used as the biorecognition element due to their binding specificity, favouring selectivity [57].

3. Immunosensor formats and immobilisation method

There are many immunosensing methods, but the most extensively used format includes direct, competitive, and sandwich methods. For the direct format, also known as non-label immunosensor, the analyte is captured by the immobilised antibody, and the detector directly records the signal detected. As for the competitive format, the sample antigen and the labelled antigen must completely bind to the immobilised antibody, and the signal is recorded. The sandwich format uses two antibodies to sandwich the antigen and often provides greater selectivity. This is because cross-reacting species seldom react and thus sandwiching in between the species increases its selectivity [71]. Moreover, indirect detection provides better sensitivity and selectivity. However, more complexity of indirect detection based immunosensor is disadvantageous due to the overall cost. In addition, practical application of the indirect detection based immunosensor is also a challenge due to the concern of reagents stability as well as cost of additional reagents and materials[72]. **Fig. 2D** illustrates the difference between a direct, indirect, sandwich, direct competitive and indirect competitive immune sensing methods.

Further, to improve the immunosensor sensitivity, the signal amplification system need be modified using novel strategies. In addition, how the biorecognition layer is immobilised onto the electrode (transducer) plays a vital role in improving the sensor sensitivity [73]. Besides, the performance of the immunosensor, such as its repeatability, stability, specificity and LoD, are considerably associated with the antigen-binding molecules (antibodies/biorecognition elements) [71]. Moreover, the immobilisation method and the

immunoreagents influence the biorecognition elements (antibodies) amount, distribution, orientation and the resulting bioactivity on a transducer. Therefore, in recent years, several immobilisation strategies have been reported. Albeit in selecting an immobilisation strategy, the Fab region is considered, ensuring that the region remains unchanged throughout the process [67]. Whilst different immobilisation methods lead to uniform or random orientation of biorecognition elements on a solid support (transducer). Thus, it is crucial to select methods that avoid the destruction or cause steric hindrance of the immobilised Fabs.

3.1. Physical adsorption

Depending on the immobilisation technique, the immobilised biorecognition elements can assume a variety of orientations. Methods such as adsorption often result in a more "flat-on" orientation in which the Fab and Fc regions lay flat on the surface. This confirmation causes hindrances in the ability of an antigen to access the antibody binding sites, which in turn results in a decrease in the binding capacity of the antigen. Albeit specific orientation is always preferable, it is not simple to achieve with adsorption because site-specific modification of antigen-binding molecules necessitates the inclusion of a unique reactive group [67].

As illustrated in **Fig. 3A**, physical adsorption method adsorbs protein onto the electrode surface via noncovalent interactions, mainly electrostatic forces, including ionic, hydrogen and hydrophobic. Furthermore, this approach provides non-covalent antibody attachment primarily "tail-on." As a result, this simple method generates only a tiny effect on bioactivity [74]. Albeit this immobilisation method is the simplest, however unfortunately, there are a few drawbacks in terms of stability which results in random orientation and weak attachment between the biomolecules and substrate. This frequently results in protein denaturation and

dissociation on the surface [75]. In addition, the drop-casting method that involves the deposition of solution droplets where the solvent is left to evaporate slowly leads to non-uniformity in the thickness of the thin film. Further, material deposition at a precisely defined location is challenging, resulting in changes in sensor variance [76]. Thus, to overcome these challenges, it has also been reported that incorporating nanoparticles into physical adsorption-based enzyme immobilisation enhances stability and orientation [77].

3.2. Polymer entrapment

Another method of immobilisation is polymer entrapment. To ensure the high stability and bioactivity of immunosensors, various polymers with a structural network are often used for the entrapment of immunoreagents. For example, high-capacity porous gel matrices and organically modified silicate sol-gel are used as they provide excellent biocompatibility and an environment for antigen immobilisation [74]. Polymers can be in a relatively flat layer adhering to the surface or by looping strands extending distally from the surface, as shown in (a) and (b), respectively, in **Fig. 3B**. Further, various densities of antibodies can be obtained by layering different thicknesses of the polymer [75]. Therefore, in recent years, significant benefits of using polymers for enzyme immobilisation have been recorded because the enzyme, mediator and additives could be deposited simultaneously. Moreover, this immobilisation is relatively easier to develop [78].

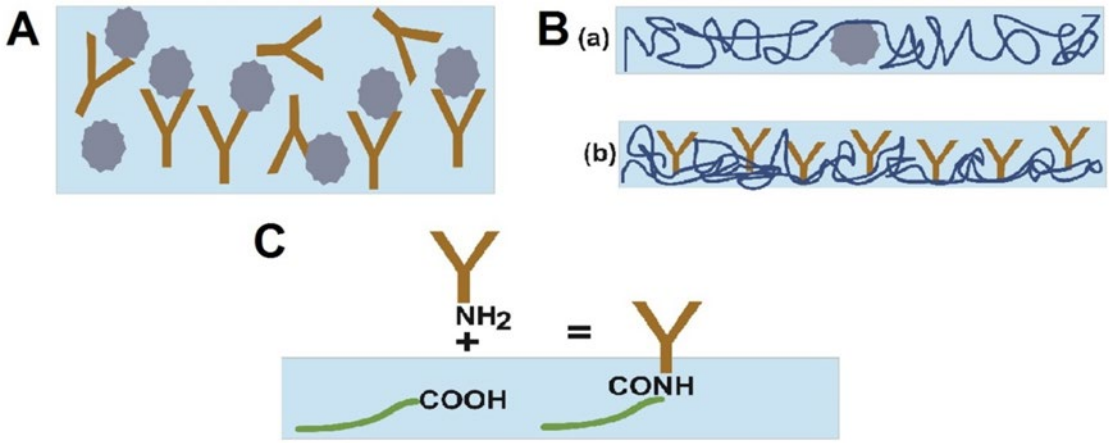

Fig. 3 (A) Physical immobilisation can cause interference with adsorption of protein on the surface due to inconsistencies; **(B)** Entrapment of immunoreagent; and **(C)** Covalent immobilisation technique.

3.3. Covalent immobilisation

The covalent immobilisation technique utilises the covalent bond, which is formed mainly between functional groups with exposed side chains of proteins and the modified surface of the transducer (**Fig. 3C**). This results in an irreversible binding which allows high surface coverage. This method randomly binds antibodies with an activated sensor surface via their amino group. Depending on the functional groups present on the sensor surface, the functional group is activated by treating with chemical esters [73,74]. For example, the amine functional group on the sensor surface could activate using chemical agents such as isothiocyanate, epoxide or aldehyde [79–81].

4. Nanomaterials-based immunosensors

As sensitivity is one of the essential factors in developing an immunosensor, it is crucial to amplify the signal that results from the antibody-antigen reaction [73]. To achieve this enhanced sensitivity, nanomaterials-based immunoassays and immunosensors that depend on antibody-antigen interactions offer a promising way of studying this aspect [82].

Nanomaterials (NMs) are small-scale chemical materials that are 1-100 nm small in dimension [47,48] and demonstrate unique electrical and optical properties. Owing to these advantages, NMs have significantly impacted various fields, especially the electronic and medical fields [83]. As seen in **Fig. 4A**, there has been a significant increase in the number of articles published related to NMs and their applications, demonstrating the importance of nanotechnology in NMs based immunosensors. Furthermore, there has been an increase in immunosensors fabrication with the involvement of NMs in the last ten years, as shown in **Fig. 4B**. **Fig. 4C** portrays the increased application of specific NMs in immunosensor fabrication.

