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REVIEW ARTICLE

Recent advances in nanomaterials-based electrochemical immunosensors and aptasensors for HER2 assessment in breast cancer

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Abstract

Human epidermal growth factor receptor 2 (HER2) is one of the key molecular targets in breast cancer pathogenesis. Overexpression and/or amplification of HER2 in approximately 15–20% of breast cancer patients is associated with high mortality and poor prognosis. Accumulating evidence shows that accurate and sensitive detection of HER2 improves the survival outcomes for HER2-positive breast cancer patients from targeted therapies. The current methods of clinical determination of HER2 expression levels are based on slide-based assays that rely on invasively collected primary tumours. Alternatively, ELISA-based detection of the shredded HER2 extracellular domain (HER2-ECD) of has been suggested as a surrogate method for monitoring disease progress and treatment response in breast cancer patients. In the past decade, biosensors have emerged as an alternative modality for the detection of circulating HER2-ECD in human serum samples. In particular, electrochemical biosensors based on nanomaterials and antibodies and aptamers have been increasingly developed as promising tools for rapid, sensitive, and cost-effective detection of HER2-ECD. These biosensors harness the high affinity and specificity of antibodies and aptamers, and unique conductive properties, biocompatibility, large surface area, and chemical stability of nanomaterials for selective and sensitive assessment of the HER2. This review provides an overview of the recent advances in the application of nanomaterials-based immunosensors and aptasensors for detection of circulating HER2-ECD. In particular, various electrochemical techniques, detection approaches, and nanomaterials are discussed. Further, analytical figures of merit of various HER2 immunosensors and aptasensors are compared. Finally, possible challenges and potential opportunities for biosensor-based detection of HER2-ECD are discussed.

Keywords Breast cancer · Human epidermal growth factor receptor-2 · Immunosensors · Aptasensors · Nanomaterials · Electrochemical techniques;

Introduction

Breast cancer is a complex, multifactorial disease characterised by a high degree of morphological and molecular heterogeneity (both intertumour and intratumour), and the common histology of ductal, lobular or mixed ductal-lobular malignancy [1]. Noticeably, it is the most common female cancer in both developed and developing countries and accounts for most cancer-related deaths among women globally. Recent global estimates report approx. 2.261 million new incidences and 0.685 million deaths from female breast cancer in 2020 [2]. Mounting evidence shows that the division of breast cancer to various therapeutic groups has helped in improving patient selection for therapy, and monitor the response of the disease to targeted treatments [3–5]. Treatment decision-making based on the presence or absence of predictive tumour markers categorizes breast cancer into three major subtypes: hormone (oestrogen or progesterone) receptor positive (ER/PR + ve)/ human epidermal growth factor receptor 2 negative (HER2 − ve) cancer, HER2 positive (HER2 + ve) cancer, and triple-negative (ER/PR/HER2-ve) cancer [6]. Structurally, HER2 is a 185 kDa transmembrane growth factor receptor comprised of an extracellular ligand binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. HER2 is known to involved in a plethora of signalling pathways, in

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particular, those related to cellular transformation and tumorigenesis [7–9]. Noticeably, HER2 overexpression by breast epithelium cells, either through gene amplification or via transcriptional deregulation, is known to confer worse biological behaviour that may include aggressive cellular growth of cancer cells, poor prognosis, high propensity to metastasis, and shorter disease-free survival [10, 11].

Measurement of HER2 status is a 'standard of practice' for the evaluation of breast cancers and selection of patients for HER2-targeted therapeutics [12–14]. Tumours that lack HER2 overexpression/ amplification (i.e. baseline expression) are clinically categorized to the HER2-negative group, while those with gene amplification and/or overexpression of HER2, which represent roughly 15–20% of all breast cancer patients worldwide, are grouped as HER2-positive tumours [15]. Currently, the clinical assessment of cellular HER2 expression/amplification in breast cancer patients is carried out on the biopsied tissues using sophisticated molecular techniques such as immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), and next-generation sequencing (NGS) based assays (Table 1) [16–18]. Immunohistochemistry is the preferred method to assess HER2 expression levels, whereas FISH, CISH, and NGS are used for determining the amplification status of the HER2 gene. Notwithstanding the advent of automated IHC, the manual IHC method continues to have the concern of inaccuracy/reliability that primarily results from variation in tissue staining protocols and differences in interpretation of the staining pattern across labs [19–21]. Also, these methods are relatively expensive, time-consuming, require invasively collected samples, and lack real-time monitoring. Apart from cellular HER2, the levels of the extracellular domain of HER2 (HER2-ECD), which is released into the circulation by ADAM (A Disintegrin And Metalloprotease) proteases-mediated cleavage of membrane-bound HER2, have been shown to associate with tumour burden in both patients with primary breast cancer as well as those wtith metastatic cancer [22, 23]. Interestingly, analysis of the circulatory levels of HER2-ECD is suggested as a minimally invasive alternate to the conventional means for assessing HER-2 levels, predict response to therapy, and monitor therapeutic response [23–25]. Molecular techniques and tools such as the enzyme-linked immunosorbent assay (ELISA) and biosensors have emerged as newer modalities of HER2-ECD analysis [18, 26–28]. Over the past decade, several biosensors have been developed for breast cancer biomarkers including HER2 analysis [29–32]. Specifically, electrochemical biosensors based on antibody/affibody and aptamer (commonly referred as immunosensors and aptasensors, respectively) have attracted wide attention for their low-cost setup, simple operation, rapid response, and high sensitivity. These biosensors use antibodies, which are defence proteins (sizes ~ 150 kDa) synthesized by the immune system, with ability to selectively bind the target antigens with a high binding constant (e.g. K_a of 10^8 L mol^{-1} or more) as biorecognition elements or detection probes [33]. Affibodies, the engineered antibody mimetics of sizes 3–20 kDa that imitate monoclonal antibodies, have also been used as promising affinity reagents in biosensing applications [34].