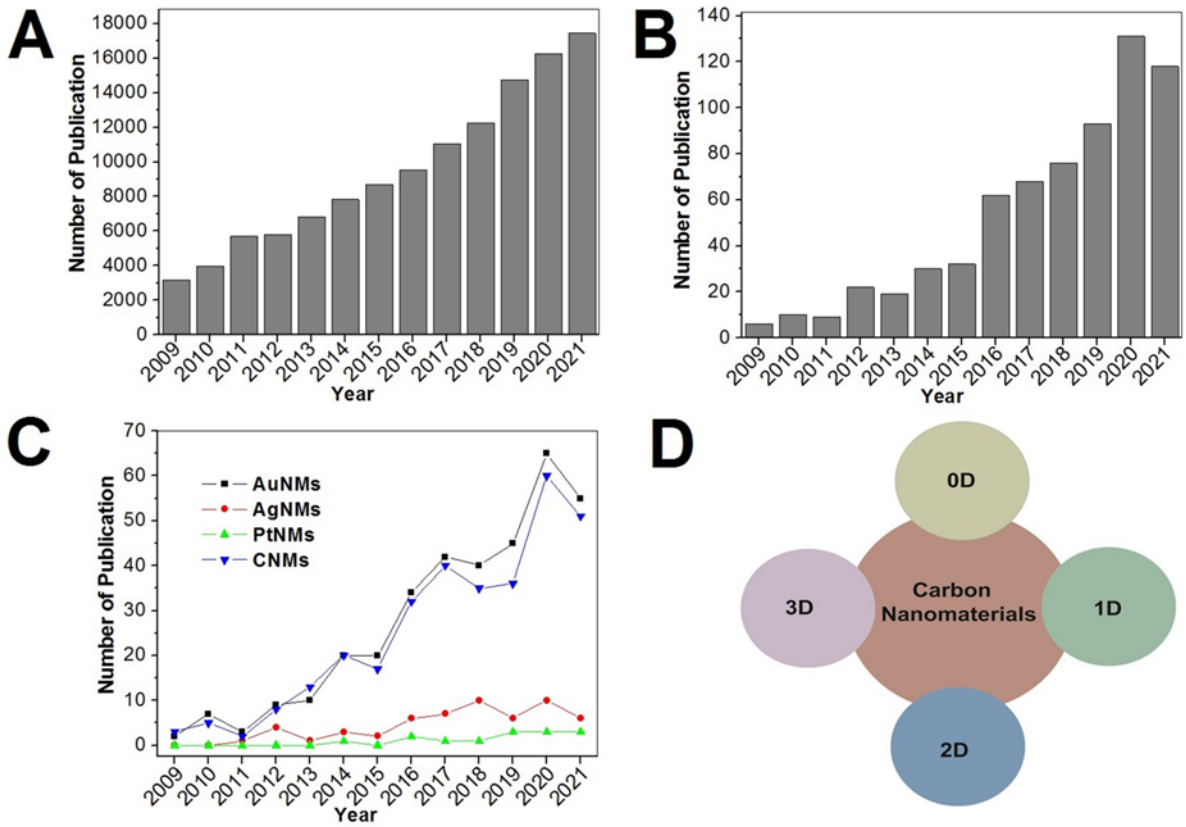

Fig. 4 (A) Articles published with the keyword "Nanomaterials", compiled from Web of Science™ database from the year 2009-2021 on 07th of April 2022; **(B)** Articles published with the keywords "Nanomaterials" and "Immunosensor", compiled from the Web of Science™ database from the year 2009-2021, on 07th of April 2022; **(C)** Articles published on the specific NMs used in the fabrication of immunosensor, obtained and compiled from Web of Science™ database from the year 2009-2021, on 07th of April 2022, on 20th of September 2021 [Keywords: "gold nanomaterials and immunosensor" (AuNMs); "silver

nanomaterials and immunosensor" (AgNMs); "platinum nanomaterials and immunosensor" (PtNMs); and "carbon nanomaterials and immunosensor" (CNMs); and **(D)** Schematic illustration portraying different dimensions of CNMs (0Ds such as fullerene, 1Ds such as carbon nanotubes, 2Ds such as graphene and 3Ds such as graphite).

This shows that the advances in nanotechnology have demonstrated outstanding potential for using NMs in fabricating different types of immunosensors to achieve specific objective especially in signal amplification of EC immunosensor [82]. In particular, the sensitivity can be increased by loading copious amounts of electroactive labels in EC immunosensing and heavy mass loading in mass immunosensing [84]. Furthermore, with the involvement of NMs in developing biosensors, analytical performance gets greatly improved [85]. In addition by utilising the electron transfer abilities, biocompatibility, and large surface area of the NMs such as platinum nanomaterials (PtNMs), carbon nanomaterial (CNMs), gold nanomaterial (AuNMs) and silver nanomaterials (AgNMs) are suitable in various immobilisation strategies for fabricating immunosensors [7,86]. Each of these NMs has attractive fundamental properties, and thus a myriad of combinations is applied in the immunosensor to improve the detection of the target analyte. Moreover, different types of NMs and their forms employed to fabricate immunosensors for sensitive detection of carcinoembryonic antigens (CEA) have been summarised in **Table 1**.

Table 1. Summary of specific NMs and their critical properties employed for the fabrication of immunosensors for sensitive detection of carcinoembryonic antigen.

Form of NMs	Properties	Applications' Objective	Detection Approach	Detection Type	Biological Samples	Detection Limit	Ref.
			AuNMs				
Gold nanoparticles (AuNPs)	enhance electrical properties	impressive linear range	EC	direct	clinical blood	0.06 pg/mL	[87]
Nano-gold	larger surface area and good biocompatibility	to enhance the large immobilization amount of anti-CEA	EC	direct	-	0.1 ng/mL	[88]
Au@Ag nanorods	catalytic, large amount of anti-CEA adsorption because of the large surface area and good biocompatibility	peroxidase mimic	ECL	direct	human plasma	30 fg/mL	[89]
Au@Pd nanorods	catalytic, high homogeneity, biocompatibility with proteins, and easy preparation	peroxidase mimic	ECL	sandwich	human serum	3 fg/mL	[90]
3D AuPd nanoflowers	simple preparation and larger specific surface area	catalytic	EC	sandwich	human serum	3.2 fg/mL	[91]
Gold colloidal nanoparticles	high surface-to-volume ratio, mediate electron transfer amid biomolecules and surface of the electrode, good biocompatibility, and high affinity of mercapto group (-SH), amino group (-NH2), and nitrile group (-CN)	improve sensitivity	EC	direct	human serum	0.1 ng/mL	[92]
AuNPs	better electrical conductivity and good biocompatibility	primary antibody immobilization	EC	sandwich	human serum	0.33 pg/mL	[93]

Cubic Au@Pt Dendritic NMs	large active surface area, good, excellent conductivity, high catalytic and biocompatibility; further cubic NMs provide high electrocatalytic efficiency compared to spherical NMs	efficiently enhance electrocatalysis	EC	sandwich	human serum	0.167 pg/mL	[94]
AuNPs	good conductivity	sensing platform	EC	sandwich	serum samples	0.17 pg/mL	[95]
Chitosan wrapped AuNPs (CS-Au NPs)	improve conductivity and facilitates electron transfer	immobilization of anti-CEA	EC	direct	whole blood and serum samples	0.251 fg/mL	[96]
nano-Au	larger surface area and good biocompatibility	immobilization of anti-CEA	EC	direct	serum samples	0.083 ng/mL	[97]
AuNPs	large surface area, splendid electrical conductivity, high biocompatibility, and excellent binding with the antibody	signal amplification	EC	direct	human serum	100 fg/mL	[30]
AuNPs	large active surface area, high electrical conductivity, excellent biocompatibility and high stability	to enhance conductivity and loading capacity	EC	direct	human serum	75 fg/mL	[98]
		AgNMs					
Au@Ag nanorods	catalytic, large amount of anti-CEA adsorption because of the large surface area and good biocompatibility	peroxidase mimic	ECL		human plasma	30 fg/mL	[89]
Silver nanoparticles (AgNPs)	catalytic	as the probe and signal amplification	EC	sandwich	serum samples	0.17 pg/mL	[95]
nano-silver	cheap, excellent conductivity and catalytic	as the probe and signal amplification	EC			0.18 pg/mL	[99]

(AgNPs)							
Silica-coated silver Nanoparticles ($Ag@SiO_2$ NPs)	excellent electrochemical characteristics, exceptional biocompatibility and good stability	to enhance the current response	EC	direct	-	0.01 ng/mL	[100]
AgNPs	large specific surface area, superior electrical conductivity and better biocompatibility	signal amplification	EC	direct	human serum	0.3 pg/mL	[101]
AgNPs	large specific surface area, high conductivity, biocompatibility, small granule diameter, and transfer photoinduced electrons	to enhance electrochemical response	EC	sandwich	clinical serum	1.0 pg/mL	[102]
AgNPs	large surface area, high electrical conductivity, excellent biocompatibility and high stability	signalling probe	EC	direct	human serum	75 fg/mL	[98]
		PtNMs					
platinum metal nanoparticles	large surface area, biocompatibility and excellent electrochemical stability	to capture antibodies and accelerate electron transfer	EC	sandwich	human serum	0.0006 ng/mL	[7]
Platinum Nanoparticle	high specific surface area, high electrical conductivity, good biocompatibility, excellent biomolecular adsorption, and good chemical stability	to enhance antibody loading capacity and to amplify the electrical signal	EC	direct	clinical serum	0.01 ng/mL	[45]
Flower-like PtAu NPs	excellent electrocatalytic activity, high electrode surface area, biocompatible matrix for antibody, thus providing	high-efficiency immunoassay platform	EC	direct	serum samples	7 fg/mL	[103]

Trimetallic NiAuPt nanoparticles	resistance against electromigration, high electrocatalytic performance,	signal amplification	EC	sandwich	human serum	0.27 pg/mL	[96]
CNMs							
Graphene and carbon nanotubes	large surface area and high electrical conductivity	to enhance electrode surface area and improve the electron transfer rate	EC	direct	human serum	60 pg/mL	[104]
Carbon nanodots (CNDTs)	large surface area, excellent electrical conductivity, peroxidase property, high aqueous solubility, low toxicity, photophysical properties, ease of synthesis, robust chemical inertness, excellent biocompatibility and cheap	peroxidase nanozyme	EC	direct	human serum	1.45 pg/mL	[17]
Carbon nano-onions (CNOs) and single-walled carbon nanotubes (SWCNTs)	Large surface area, high electrical conductivity, high aspect ratio and excellent biocompatibility	signal amplification	EC	direct	human serum	100 fg/mL	[30]
Carbon quantum dots (CQDs)	large modifiable active surface area, unique optical properties, high electrical conductivity, controllability, biocompatibility, low toxicity and environmentally friendly material	ECL signals	ECL	sandwich	human serum	1.67 pg/mL	[105]
3D porous structures of graphene	large active surface area and excellent electrical conductivity	large surface area	EC	direct	serum samples	5.0 pg mL^{-1}	[22]

reduced graphene oxide (rGO)	high electrical conductivity, large surface area, excellent biocompatibility, non-toxicity, and functional groups to bind various biomolecules	to bind the antibody	EC	direct	human serum	0.05 ng/mL	[106]
graphene quantum dots (GQDs)	excellent electrical properties and fast electron transfer,	amplify the electrochemical activity	EC	direct	human serum	10 pg/mL	[107]

4.1. Platinum nanomaterials

In the last decade, there has been a tremendous increase in the utilisation of platinum nanomaterials (PtNMs) in the fabrication of immunosensor. This is not surprising as PtNMs possess attractive properties, including large surface area, excellent electrical conductibility, biocompatibility and good catalytic properties. These properties help in improving sensor sensitivity and stability [85]. Further, while developing immunosensors, PtNMs are used as a substrate material to incubate the capture antibody [7]. An example of the incorporation of PtNMs in immunosensor has been shown in a study by Wang *et al*., where the specific detection of CEA was successfully carried out using a dual-mode biosensor. This study developed the EC analysis combined with colorimetric analysis technology to quantify tumour makers accurately. The developed method improved the accuracy of the clinical detection of CEA in comparison to a single response system [108]. Similarly, in another study by Lv *et al*., an EC immunosensor has been developed based on a cubic Au@Pt dendritic NMs functionalised nitrogen-doped graphene loaded with copper ion (Au@Pt DNs/NG/Cu^{2+}) modified glassy carbon electrode (GCE) for the ultrasensitive, and early detection of CEA. Interestingly, it was observed that the electrocatalysis for the reduction of hydrogen peroxide (H_2O_2) was enhanced efficiently due to the nanocomposite, owing to its large surface area and greater EC properties. In addition, the modified GCE demonstrated exceptional adhesivity and conductivity, providing a potential alternative for determining CEA in actual samples [94]. Due to their attractive outcome when combined with other materials, PtNMs have gained remarkable interest in the development of immunosensor. A summary of some recent development of immunosensors with the incorporation of PtNMs can be seen in **Table 2**, which demonstrates a remarkable detection range of the immunosensor.

4.2. Carbon nanomaterials

Carbon nanomaterials (CNMs) have currently gained significant attention recently due to their electrical and mechanical properties, high surface-to-volume ratio, electrical conductivity, excellent chemical stability, biocompatibility, and robust mechanical strength [47,109]. Various types of CNMs ranging from zero to three-dimensional structures are shown in **Fig. 4D**. Their typical structure includes fullerenes, carbon dots (CDs), carbon nanotubes (CNTS), nanofibers, nanodiamonds, and graphene [110]. Interestingly, CDs have attracted attention of the scientists and researchers involved in photophysical studies as well as imaging targets in live cells due to their various appealing properties including easy manipulation, chemical inertness and property to disperse in water. For example, Lu *et al*., fabricated different types of CDs for emission of three types of colours [111], whilst Zhou *et al.,* designed novel chemical sensors to study targets in live cells [112]. Moreover, due to the unique property of CNMs, the evolution and development of EC sensors and biosensors have been greatly influenced, especially regarding a modern EC system [113]. An example of widely used CNMs is multi-walled carbon nanotubes (MWCNT), which have gathered much interest due to their attractive features of outstanding electrical conductivity, exceptionally high mechanical strength, and remarkable chemical stability [114]. Additionally, MWCNTs are an excellent supporting catalyst as they possess benefits like significant effective surface area and reactive functional groups that allow for loading high-density redox species and an enhancement in EC currents [115,116]. For example, in a study by Jian *et al*., β-cyclodextrin/multiwalled carbon nanotubes (β-CD/MWCNTs) were fabricated for the quantitative determination of CEA. In this study, the sensitivity was improved by the enhanced electroconductivity and the immobilisation of the primary and secondary antibodies [117].