Table 1 List of commercial HER2 testing kits [18]

Assay type	Trade name	Manufacturer	Approval
IHC	HercepTest™	DAKO	FDA, 1998
IHC	PATHWAY®	Ventana Medical Systems Inc	FDA, 2000
IHC	InSite®	Biogenex Laboratories Inc	FDA, 2004
IHC	Bond Oracle™	Leica Biosystems	FDA, 2012
FISH	PathVysion®	Abbott Molecular Inc	FDA, 2001
FISH	PharmDx™ Kit	DAKO	FDA, 2005
CISH	SPoT-Light®	Life Technologies Inc	FDA, 2008
CISH	INFORM HER2 dual ISH DNA probe cocktail	Ventana Medical Systems Inc	FDA, 2011
CISH	PharmDx™	DAKO	FDA, 2011
CISH	VENTANA HER2 Dual ISH DNA Probe Cocktail	Ventana Medical Systems Inc	FDA, 2020
NGS	FoundationOne CDx	Foundation Medicine, Inc.	FDA, 2017
Immunoassay[†]	Bayer Immuno 1™	Bayer Diagnostics	AMA, 2002
ELISA	c-ErbB2/c-neu assay kit	Calbiochem	–
ELISA	Human ErbB2 (HER2) Kit	Triton Biosciences, Inc.	–
ELISA	ADVIA Centaur HER2/neu assay	Siemens Healthcare Diagnostics	–

IHC immunohistochemistry, *FISH* fluorescence in situ hybridization, *CISH* chromogenic in situ hybridization, *NGS* next-generation sequencing, *FDA* US Food and Drug Administration, *AMA* American medical association

[†] Magnetic particle separation-based automated immunoassay

Also, advances in recombinant antibody production (animal-free), with exceptional batch-to-batch reproducibility, and easy and rapid production have supported development of efficient and economic HER2 biosensors. Aptamers, which represent short, single-stranded RNA or DNA molecules, have also gained popularity as efficient biorecognition elements for biosensing applications because of their economic in vitro synthesis, easy chemical modification, and easy maintenance and storage [35–40]. In past decades, multiple DNA/RNA aptamers have been identified that bind selectively and with high affinity to human HER2 (Table 2). Progressive development in identification of better affinity aptamers, antibodies and engineered antibodies to disease biomarkers such as HER2, with concurrent advances in nanotechnology, has fuelled development of nanomaterials-based biosensors. In particular, nanomaterials of different geometry (e.g. nanosheets, nanotubes, and quantum dots), morphology (spherical, flat, needle, or random orientations), composition (single material or composites), properties (piezoelectric, magnetic, or photosensitive), and agglomeration states (dispersed or clusters) have been used in electrochemical biosensors to benefit from improved analytical performance, better biological compatibility, chemical stability, conductive properties, and large surface area of nanomaterials [29, 41, 42].

In summary, nanomaterials-based electrochemical immunosensors and aptasensors are increasingly developed over the past decade for analysis of circulating HER2 levels in breast cancer patients. This review outlines recent developments in electrochemical biosensing of HER2-ECD and discusses various electrochemical techniques used for HER2 assessment. The advantages of using nanomaterials in HER2 biosensing, when applied in different contexts, are also discussed. Further, key parameters of the analytical performance of HER2 immunosensors and aptasensors are highlighted. Finally, possible challenges and potential opportunities for HER2-biosensors to adopt into clinical practices are proposed.

HER2 immunosensors and aptasensors

Biosensors

Biosensor is a "device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction" [62]. These functional integrative devices consist of two major components—a biorecognition element that perceives the physical signal, and a transducer that converts the biorecognition information into a measurable outcome [62]. Biosensors can be classified into various groups based on the nature of biorecognition elements (e.g. enzyme, antibody, nucleic acid, cells and aptamer) as well as the types of transducers (e.g.

Table 2 Sequences of various aptamers reported in development of different electrochemical biosensors for HER2 detection

S. No.	Aptamer sequence (5′-3′)	Ref.
1	AATTAAGCC GCGAGGGGAGGGATAGGGTAGGGCGCGGCT; GCAGCGGTGTGGGGGCAGCGGTGTGGGGGCAG CGGTGTGGGG	[43]
2	AAAGTAAAAGAACTGATCAGCACGGGATGGGATAGGGAGGGGAGTGTTGAAAA	[44]
3	AACCGCCCAAATCCCTAAGAGTCTGCACTTGTCATTTTGTATATGTATTTGGTTTTTGGCTCTCACAGACACAC TACACACGCACA	[45]
4	GGGAGAUACCAGCUUAUUCAAUUUGGAUGGGGAGAUCCGUUGAGUAAGCGGGCGUGUCUCUCUGCCGC CUUGCUAUGGGGAGAUAGUAAGUGCAAUCU	[46]
5	GCAGCGGTGTGGGG	[47]
6	GGCGCUCCGACCUUAGUCUCUGUGCCGCUAUAAUGCACGGAUUUAAUCGCCGUAGAAAAGCA UGUCAAAGCCGGAACCGUGUAGCACAGCAGA	[48]
7	TGGGGCCTGGATACGGATTGGTAAGGATTAGTAGGGGGCATAGCT	[49]
8	GGGCCGTCGAACACGAGCATGGTGCGTGGACCTAGGATGACCTGAGTACTGTCC	[50]
9	AACCGCCCAAATCCCTAAGAGTCTGCACTTGTCATTTTGTATATGTATTTGGTTTTTGGCTCTCACAGACAC ACTACACACGCACA	[51]
10	GGGCCGTCGAACACGAGCATGGTGCGTGGACCTAGGATGACCTGAGTACTGTCC	[52]
11	GCAGCGGTGTGGGGGCAGCGGTGTGGGGGCAGCGGTGTGGGGTTTTT	[53]
12	GCAGCGGTGTGGGGTATCGTTAATTCGGTCG	[54]
13	GCAGCGGTGTGGGG	[55–57]
14	TTTTTAATTAAGCCGCGAGGGGAGGGATAGGGTAGGGCGCGGCT	[58]
15	GGGCCGTCGAACACGAGCATGGTGCGTGGACCTAGGATGACCTGAGTACTGTCC	[59]
16	AAAAGTTGTGAGGGGAGGGATAGGGTAGGGCACGACTAGTCAAGAAAATG	[60]
17	ATTAAGAACCATCA CTCTTCCAAATGGATATACGACTGGG	[61]

The shown aptamer sequences adopt unique tertiary confirmations that allow them to selectively bind to HER2

electrochemical, optical, thermal and piezoelectric) [63, 64]. For example, most of HER2 biosensors are based on the use of aptamer and antibodies as biorecognition elements/detection probes, and rely on electrochemical transducers to transform biochemical information (e.g. HER2 presence/absence) into current or voltage-based signals.