Besides, graphene nanomaterials (GNMs) have also attracted the attention of scientists and researchers across the globe [118,119]. Graphene is an allotrope of carbon which is a flat monolayer of sp^2-bonded carbon atoms tightly packed into a 2D-honeycomb lattice structure [66,73,120]. Interestingly, it is the thinnest material in the universe. It has a large surface area of 2630 m^2/g, which is significantly larger than that of graphite and carbon nanotubes, with surface area values of 10 m^2 and 1315 m^2/g, respectively [66,85]. Moreover, graphene possesses more advantageous properties compared to other materials due to its shape and chemical structure [88,121]. In addition, they have a large surface area, which aids in the immobilisation of large amounts of antibodies and outstanding electrical conductivity; thus, graphene and graphene-related materials have been extensively used in EC biosensors [121,122]. These materials, especially graphene sheets, aid in amplifying the detection response [70,75]. Graphene-based immunosensors immobilise the antibody molecules via covalent immobilisation and exhibit remarkable sensitivity and selectivity towards cancer biomarkers detection [39,123]. Another prominent feature of graphene is its high electron transfer rate and its high carrier mobility [124,125]. The unique properties GNMs, particularly graphene oxide, are excellent for EC applications such as sensor and biosensor development [125,126]. This can be seen in a study by Jozghorbani *et al*. in which a label-free EC immunosensor was developed based on the immobilisation of anti-CEA on reduced graphene oxide modified GCE (rGO/GCE). The proposed immunosensor showed remarkable selectivity and specificity towards its target molecule [106]. In the tabulated summary of some of the recent development of immunosensor with regards to the incorporation of GNMs, it can be seen that the inclusion of graphene, notably reduced graphene oxide, brings about a reasonable LoD that is viewed as attractive to many researchers [127,128]. Examples of GNMs incorporated immunosensor properties are portrayed in **Table 2**.

4. 3. Gold nanomaterials

In recent years, gold nanomaterials (AuNMs) and their nanocomposites have been extensively studied, and they are used to modify electrode surfaces in the fabrication of biosensing platforms for biomarker detection [128]. AuNMs are one of the most commonly used NMs for the fabrication of EC immunosensor [73], because of their remarkable characteristics, which elevate the electron transfer properties, including biocompatibility and electrical conductivity [8]. Therefore, AuNMs based on varying shapes serve as favourable conductive materials to enhance the response and improve immunosensor sensitivity [129]. Furthermore, besides their micro and macro sizes, AuNMs possess other properties like their inertness, improving their usability in multiple chemical processes. In addition, AuNMs allow electronic mobility with a spatial length scale, a unique electronic property for application in localised surface plasmon resonance (LSPR) [84]. For example, in a study by Begum *et al*., an ultrasensitive EC immunosensor was successfully constructed for the quantitative detection of CEA using Au@SiO_2/Cu_2O as the signal label. The developed sensor displayed remarkable biocompatibility, sensitivity as well as excellent conductivity. Furthermore, the study showed that the incorporation of the SiO_2 nano-frames not only minimises AuNMs and Cu_2O agglomeration and offers high biocompatibility for secondary antibody attachment [130]. Further, studies on AuNMs-based immunosensors are illustrated in **Table 2**.

4. 4. Silver nanomaterials

Silver nanomaterials (AgNMs) are one of the low-cost noble nanomaterials [100,101]. Moreover, AgNMs also have unique physical and chemical properties such as large surface to volume ratio, high electronic conductivity, high chemical stability, large catalytic activity and excellent biocompatibility [99,101,131]. Thus, due to these unique properties AgNMs have been widely employed in medical and healthcare [132]. Likewise, application of AgNMs and

their composites has greatly increased in the biosensor and immunosensors as part of strategies for the accurate and sensitive detection of the disease biomarkers including CEA [99,100,102]. For example, Wang *et al*., used Ag-$CoFe_2O_4$-GO nanocomposite to modify GCE for having novel interface with large specific surface area, strong redox capacity, faster electron transfer and good biocompatibility. Further, immunosensor was developed over modified (GCE/Ag-$CoFe_2O_4$-GO) interface for the label-free electrochemical detection of the tumour marker CEA. Developed immunosensor demonstrated a wide linear range for the detection of the CEA from 1 pg/mL to 80 ng/mL with low LoD 0.3 pg/mL. In addition, immunosensors portrayed excellent stability, good selectivity and stability. Furthermore, RSD [%, *n* =5] within the range of 1.96 to 3.26 illustrated the potential of the immunosensor to detect the CEA in real clinical samples [101]. Few examples of AgNMs-based immunosensors are enlisted in **Table 2**.

Table 2. Examples of PtNMs, CNMs, AuNMs and AgNMs incorporated in the development of immunosensors.

Different NMs modified electrode	**Detection method**	**Detection range**	**Limit of Detection**	**Ref**
Au@PtPd MPs	CA	50 fg/mL to 100 ng/mL	16.7 fg/mL	[8]
$ZnMn_2O_4$@rGO	DPV	0.01 to 50 ng/mL	1.93 pg/mL	[24]
GNP-MnO_2/Fe_3O4@Au	LSV and EIS	0.001 to 100 ng/mL	0.10 pg/mL (LSV) 0.30 pg/mL (EIS)	[133]
Ag-Co3O4@NrGO	DPV	0.001 to 200 ng/mL	0.18 pg/mL	[99]
rGO/GCE	CV and EIS	0.1 to 5 ng/mL	0.05 ng/mL	[106]
Au@SiO_2/Cu_2O	CA	0.01 pg/mL to 80 ng/mL	0.0038 pg/mL	[130]
Ag/MoS_2/rGO	AC impedance	0.01 pg/mL to 100 ng/mL	1.6 fg/mL	[134]
PB@PDA NPs	PEC	0.001 to 100 ng/mL	54.9 fg/mL	[135]

Abbreviations: Au@PtPd MPs, mulberry-like Au@PtPd porous nanorods; Ag/g-C_3N_4/Au@SiO_2/Cu_2O, Ag modified 2D aminated nitrogen carbide nanosheets with $AuSiO_2$/Cu_2O; GNP-MnO_2/ Fe_3O_4@Au, manganese dioxide on graphene nanoplatelets with core-shell Fe_3O_4@Au nanoparticles; and PB@PDA NPs, polydopamine (PDA)-coated Prussian blue (PB) nanoparticles.

5. CEA detection approaches using immunosensors

The unique properties of NMs are synchronised with the employed detection approach. In addition application of NMs enhance the sensitivity of immunosensors [136–138]. The most common detection approach in immunosensor includes (a) PEC, (b) ECL and (c) EC. Therefore, based on the detection approaches, there are three main types of nano-immunosensors which includes PEC, ECL and EC-based nanoimmunosensors [139–141]. PEC technique-based immunosensors provide high selectivity at a low cost due to simple instrumentation, albeit the LoD and linear detection range highly depends on the selected photo-excited material. While high sensitivity, rapid response and low background signal are essential characteristics of ECL technique-based immunosensors. However, EC immunosensors demonstrate excellent selectivity, sensitivity and rapid response with low-cost. Moreover, EC immunosensors are based on the most straightforward classic technique. Further, both ECL and EC techniques based immunosensors have the possibility of miniaturisation and portability. In addition, each the nano-immunosensors are briefly described in the following sections.

5.1. Photoelectrochemical nanoimmunosensor

Many PEC immunosensors have been developed in recent years, showing promising properties of good storage stability and reproducibility [142]. PEC immunosensors possess characteristics of both spectroscopic and EC techniques and help measure photocurrent generated [141]. This method dramatically affects photocurrent changes and uses charge

transfer current and photoexcited material as a detection signal [142]. PEC immunosensors have simple instrumentation with high selectivity at a low cost compared to conventional EC methods [39,143]. Thus, many researchers prefer the detection quality exhibited by PEC immunosensors. For instance, in a study by Liu *et al*., a PEC immunosensor was successfully developed for the sensitive detection of CEA based on g-C_3N_4/CdSe nanocomposite. In this study, g-C_3N_4/CdSe nanocomposite was employed over fluorine-doped tin oxide (FTO) electrode, which was treated with poly dimethyl diallyl ammonium chloride. The FTO electrode showed a small photocurrent signal response compared to g-C_3N_4/CdSe nanocomposite modified FTO electrode. This could be due to matching of the g-C_3N_4 direct band gap with CdSe QDs energy level [143]. In addition, several such studies have been carried out in recent years; examples of a few NMs-based PEC immunosensors are illustrated in **Table 3**.