Detection of biomolecules using an electrochemical biosensor, in general, involves selecting and preparing electrodes, decorating their surface with biorecognition elements/signal probes performing the biorecognition event, and measuring the generated current (amperometric detection), potential/charge accumulation (potentiometric), change in the conductivity of medium (conductometric), or the electrical resistivity/conductivity of the medium (impedance) [65]. Three-electrode systems consisting of a working electrode (WE, transducer with immobilized biorecognition molecule), a reference electrode (RE, maintain a known/stable potential), and a counter/auxiliary (CE, establish connection to electrolytic solution) are used for electrochemical measurements. Screen-printed electrodes (SPEs) made of PVC, ceramic or aluminium base printed with gold, carbon, or platinum inks are preferred for most point-of-care analyses due to its low maintenance, single-use application, and low-cost analysis [66]. In SPEs, a ring-shaped layer (e.g. 4 mm diameter) is printed around the working electrode to constitute the reservoir of the electrochemical cell for most in situ analyses with low volume (e.g. 50 μL) of samples. Use of both conventional three-electrode system with standard electrodes (e.g. 4 mm glassy carbon WE, platinum wire CE and Ag/AgCl RE) as well as SPE has been reported in the development of HER2 immunosensors and aptasensors.

Electrochemical techniques

Conductometric, amperometric, and electrochemical impedance spectroscopy (EIS) are the most commonly used electrochemical techniques reported in HER2 biosensor development (Table 3 and Table 4). Conductometric techniques, in general, measure the conductivity of the solution (ionic) and determine the concentration of target analyte based on the differences in the electrical conductivity of the electrolytic solution in conditions of presence and absence of the target analyte. Contrarily, amperometric techniques measure the change in current upon oxidation/reduction in the assay medium. Amperometry and voltammetry are the two widely used amperometric techniques. Essentially, when the change in the current upon oxidative/reductive reaction is measured at a constant potential, it is referred to as amperometry, whereas measurement of current over a linear potential range is termed voltammetry. Various voltammetry techniques, such as cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and the square-wave voltammetry (SWV), are frequently reported in biosensor development [65, 67, 68]. Briefly, CV and LSV measure current by sweeping the applied potential (at the working electrode) in both forward and reverse directions (e.g. CV), or linearly in one direction (e.g. LSV) at a fixed scan rate to determine the electrochemical behaviour (oxidation/reduction) of the substrate, reaction intermediates, anodic limits, and reaction reversibility [69].Usually, CV along with DPV or SWV is used to determine the nature of the redox reactions occurring in the solution, and quantitatively analyse the analytes. On the other hand, DPV measure current by applying amplitude potential pulses on a linear ramp potential [70]. A base potential at which no oxidation/reduction occurs in the system is applied to the electrode, which is increased between pulses with equal increments, and the current is sampled just before the start and at the end of a potential step to record differential current. High signal-to-noise ratio to obtain better sensitivity is the key advantage of DPV [71]. Similarly, SWV, an advanced form of the DPV, has been reported to achieve high sensitivity and speed of analytical measurement. It involves stepping the potential of the working electrode through a series of forward and reverse pulses, where the forward step is determined by the square amplitude and the reverse step is determined by subtracting the square increment from the square amplitude [72, 73].

Yet, another commonly used method in HER2 biosensors is the EIS technique which measures the change in the impedance of system upon binding of biological entities such as proteins or aptamer to the electrode surface. Electrochemical impedance, a measure of the ability of a circuit to resist the flow of electrical current, is measured by applying AC potential (over a wide range of frequencies) to an electrochemical cell and then recording the impedance spectrum. EIS utilises electroactive species such as $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ redox couple which oxidises and reduces at the working electrode, allowing easy measurement of increasing charge transfer resistance of the circuit upon layer-by-layer formation of biorecognition complex on the electrode [74].

Biosensing approaches

Development of electrochemical immunosensors and aptasensors for HER2 has been reported on two approaches—label-fee and labelled-based detection methods. The label-free methods utilize the intrinsic properties of HER2 that impose changes in electrode impedance and allow label-free detection. Contrarily, the label-based methods use labels such as enzymes, fluorescent tags, and nanoparticles attached/coated to biomolecules/electrode surface to enhance assay sensitivity through the production of amplified signal. Comparatively, the label-free approaches are cost-effective, and may favour adoption in low-cost point-of-care diagnostics. However, technical challenges imposed by dynamics of the interface, low signal-to-noise ratio, and limited specificity displayed by label-less methods compared to label-based (e.g.

Table 3 Overview of biosensor components, detection methods, and analytical parameters of nanomaterials-based immunosensors reported for electrochemical analysis of HER2-ECD

Detection method	Working electrode	Biorecognition element	Signalling probe	Electroactive moiety	Linearity ($ngmL^{-1}$)	LOD ($ngmL^{-1}$)	Precision (RSD)	Stability (days)$^{\alpha}$	Assay time $^{\beta}$	Ref.
AMP	Gold	SiNPs-CAb	DAb-HRP	4-TBC/H_2O_2	0–50	0.00137	6.2%	n.r.	30 min	[92]
AMP	SPC	CAb	DAb-HRP	HQ/H_2O_2	1–200×10^3	1000	8–14%	21	22 min	[104]
LSV	SPC	AuNPs-CAb	Biotin-DAb-Strep-AP	3-IP/Ag^+	15–100	4.4	3.2–5.7%	n.r.	3 h	[105]
LSV	SPC	MWCNT/AuNPs-CAb	Biotin- DAb-StrepAP	3-IP/Ag^+	7.5–50	0.16; 8.5	2.8–6.5%	n.r.	> 2 h	[78]
LSV	SPC	AuNPs-CAb	Biotin- DAb-Strep-AP	3-IP/Ag^+	9.8–50	2.9	n.r.	n.r.	4 h	[106]
CV	GC	AuNPs/Cu-MOF-CAb	DAb/CZTS NPs/Pt/g-C_3N_4	$[Fe(CN)_6]^{3-/4-}$	0.01–1×10^{-3}	3×10^6	0.3%	49	2.5 h	[107]
CV	SPC	CAb	Biotin- DAb-Strep-HRP	TMB/H_2O_2	20–200	4	0.7%	7	5 h	[108]
CV	SPC	AuNP/CeO_2	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.001–20	0.0349	n.r.	n.r.	5 h	[91]
CV	GC	Fe_3O_4/MWCNT	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.001–100	0.0003	6.1%	15	3 h	[96]
DPV	GC	rGO/Au-AgNS-CAb	DAb/Thi-Ni-PtNi HNCs	Ferrocene/thionine	0.01–100	0.0033	2.3%	15	> 13 h	[109]
DPV	SPC	CDs-CAb	Biotin-DAb/ Strep-QD	Cd^{2+}	10–150	2.1	7.1%	7	2 h	[79]
DPV	Gold	AuNPs/ Fe_3O_4 NPs- CAb	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.01–100	9.9×10^{-4}	2.4–4.4%	21	30 min	[95]
DPV	GC	AuNPs- Fe_3O_4- Ab	Label-free	Ag^+	0.0005–50	2×10^{-5}	2.4–6.1%	7	2 h	[110]
SWV	Gold	HER2 specific peptide	AuNP- dC20- DAb	MoP	0.001–1	0.0005	1.5–2.6%	n.r.	16 h	[81]
SWV	Gold	CAb	DAb-AuNP-RCA primer	MoP	1–200×10^{-3}	9×10^{-5}	2.5–3.4%	n.r.	10 h	[80]
SWV	SPC	HER2 specific peptide	DAb-QDs	Pb^{2+}	1–100	0.28	4.7%	n.r.	4 h	[111]
EIS	CIL	AuNPs/MWCNT-CAb	Label-free	$[Fe(CN)_6]^{3-/4-}$	10–110	7.2	1.8%	n.r.	35 min	[98]
EIS	SPG	AuNPs-Affibody	Label-free	$[Fe(CN)_6]^{3-/4-}$	0–40	6	8–10%	n.r.	1 h	[76]
EIS	SPC	AuNPs	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.01–100	0.01	1.1%	22	17 min	[112]

AMP amperometry, *LSV* linear sweep voltammetry, *CV* cyclic voltammetry, *DPV* differential pulse voltammetry, *SWV* square-wave voltammetry, *EIS* electrochemical impedance spectroscopy, *SPC* screen-printed carbon, *GC* glassy carbon, *SPG* screen-printed gold, *CIL* carbon ionic liquid electrode, *CAb* capture antibody, *DAb* detection antibody, *SiNPs* silica nanoparticles, *HRP* horseradish peroxidise, *Strep* streptavidin, *AP* alkaline phosphatase, *AuNPs/Cu-MOF* gold nanoparticles decorated copper-organic framework, *CZTS NPs/Pt/g-C_3N_4* Cu_2ZnSnS_4 nanoparticle quaternary chalcogenide with platinum (Pt)-doped g-C_3N_4 composite, *CeO_2* cerium oxide, *Fe_3O_4* iron oxide, *rGO* reduced graphene oxide, *Au-AgNS* Au-Ag nano shuttles, *Thi-Ni-PtNi HNCs* Ni@PtNi yolk-shell hollow nanocages-thionine, *CD* carbon dots, *QD* quantum dots, *dC20* polycytosine DNA sequence, *4-TBC* 4-tert-butylcatechol, *HQ* hydroquinone, *3-IP* 3-indoxyl phosphate, *TMB* tetramethyl benzidine, *1-NP* 1-naphthyl phosphate, *MoP* molybdophosphate, *LOD* limit of detection, *RSD* root-mean-square deviation

$^{\alpha}$ The reported stability period correspond to period of insignificant loss in signal (i.e. stability > 90%)

$^{\beta}$ Overnight incubations were considered as a 12-h period for comparison purposes. Time intervals are approximate estimates

sandwich assay) assay formats limit wider application of these methods [75]. Most studies on development of label-free electrochemical immunosensors and aptasensors for HER2 are reported making use of the immunocomplexes formed between antibody andHER2, or aptamer and HER2 at the electrode surface to increase the electron transfer resistance at the interface, thus allowing EIS-based detection of the binding event. For example, Ravalli et al. developed a label-free impedimetric immunosensors for HER2 detection via layer-by-layer assembly of gold nanoparticles, anti-HER2 affibody, a self-assembled monolayer of 6-mercapto-1-hexanol, and HER2 protein onto screen-printed graphite electrode [76]. Impedance spectra were recorded (as Nyquist plots) after each assembly step, as well as at different concentrations of HER2 using $[Fe(CN)_6]^{3-/4-}$ redox couple (Fig. 1a). A similar work by Rostamabadi et al. reported construction of a label-free electrochemical aptasensor using glassy carbon electrode that carried reduced graphene oxide, single-walled carbon nanotubes, and gold nanoparticles coated onto the electrode interface to immobilize aptamer that selectively recognizes HER2 and leads to an increased charge transfer resistance (Rct) of the electrode [51]. Also, Gu et al. reported label-free aptasensing

Table 4 Overview of biosensor components, detection method, and analytical parameters of nanomaterials-based aptasensors reported for electrochemical analysis of HER2-ECD