5.2. Electrochemiluminescence nanoimmunosensor

Electrochemiluminescence (ECL) is a quantitative method for measuring antibodies and antigens associated with changes in the ECL signal before and after immunoreaction [74]. This is a powerful and effective advanced analytical technique used in a variety of disciplines including (i) diagnostic and medicines [144–148], (ii) analysis and visualising [149,150], (iii) forensic investigation [151,152], (iv) food analysis [153,154], (v) environmental analysis [155,156], and (vi) safety and security [157,158]. As a result of its high sensing efficiency, flexibility, simple optical setup and significant temporal and spatial control, studies on ECL-based immunosensors have increased in recent years [159]. Furthermore, ECL-based immunosensor demonstrate unique properties such as high selectivity, sensitivity, reproducibility, simplicity, rapid response, wide dynamic range, and low background signal [48]. Various luminophore and co-reactant systems on various electrode materials have been studied to evaluate the pair with high ECL intensity [38]. This technique uses light emitted by

the species at the electrode in the presence of high applied voltage [57]. Owing to these advantages, this technique has been widely used primarily in the clinical analysis [54].

However, although ECL-based immunosensors are commonly used, this technique poses a few disadvantages: it is complex and often requires additional bulky instrumentation such as optical devices and special image recognition software [142]. Traditionally, ECL relies on a single signal output likely influenced by various external and internal factors such as electrode modification, instrumental performance and ambient conditions [38]. Furthermore, a single-signal assay is insufficient for disease diagnosis or prognosis because some diseases are associated with many biomarkers. To address the difficulties mentioned above, various ECL techniques with multiple-signal outputs such as radiometric ECL multiplex immunoassays (MIA) have been developed [146]. In addition, these have gained remarkable attention due to the growing demand for immunoassays with high accuracy and efficiency in clinical diagnosis [159,160]. For example, in research by Zhang *et al*., an ECL immunosensor was successfully developed using multi-functionalised flower-like Au@BSA nanoparticles to detect CEA with high sensitivity. In addition, the immunosensor achieved tremendous stability, excellent reproducibility and selectivity. According to the findings, the proposed immunosensor could provide a unique strategy for sensitive tumour marker detection in clinical immunoassays due to the remarkable biocompatibility coupled with the attractive electroconductivity and the large surface area of the composite used in the purposed ECL immunosensor [161]. Furthermore, when coupled with various NMs, ECL exhibits great potential for the sensitive immunosensor development [162]. A summary of the recent examples of ECL-based immunosensor fabricated for CEA detection is illustrated in **Table 3**.

5.3. Electrochemical nanoimmunosensor

Electrochemical immunosensors are the most commonly studied sensors among researchers, as illustrated by several publications in the last decade. This type of immunosensor is based on the measurement of electrode potential of a redox reaction, interfacial capacitance, or impedance [13,163,164]. As a result of the formation of antigen-antibody complexes, which displays significant difference in magnitudes, it aids in measuring a change in current, voltage or impedance [74]. Furthermore, these sensors demonstrate excellent selectivity and sensitivity and rapid response [5,7,25,58], which are essential properties in designing biosensors [165]. The EC immunosensors also carry the advantages of being cost-effective, possible miniaturisation and portability [165,166].

Electrochemical immunosensor could be categorised into four types based on their transduction mode which include amperometric, potentiometric, conductometric and impedimetric immunosensors [13,53]. Typically, EC immunosensors use a three-electrode system which consists of a working electrode, a reference electrode and a counter (auxiliary) electrode [167]. The fabrication of EC immunosensors is done via the immobilisation of a recognition element onto the electrode surface. Thus, this method relies on the binding between the antigen-antibody to measure the difference in currents and voltage [35]. In research by Singh *et al*., an EC disposal immunosensor was successfully created by immobilising anti-CEA on Ag@SiO_2 nanoparticle-coated defined ITO solid at the substrate to detect CEA. The study aimed to combine the benefits of silver nanoparticles with silicon dioxide (SiO_2) on an ITO at the substrate to create a new EC immunosensor for the sensitive and selective detection of CEA. The proposed immunosensor yielded an improved linear range and a remarkable detection limit [100]. In addition, metal nanoparticles can be directly electrodeposited on immunosensing surfaces to generate the EC signal indication or act as an

ideal anchor to attach biomolecules as sensing components[168]. This can be done by providing a biocompatible microenvironment to the biomolecules by using noble metal nanoparticles that substantially improve the surface-to-volume ratio of an immobilized biomolecule on the electrode surface, both of which adversely allow electrical signal enhancement [100,169]. Some of the recent examples of the NMs-based EC immunosensor fabricated for CEA detection are summarised in **Table 3**.

Table 3. Recent studies on NMs-based PEC, ECL and EC immunosensor for CEA detection.

Nanocomposite	Detection Approaches	Linear Range	Limit of Detection	Sensitivity	Specificity / Selectivity	Reproducibility	Sample Analysis	Ref
$ZnMn_2O_4$@rGO	EC	0.01 to 50 ng/mL	1.93 pg/mL	improved	high	excellent	human serum	[24]
Au@Pd nanorods	ECL	10 fg/mL to 100 ng/mL	3 fg/mL	high	good	good	human serum	[90]
Ag@SiO_2 NPs	EC	0.5 ng/mL to 10 ng/mL	0.01 ng/mL	high	outstanding	good	-	[100]
rGO/GCE	EC	0.1 to 5 ng/mL	0.05 ng/mL	high	acceptable	acceptable	human serum	[106]
Au@SiO_2/Cu_2O	EC	0.01 pg/mL to 80 ng/mL	0.0038 pg/mL	improved	good	good	human serum	[130]
WO_3/Au/CdS	PEC	0.01 to 10 ng/mL	1 pg/mL	high	good	good	human serum	[170]
g-C_3N_4/CdSe	PEC	10 ng/mL to 100 μg/mL	0.21 ng/mL	high	excellent	acceptable	-	[144]
Au@BSA	ECL	0.001 to 200 ng/mL	0.0003 ng/mL	high	good	acceptable	human serum	[161]
2D-ReS_2 nanosheets	PEC	0.0005 to 10.0 ng/mL	0.468 pg/mL	high	good	good	human serum	[171]
PEI-GO-CQDs/Au-NPs	ECL	0.005 to 500 ng/mL	0.00167 ng/mL	high	acceptable	good	human serum	[172]

Abbreviation: BSA, bovine serum albumin; and PEI-GO-CQDs/Au-NPs, carbon quantum dots (CQDs) on poly(ethylenimine) functionalised graphene oxide (PEI-GO).

6. Nanomaterials-based immunosensors for the sensitive detection of CEA

Often, a combination of NMs is used to fabricate immunosensors because different NMs synergistically amplify the signals generated and provide better sensitivity to the immunosensor [105]. Therefore, numerous studies have been made on various combinations of NMs-based immunosensors such as ECL immunosensors [70,75,161,171,173] and PEC immunosensors [142–144,170] to detect CEA with high sensitivity. In particular, EC immunosensors have earned immense interest in designing highly sensitive and selective immunosensors for biomarker detection, including CEA, due to their remarkable advantages, such as their high sensitivity, cost-effectiveness, power requirement and potential automation [73]. Thus, the following section will discuss examples of immunosensors for single tumour biomarkers and immunosensors fabricated for simultaneous detection of CEA and other biomarkers.