Detection method	Working electrode	Biorecognition element	Signalling probe	Electroactive moiety	Linearity ($ngmL^{-1}$)	LOD ($ngmL^{-1}$)	Precision (RSD)	Stability (days)	Assay time[α]	Ref.
Capacitance	Gold (μID)	Aptamer	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.07–7	0.07	5%	n.r.	2 h	69
Capacitance	Gold (μID)	Aptamer	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.2–2	0.2	n.r.	n.r.	1 h	71
CV	Gold	HER2-specific peptide	Aptamer	MoP	0.01–5	0.005	3.9%	n.r.	n.r.	74
CV	Gold	Aptamer	Label-free	$MB/[Fe(CN)_6]^{3-/4-}$	0.07–700	0.07	2.7-fold	5	30 min	77
CV	Gold	HER2-specific peptide	P_i-Aptamer- MnO_2NS	MoP	0.0001–0.5	5×10^{-5}	0.1–0.8%	n.r.	3–4 h	81
CV	GC	AuNPs-HCNT-peptide	Aptamer	MoP	0.0005–1	–	3.9%	n.r.	4 h	84
DPV	Gold	TDN-Aptamer	Mn_3O_4-PdPt-Aptamer-HRP	HQ/H_2O_2	0.1–100	0.08	3.2–7.9%	15	1.5 h	68
DPV	Gold	TDN-Aptamer	AuNR-Pd-Aptamer-HRP	HQ/H_2O_2	10–200	0.15	2.7%	n.r.	1 h	75
DPV	SPC	Aptamer	Label-free	$MB/[Fe(CN)_6]^{3-/4-}$	10–60	3	2–3%	5	4 min	76
DPV	Gold	AuNP	FcNS-HRPNS	$o\text{-PD}/H_2O_2$	10–150	4.9	4.5–7.3%	n.r.	5 h	82
DPV	GC	rGO- Chitosan-Aptamer	Label-free	$MB/[Fe(CN)_6]^{3-/4-}$	0.5–75	0.21	3.9–4.3%	4	1 h	70
SWV	GC	AuNP	AuNP-Aptamer	Ag^+	0.0001–1	37×10^{-6}	< 5%	n.r.	>24 h	86
EIS	Gold	AuNP-Aptamer	Label-free	$[Fe(CN)_6]^{3-/4-}$	10^{-5}–100	5	3%	n.r.	15 min	72
EIS	Gold	HER2-specific peptide	Aptamer	MoP	0.001–0.1	4.7×10^{-5}	n.r.	n.r.	2 h	73
EIS	Gold	CDs-ZrHf-MOF-Aptamer	Label-free	$[Fe(CN)_6]^{3-/4-}$	10^{-4}–10	19×10^{-6}	3.4%	15	10 min	78
EIS	WS_2NW-TM	Aptamer	GOD-AuNPs	$[Fe(CN)_6]^{3-/4-}$	0.5–10	0.36	1.5–3.2%	n.r.	4.5 h	79
EIS	GC	rGO- SWCNTs-AuNPs	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.0001–1	5×10^{-5}	3.9–5.7%	30	40 min	80
EIS	Gold	MnFePBA-AuNP-Aptamer	Label-free	$[Fe(CN)_6]^{3-/4-}$	0.001–1	25×10^{-5}	4.4%	15	2–4 h	83
EIS	SPG	Aptamers	Label-free	$[Fe(CN)_6]^{3-/4-}$	$0.001–10^3$	0.172	n.r.	n.r.	> 18 h	87

CV cyclic voltammetry, *DPV* differential pulse voltammetry, *SWV* square-wave voltammetry, *EIS* electrochemical impedance spectroscopy, *GC* glassy carbon, *SPC* screen-printed carbon, *WS_2 NW-TM* tungsten nanowire/titanium-mesh, *SPG* screen-printed gold, *HER2* human epidermal growth factor receptor 2, *HCNT* hexagonal carbon nitride tubes, *TDN* tetrahedral DNA nanostructure, *CDs-ZrHf-MOF* ZrHf metal–organic framework embedded with carbon dots, *rGO* reduced graphene oxide, *GOD* glucose oxidase, *nFePBA-AuNP* bimetallic MnFe Prussian blue analogue coupled to gold nanoparticles, *MoP* molybdophosphate, *HQ* hydroquinone, *MB* methylene blue, *OPD* o-Phenylenediamine

[α] The assay time does not include transducer preparation and probe coating time. Time intervals are approximate estimates

of HER2 using metal nanoparticles and metallic organic framework (MOF) as a substrate for immobilization of aptamers (Fig. 1b) [59]. Two studies reported HER2 analysis in spiked human serum samples based on the capacitance response. The reported capacitive aptasensors measured the change in the total negative charges present around capacitive interdigitated gold microelectrode (carrying initially only aptamers) upon HER2 binding to quantify HER2 levels [45, 50].

On the other hand, biosensors based on labelled affinity reagent, in general, follow the conventional immunoassay format in which a variety of labelling agents are used to label the target molecule. For example, most of the label-based HER2 immunosensors were developed based on sandwich protocols in which the tethered biorecognition element (e.g. primary antibody) on electrode surface binds HER2, which is then quantified with the aid of secondary antibody labelled to enzymes like horseradish peroxidase (HRP), alkaline phosphatase (AP),

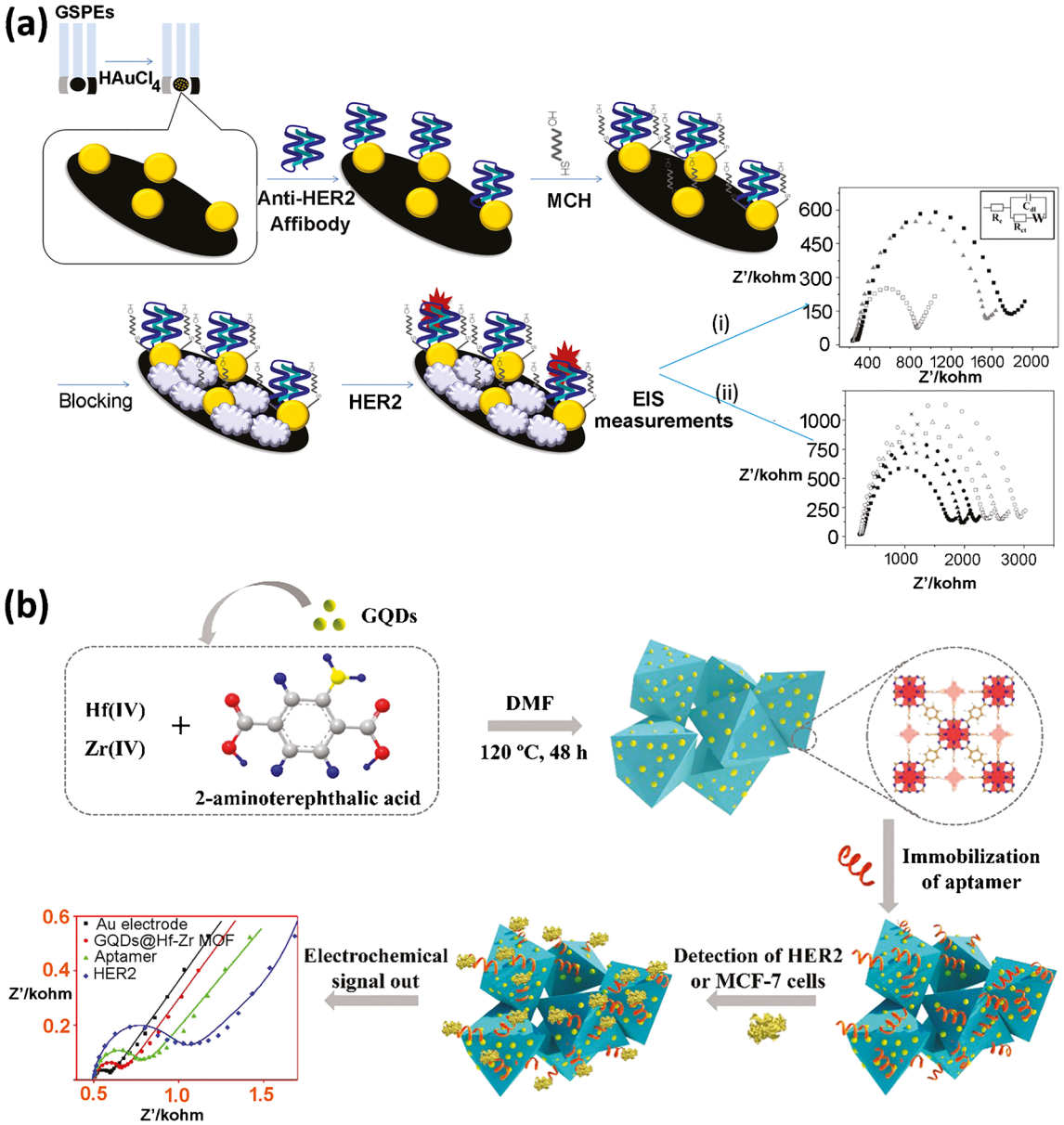

Fig. 1 Schematic representation of label-free electrochemical HER2 biosensors. Image (**a**) shows construction of a gold nanoparticles-based immunosensor on graphite screen-printed electrodes. Anti-HER2 affibody was coated onto modified electrode via gold-thiol bond, followed by coating of self-assembled monolayer of 6-mercapto-1-hexanol (MCH), blocking with non-fat milk, selective binding of target (HER2-ECD), and electrochemical measurement using EIS. Nyquist plots drawn after each assembly step (i), and at different HER2 concentrations (ii) in 0.01 M $[Fe(CN)_6]^{3-/4-}$ equimolecular mixture in 0.1 M PBS, pH 7.4 are shown in the inset. Image (**b**) shows sequential fabrication of a novel nanocomposite CDs@ZrHf-MOF-based aptasensor on gold electrode. The nanocomposites were synthesized by embedding the amino-functionalized carbon dots (CDs) into bimetallic ZrHf- MOF and dropped onto gold electrode, followed by anchoring of aptamers to nanocomposite, and detection of HER2-ECD through EIS. Images are adopted from reference [59, 76] with permission from publisher

or nanoparticle tags (e.g. gold nanoparticles, quantum dots, and metal oxides). These labels catalyse certain reaction to alter the charge transfer process at electrode interface, leading to the increased electrochemical signal production and decreased background noise (Table 3). A depicted in Fig. 2 a, Chen et al. developed a HER2 immunosensor on gold electrode having DNA tetrahedron (a tetrahedral nanostructure constructed from self-assembles DNA double helices) with expanded aptamer as an interface for HER2 binding, and a bioconjugate of gold nanorod with aptamer and horseradish peroxidase enzyme as a signal probe [77]. In another similar approach, Freitas et al. reported an electrochemical immunosensors that use gold nanoparticles and multiwall carbon nanotube immobilized antibodies to capture HER2 and biotinylated secondary antibodies with streptavidin-alkaline phosphatase to catalyse substrate (3-indoxyl phosphate)-mediated oxidation of silver nitrate to Ag^{2+}, which is sensed as amplified current signal (Fig. 2b) [78]. Other label-based biosensors reported use of MnO_2 nanosheets, Mn_3O_4 nanozyme, and quantum dots (QD) based labels to amplify electrochemical current [43, 56, 79].

Also, a few studies reported label-based HER2 biosensing through molybdate-based DNA generated electric current [54, 56, 57, 80]. The molybdate-based methods are based on the reaction of free phosphate ions and/or phosphate in the backbone of DNA/aptamer with molybdate to form redox-active molybdophosphate that results in enhanced signal generation. Among these methods, polycytosine DNA-generated electric current and rolling circle amplification (RCA) were the prominent approaches. These methods combine immunoreactions with DNA-based signal amplification (Fig. 3). For instance, Li et al. developed a polycytosine DNA-based immunosensors for HER2 via a sandwich immunocomplex between electrode-coated HER2 peptide and bioconjugate of AuNPs, antibody, and polycytosine DNA sequence (dC20), which generated an enhanced electrochemical current from the reaction of molybdate with DNA phosphate backbone [81]. Multiple label-based electrochemical aptasensors based on the DNA generated electric current method are reported in the literature [54, 56, 57].

To further improve the assay sensitivity, a few studies also demonstrated the use of the rolling circle amplification (RCA) technique in HER2 biosensing. In RCA methods, a DNA tag serves as a primer to trigger rolling circle amplification and results in the formation of hundreds of DNA sequences on the electrode surface. Additions of molybdate to DNA containing solutions result in the formation of redox-active molybdophosphate that generate redox electric current [82, 83]. An electrochemical immunosensor based on the RCA method is reported by Shen et al., wherein the authors modified a gold electrode with HER2-affinity peptide to serve as biorecognition element and a bioconjugate of gold nanoparticles, secondary antibody, and DNA primer to serve as a signal probe in HER2 immunosensing [80].