6.1. Nanomaterials-based immunosensors for CEA detection

Recently, Nakhjavani *et al.* reported an EC immunosensor based on the gold and silver bio/nano-hybrids and thiolated-graphene oxide (T-GO) for ultra-sensitive detection of CEA **(Fig. 5A)**. In this study, firstly, the electrode surface was modified with T-GO to increase the active surface area to enhance the loading capacity of the CEA monoclonal antibody (CEA mAbs). AuNPs were first coated with streptavidin (STP) before introducing the composite to the electrode surface. Then, to achieve the highest sensitivity, a sandwich approach was implemented using the STP-AuNPs conjugated with CEA mAbs and horseradish peroxidase (HRP) to amplify the signal obtained. Finally, the detection of varying concentrations of CEA was carried out using differential pulse voltammetry (DPV) in phosphate buffer saline (PBS) containing hydroquinone (HQ) and hydrogen peroxide (H_2O_2). An excellent detection limit of 75 fg/mL and a linearity range of 100 fg/mL to 5 pg/mL were obtained. The study concluded

that the engineered immunosensor showed excellent stability, repeatability, specificity, and selectivity contributed by the AuNPs coated streptavidin, which provided a more oriented binding site, resulting in a dense loading capacity for mAbs. The excellent outcome was also greatly influenced by using HQ and H_2O_2 in the PBS, as this combination facilitates the electron transfer, further amplifying the EC signals in the final step [98].

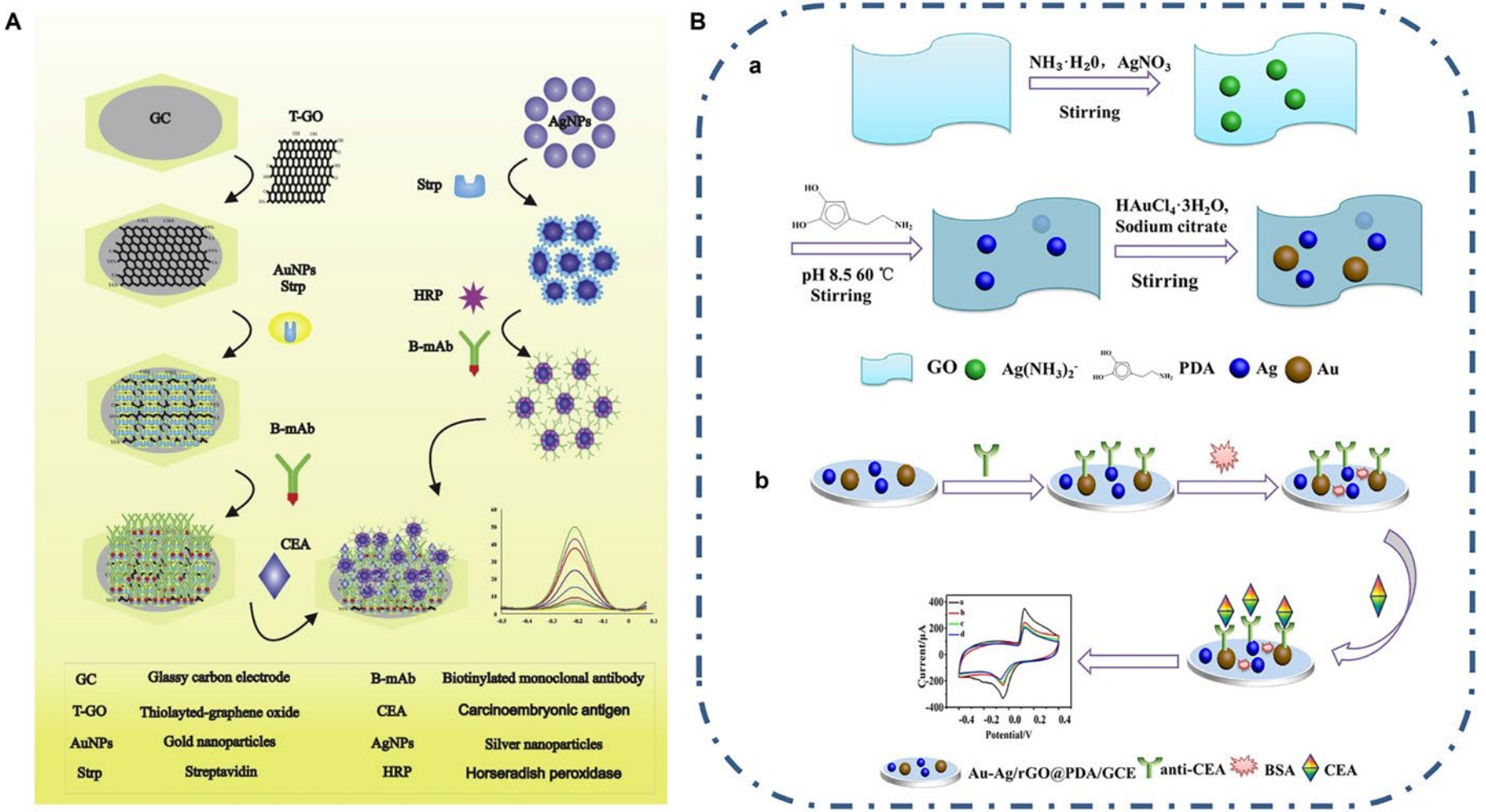

Fig. 5 (A) Schematic illustration of the electrochemical immunosensor for the ultra-sensitive detection of CEA based on the gold and silver bio/nano-hybrids and thiolated-graphene oxide (T-GO). Reprinted from reference [98] with permission from Elsevier; and **(B)** Schematic illustration of **(a)** preparation of Au-Ag/rGO@PDA nanocomposite and **(b)** Au-Ag/rGO@PDA/GCE based immunosensor for the CEA detection. Reprinted from reference [174] with permission from Elsevier.

In a separate study, Yang *et al.* designed a mono-step and environmental-friendly EC immunosensor for CEA quantitation based on the dual signal amplification strategy (**Fig. 5B**). The authors used gold nanoparticles combined with silver and reduced graphene oxides on polydopamine (Au-Ag/rGO@PDA) nanocomposite (**Fig. 5B(a)**). A conventional three-electrode system was used: a GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. Firstly, the electrode surface modification with Au-Ag/rGO@PDA was done before the incubation in a solution of anti-CEA. Next, to eliminate unbounded non-specific active sites on the electrode surface, the electrode was incubated in bovine serum albumin (BSA). Then finally, the electrodes were incubated in different concentrations of CEA standard solution. The CEA detection was carried out using cyclic voltammetry (CV) in a PBS containing a mixture of KH_2PO_4, Na_2HPO_4 and KCl **(Fig. 5B(b))**. The group recorded a detection limit of 286 fg/mL and a linear range of 0.001 to 80 ng/mL. The result indicated the quantitative ability of the immunosensor to detect CEA. Furthermore, the authors reported that the designed immunosensor displayed excellent detection performances packed with good selectivity, reproducibility and stability [174].