Nanomaterials-free biosensors

Notwithstanding the multiple advantages of nanoparticles and nanocomposites in biosensing applications, one of the major challenges in their use is the immobilization strategy used for conjugating nanoparticles to biomolecules. Because the stability, selectivity and the binding affinity of a biorecognition

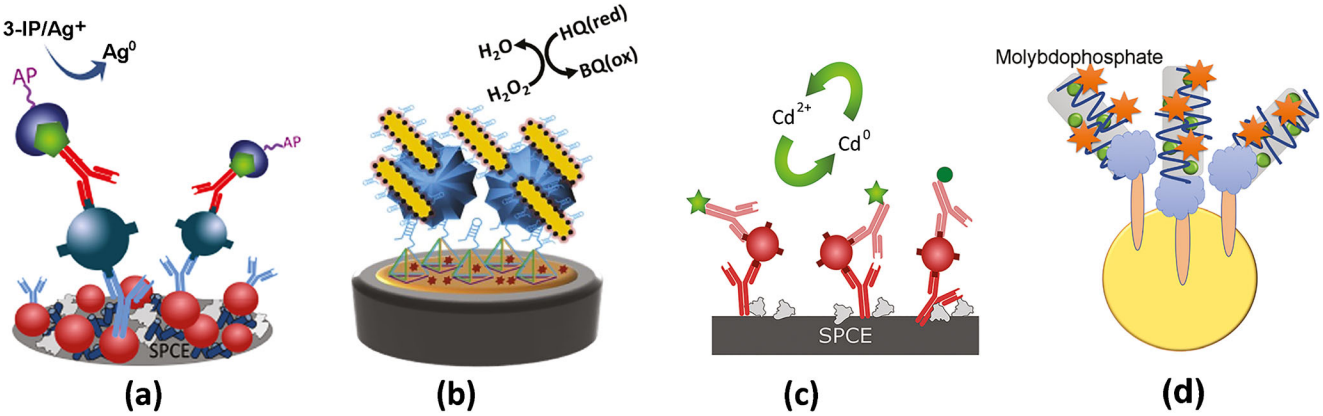

Fig. 2 Representative examples of electrochemical labels used in HER2 biosensing. Images show HER2 biosensors developed based on alkaline phosphatase (**a**), horseradish phosphatase (**b**), quantum dot (**c**), and MnO_2-based labels (**d**). The immunosensor in (**a**) is fabricated by dropping AuNPs and MWCNTs on SPCE, followed by immobilization of capture antibody, blocking (with 2% b-casein) electrode surface, capturing HER2, and detection with AP-labelled detection antibody. The aptasensor in (**b**) is constructed by tethering DNA tetrahedron with expanded aptamer on the gold electrode to capture HER2 and detect through bioconjugate of gold nanorod@Pd superstructures-aptamer-horseradish peroxidase (GNR@Pd SSs-Apt-HRP) signal probe. Immunosensor in (**c**) is designed to capture HER2 through SPCE immobilized capture Ab, followed by detection through biotinylated detection antibody and streptavidin-quantum dots bioconjugate. Aptasensor in (**d**) is constructed to capture the HER2 onto gold electrode through HER2-specific peptide and detect using MnO_2 nanosheets functionalized with phosphate and HER2 binding aptamer which react with molybdate to form redox-active molybdophosphate which results in dual signal amplification. Images are adopted from reference [77, 78] with permission from publishers

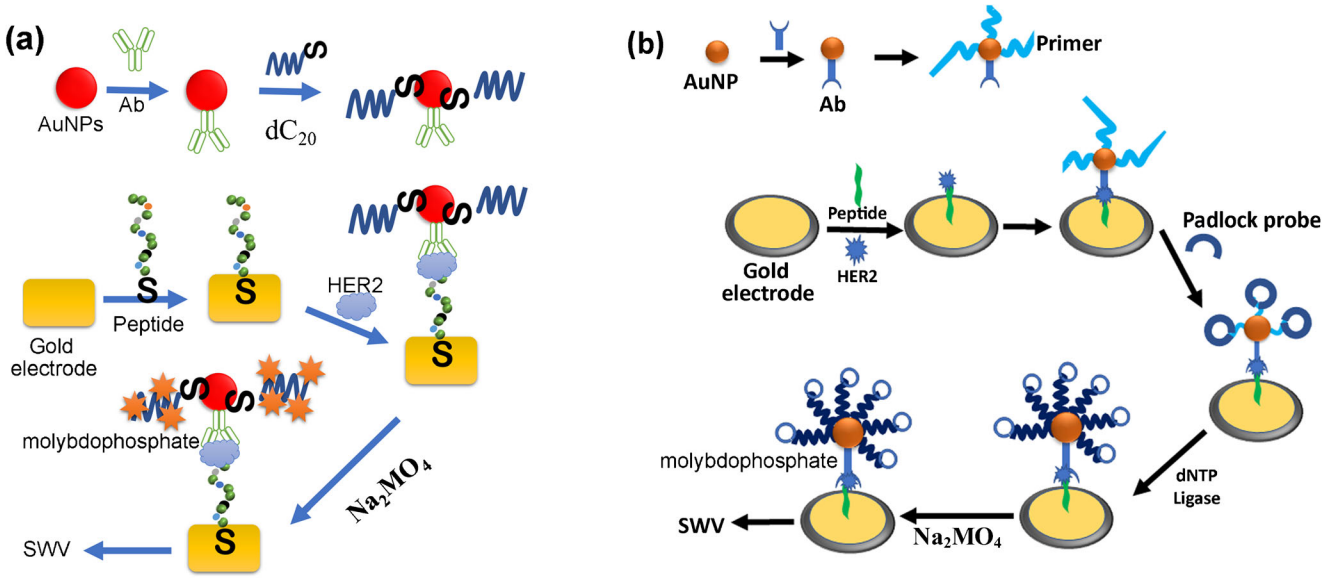

Fig. 3 Schematic representation of molybdate-based (label-based) methods of HER2 biosensing using DNA generated electric current (**a**), and rolling circle amplification (**b**). The phosphate group present on phophodiester backbone of DNA react with molybdate to form molybdophosphate, which is precipitated on the electrode surface and serves as redox mediator to give measurable electrochemical signal. Image (**a**) is adopted from reference [57] with permission from Elsevier, and image (**b**) is re-drawn from reference [80]

molecule or the enzyme towards its target is principally dependent on its native structure, immobilization that alter, reduce or restrict the freedom of the conformational change or exposure to binding sites may lower the advantages of biomolecule immobilization on nanoparticles. Therefore, weighing the advantages and disadvantages of using nanomaterials in biosensing applications at multiple levels such as sensing cost, preparation time and efforts, stability, and sensitivity is very crucial. A few studies on HER2 biosensing reported immunosensors and aptasensors development without the use of nanoparticles. As shown in Fig. 4, the covalently immobilized aptamer/HER2-specificity peptide (biorecognition molecules) on electrode surfaces was used to capture target HER2, and assess the binding events based on electric current produced from electrochemical reduction of the ferricyanide redox indicator or DNA generated electric current [53, 57, 81].