In yet another study, a sandwich-type EC immunosensor (**Fig. 6A**) was developed based on Au triangular nano prisms (AuTNPs) as the substrate materials and amino-functionalised molybdenum disulfide nanoflower (MoS_2NFs/Au@AgPt YNCs) as labels of Ab_2. This sensor allowed the quantitative detection of CEA. To improve the sensitivity of the immunosensor, trimetallic yolk-shell Au@AgPt nanocubes (Au@AuPt YNCs) were loaded onto MoS_2NFs/Au@AgPt. YNCs were used as the labelled secondary antibody (Ab_2). On their own, MoS_2NFs have poor biocompatibility and conductivity; however, due to the interaction between these two, the combination of the prepared nanocomposite was greatly improved and

was able to act as carriers to immobilise the Ab_2 through the stable metal-N covalent bond between the -NH_2 and AuAgPt YNCs on the Ab_2. In this study, AuTNP was used as the substrate material of the immunosensor, and for the signal amplification mechanism, 3,3,5,5-Tetramethylbenzidine (TMB) was used as the active substrate material. Firstly, the primary antibodies (Ab1) were incubated onto the modified GCE, followed by immobilization of BSA to block the non-specific binding sites. Before the dropwise addition of MoS_2NFs/Au@AgPt YNCs Ab_2 onto the electrode, the electrode was incubated in CEA solution to ensure the specificity of Ab_1 with CEA. CEA was detected using an amperometric (i-t) approach in a solution of PBS containing H_2O_2 as an electroactive substance. A detection limit of 2.09 fg/mL and a linear range of 10 fg/mL to 100 ng/mL were obtained. The interaction between the Au TNPs and the MoS_2 NFs/Au@AgPt YNCs showed great sensitivity, selectivity, and acceptable stability. The EC response signal of the various NMs was compared using amperometry and linear sweep voltammetry (LSV) in a solution of PBS containing H_2O_2. The results displayed that the signal amplification of the immunosensor was predominantly obtained by the MoS_2 NFs/Au@AgPt catalysing the reduction of H_2O_2. Further, the authors reported satisfactory results of the fabricated sandwich EC immunosensor using AuTNPs. The sensor thus displayed good reproducibility and sensitivity [175]. Other literature about different combinations of nanocomposite and EC detection methods has been summarised in **Table 4**.

Table 4. Summary of recent literature about different combinations of nanocomposite based and EC immunosensors for the detection CEA.

Nanomaterials or Nanocomposite	Detection Technique	Linear Range	Limit of Detection	Ref
CeO_2-MoS_2-Pb^{2+}-Ab_2	SWV	0.001 to 80 ng/mL	0.3 pg/mL	[2]
CNTs-COOH/rGO/Ag@BSA/PEDOT	LSV	0.002 to 50 ng/mL	0.1 pg/mL	[5]
3DPt/HGO	DPV	0.001 to 150 ng/mL	0.0006 ng/mL	[7]
Au@PtPdMPs porous nanopods	CA	50 fg/mL to 100 ng/mL	16.7 fg/mL	[8]
GNP-MnO2/Fe3O4@Au	LSV and EIS	0.001 to 100 ng/mL	0.10 pg/mL (LSV) 0.30 pg/mL (EIS)	[133]
AgNPs-MWCNTs/MnO_2	CA	0.0001 to 0.5 ng/mL	0.03 pg/mL	[117]
$PdAgCeO_2$ MNS	DPV	0.001 $ngmL^{-1}$ to 40 $ngmL^{-1}$	0.5 $pgmL^{-1}$	[176]
HRP-Au@AgNPs	DPV	0.0001 to 100 $ngmL^{-1}$	0.05 pg/mL	[177]
Aq/PGMA-g-MWCNTs and Fc/PGMA-g-MWCNTs	DPV	163 $fgmL^{-1}$ to 163 $ngmL^{-1}$	56.1 fg/mL	[178]
Ag NPs-PANI@MnO_2	DPV	0.0005 to 80 $ngmL^{-1}$	0.17 pg/mL	[179]
AgNPs@CS-Hemin/rGO	CA	20 $fgmL^{-1}$ to 200 $ngmL^{-1}$	6.7 fg/mL	[180]

Abbreviations: HRP, Horseradish peroxidase; 3DPt/HGO, Three-dimensional porous graphene oxide supported platinum metal nanoparticles; PGMA, surface-initiated poly(glycidyl methacrylate); MWCNTs, Multiwalled carbon nanotubes; PANI, Polyaniline; CS-Hemin/rGO, microporous carbon spheres loading silver nanoparticle spaced Hemin/reduced graphene oxide; SBA-15, Santa Barbara Amorphous mesoporous silica; $PdAgCeO_2$ MNS, PdAgCeO2 mesoporous nanospheres; Au@PtPdMPs, mulberry-like Au@PtPd porous nanopods; CNTs-COOH, carboxylated single-walled carbon nanotubes; PEDOT, poly(3,4-ethylenedioxyhiophene); BSA, bovine serum albumin; SWV, square wave voltammetry; LSV, linear sweep voltammetry; DPV, differential pulse voltammetry; and CA, *Chronoamperometry.*

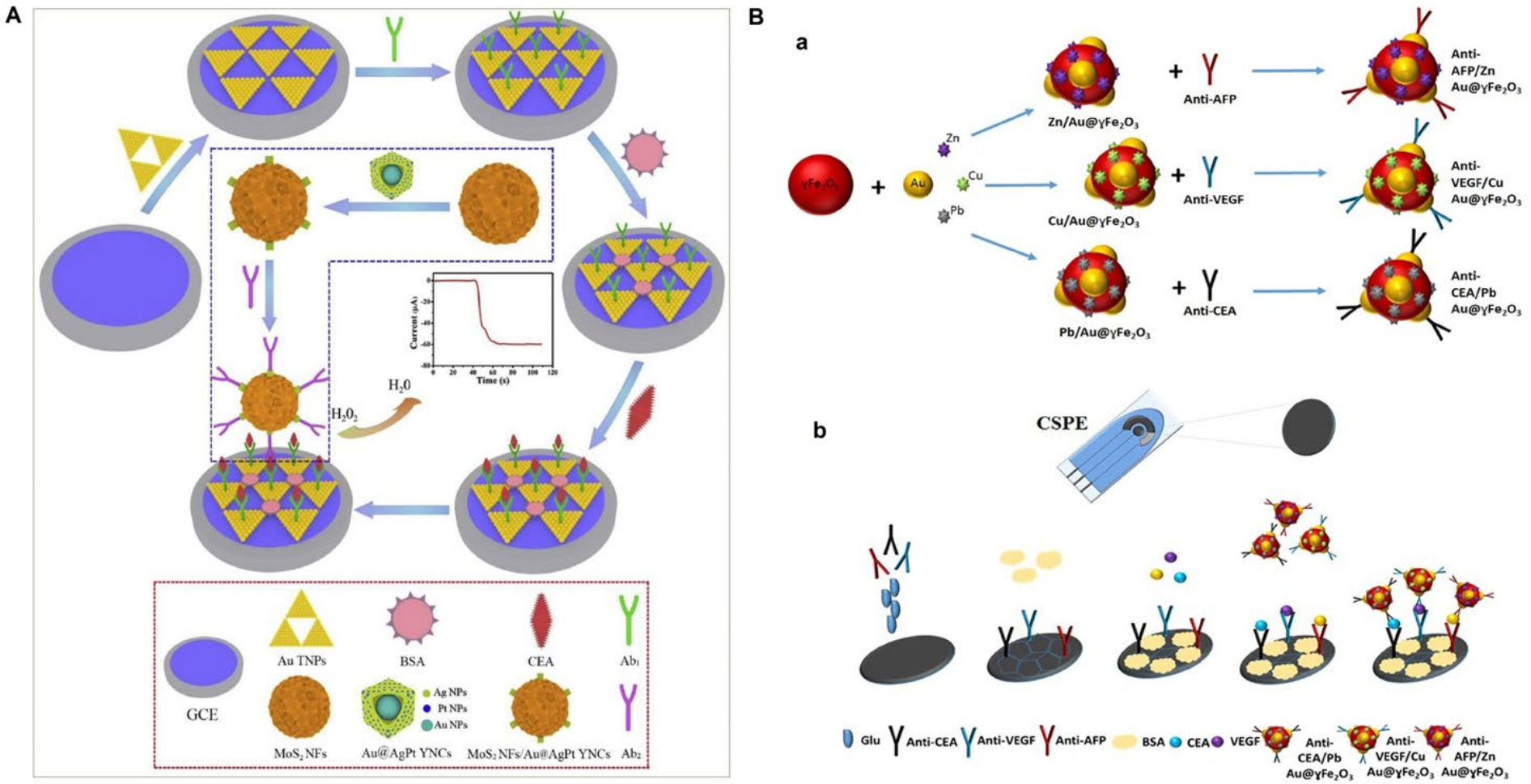

Fig. 6 (A) Schematic illustration of the preparation of the sandwich-type electrochemical immunosensor. Reprinted from reference [175], with permission from Elsevier; and **(B)** Schematic illustration of **(a)** hybrid nanostructure synthesis and antibody modification and **(b)** sandwich-type immunosensor development. Reprinted from reference [184], with permission from Elsevier.

6.2. Nanomaterials-based immunosensors for simultaneous CEA and other biomarkers detection

A continuous effort has been put forward toward developing treatment strategies to detect different clinical biomarkers simultaneously sensitively [181,182]. Interestingly immunosensors for simultaneous detection of CEA and other tumour biomarkers have also been fabricated. For example, Jiang *et al*., fabricated a fluorescent-based aptasensor to detect CEA and alpha-fetoprotein (AFP). Both CEA and AFP are essential biomarkers for the clinical investigation of commonly occurring cancers. In this approach, a fluorescence platform (FL) with AgNCs as the FL signal source was used for the multiplex detection of CEA and AFP. A system of polydopamine nanosphere@silver nanocluster (PDAN@AgNCs) was employed to help observe the quenching and recovery of fluorescence. In addition, AgNCs with other emissions were prepared using two aptamers' sequences with an affinity towards AFP and CEA. The presence of respective biomarkers allowed the formation of antigen-aptamers complexes. Furthermore, the release of AgNCs from the PDAN@AgNCs system resulted in fluorescence recovery. Thus, promising preliminary results were obtained for detecting CEA and AFP in human serum [183].

Similarly, in another study, Kalyoncu *et al*. fabricated a sandwich immunosensor that could simultaneously detect three biomarkers: AFP, vascular endothelial growth factor (VEGF) and CEA (**Fig. 6B**). Differential pulse voltammetry was employed to detect AFP, VEGF and CEA over screen-printed carbon electrodes immobilised with anti-VEGF, anti-AFP and anti-CEA. Moreover, individual nanoparticles, including CuAu@ɣFe_2O_3, ZnAu@ɣFe_2O_3 and PbAu@ɣFe_2O_3, were synthesised to label the antibodies respectively: anti-VEGF, anti-AFP and anti-CEA **(Fig. 6B(a))**. As a result, VEGF, AFP and CEA biomarkers were concurrently detected under applied potential using DPV oxidation peaks of Cu, Zn and Pb metals **(Fig. 6B(b))**.

Therefore, such promising systems can be developed for the inexpensive and convenient multiplex detection of different tumour biomarkers [184].

7. Commercialization of immunosensors

For any sensor to be scientifically accepted and commercially developed, it must be technologically enhanced to be easily assembled and mass-produced with a device exhibiting uniform sensitivity, selectivity, specificity, and reproducibility. Several scientific patents have limited the application of numerous immunosensors over an industrial scale. Many products and materials are patent protected and, therefore, cannot be commercialized. Also, before such immunosensors are made commercially available, their industrial scale preoptimization, validation of target biomarkers, and interference studies are necessary [185–187]. Moreover, most laboratory-tested methods have a specific linear range within which a given immunosensor operates and gives accurate results [188]. Often, immunosensors do not give accurate results when the target analyte is present at more or lesser concentrations than the reference range in which the sensor can operate. Therefore, further optimization of individual sensors is essential before commercialization and widespread availability.

In addition, other external factors, like the matrix effect, hinder the commercial applicability of immunosensors. Most biosensors have been developed on a laboratory scale and allow for detecting a target analyte, usually in highly diluted samples [189]. Therefore, with reoptimization, the sensitivity of biosensors must be enhanced such that they can detect target biomolecules even from a given undiluted matrix. Immunoassays rely entirely on costly antibodies, and the instability of antibodies can reduce production yields and efficacy [190]. Also, in the case of enzyme-based immunosensors, they have a lower shelf life because of the

reduced activity of enzymes over time [191]. Cost-effective, reliable multiplex cancer assays in complex biological samples remain a major challenge [192]. Thus, nanomaterial or nanocomposite-based biosensors are promising alternatives that will demonstrate better shelf life and, owing to their larger surface area, will aid in improving the sensitivity and selectivity of the detection, thereby making the immunosensors more robust. Furthermore, more studies and development of such highly stable and sensitive immunosensors coupled with present imaging technologies will revolutionize the simultaneous detection of multiple clinical biomarkers including CEA.

8. Concluding remarks and prospects

Nanomaterial-based immunosensors have attracted tremendous interest from the scientific community due to their high selectivity and sensitivity in developing sensitive detection tools for clinical diagnoses, like detecting CEA biomarkers. This review discussed the trends in promising immunosensors that allow for CEA detection. Recent studies show that nanomaterial integrated immunosensors improve sensor signal generation and enhance the overall sensitivity while detecting CEA oncomarkers. The diversity of such immunosensors will permit for real-time diagnosis of CEA biomarkers. In particular, the inclusion of AuNMs, AgNMs, PtNMs and CNMs are widely popular owing to the large specific surface area of the nanomaterials, their high electrocatalytic efficiency, notable electronic, optical, biocompatibility and excellent binding properties with biorecognition elements (antibodies). As a result, NMs based immunosensors demonstrate higher sensitivity for the clinical diagnosis of CEA using different strategies: (a) immobilisation of large amount of anti-CEA, (b) enhancing electrochemical response, (c) increasing current response, (d) electrocatalysis, and (e) signal amplification. NMs

and metal nanoclusters are often employed as a tag for concurrent detection of CEA and other tumour biomarkers. However, further research is required to investigate the commercial application of these nanomaterial-based immunosensors developed in laboratory settings. This is a crucial step for the large-scale commercialisation of such sensors that are inexpensive and demonstrate high-quality specifications.

Regardless, over the years, many scientists and researchers have successfully experimented with and developed immunosensors using various methods, including PEC, ECL and EC. Among the many immunosensors developed over the years, nanomaterial-based electrochemical immunosensors have received the most recognition in the medical field owing to their simplicity, cost-effectiveness, rapid response, robustness and miniaturisation. Electrochemical immunosensor provides great promise in terms of the development for point-of-care application in the detection of CEA on top of the detection of various types of contaminants. However, many CEA detection methods are often time-consuming, labour intensive and require bulky instrumentation. Thus, a more simplified methods-based construction of immunosensors is required. In addition, further work is needed to develop multiple biomarker-based modalities for the reliable and accurate detection of tumour biomarkers. In addition, the rapid detection of tumour markers remains a challenge owing to the multiple factors involved in the progression of this disease. Therefore, further works need to be done to understand better the analytical performance factors of the biosensors must be improved before the method is applied in clinical settings. Furthermore, non-invasive methods combined with diagnostic imaging for early and rapid detection of cancer biomarkers, including CEA, must be further explored.

Also, besides using immunosensors in terms of protein biomarker detection, such as CEA, immunosensors have also been used to detect various other analytes. For instance, NMs fabricated immunosensors have been gaining attention in the food and environmental field, demonstrating the increase in the demand for the development of nanomaterials or nanocomposite-based fabrication of sensitive immunosensor. Currently, research works are focused on developing NMs based eco-friendly disposable immune sensing strips for clinical analysis and ultrasensitive immune sensing devices - a step forward in achieving the goals of WHO and the UN.

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Bazilah Awang Abd Manaf: completed the literature survey and prepared the original draft; **Shyang Pei Hong:** writing-review and editing; **Mohammad Rizwan:** literature survey**,** original draft preparation, writing-review and editing; **Fareeha Arshad:** prepared the original draft, writing-review and editing; **Christopher Gwenin:** writing-review and editing; **Minhaz Uddin Ahmed:** conceptualization, writing-review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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