Nanomaterials used in HER2 biosensing

The high surface area, biocompatibility, electronic properties and electrocatalytic activity of nanomaterials has promoted their use as biosensor components. Nanomaterials of different geometry, morphology, composition, properties, and agglomeration states have been reported used in the development of HER2 biosensors. For example, gold nanoparticles, quantum dots, and carbon nanotubes have been widely used as tailoring material on the electrode surfaces to provide sufficient binding sites for biorecognition elements, or as labels to allow signal amplification, and/or as an enrichment material to separate HER2 from complex samples [84–86]. Use of nanomaterials in biosensing applications has helped to achieve improved sensitivity and selectivity through enhanced immobilization of biorecognition elements, increasing the electrochemical signal production, and decreasing the background noise (i.e. increase in the signal-to-noise ratio) [87, 88].

Nanomaterials as electrode materials to construct sensing platform

The surface architecture of working electrodes that brings samples and sensing element together plays a crucial role in overall performance of the electrochemical biosensors. In particular, sensitivity may largely depend on electrode surface modification. Gold nanoparticles of spherical or rod-shaped are commonly reported for modifying the electrode-electrolyte interface in HER2 biosensors. Their unique properties, including high surface-to-volume ratio, ability to directly transfer electrons between a wide range of electroactive biological species and the electrode, ability to provide a stable surface for biomolecular immobilization, and the freedom to control size and surface morphology, have favoured decorating electrode surfaces for enhanced performance [89]. Nanocomposite film of AuNPs on electrode surface can be formed using electrodeposition as well as physical deposition [60]. Further, the biorecognition elements can be immobilized onto the oxidized gold nanoparticles surface through a covalent thiol–gold bond (at higher pH) [90]. As another commonly used nanomaterials in HER2 biosensors, cerium oxide (CeO_2) nanoparticles have been used to

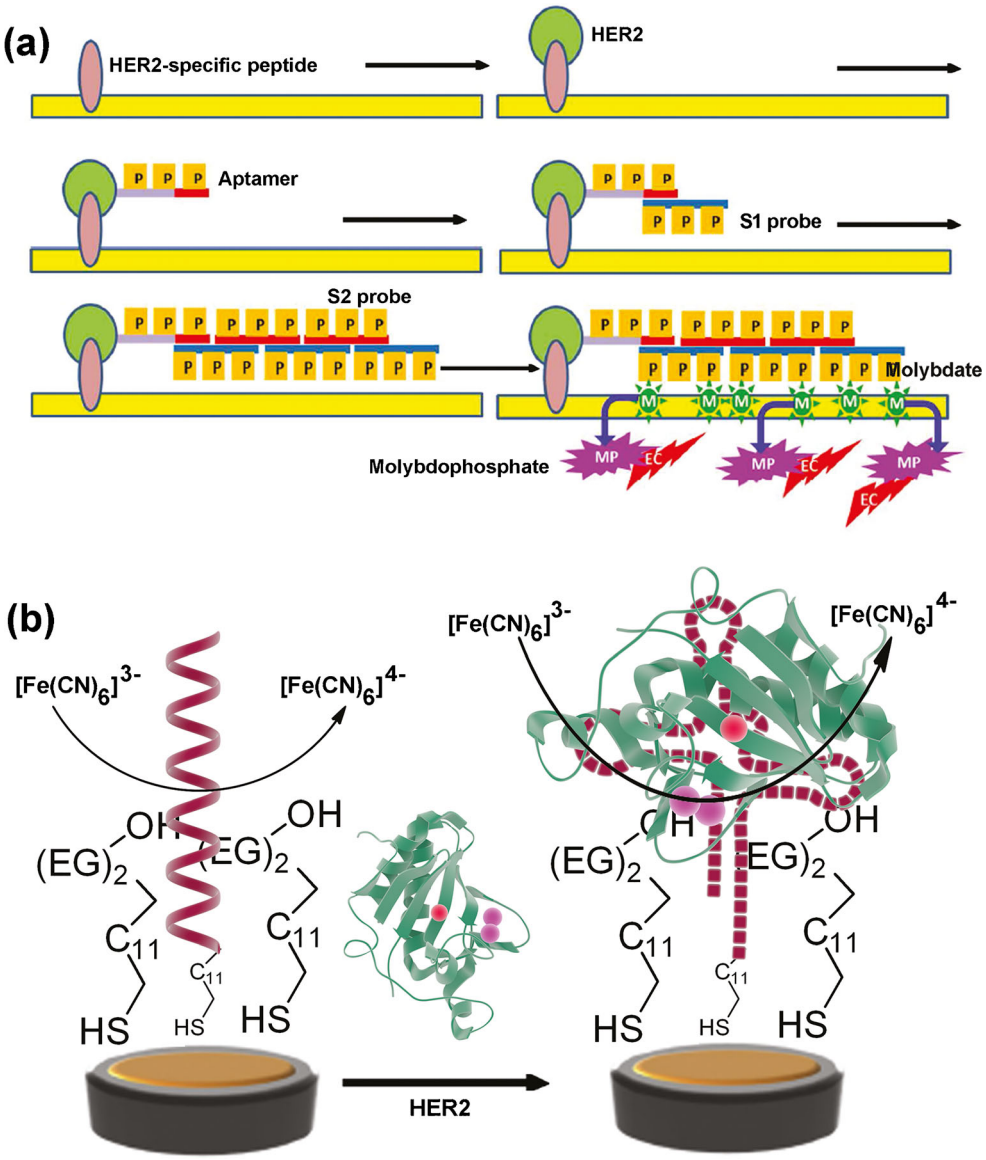

Fig. 4 Schematic illustration of the nanomaterials-free HER2 biosensing methods. Depicted immunosensor (**a**) used HER2-specific peptide (CKLRLEWNR) as biorecognition element, and the covalently coupled aptamer as capture probe in the aptasensor (**b**). Images are adopted from references [53, 54] with permission from publisher

immobilize anti-HER2 antibody (using APTMS and PEG-NHS-Maleimide chemistry) and decorate over the screen-printed carbon-gold nanoparticles electrode surface. Binding of HER2 to CeO_2 NP-Ab bioconjugate inhibited electron transfer, which is sensed as decreased current peak [91]. Another study reported use of chitosan-coated SiNPs for their remarkable high surface areas as support for immobilization of anti-HER2 antibody in a microfluidic-based immunosensor [92].

MOFs, the porous coordination polymer structures made of metal ions coordinated to organic ligands in three-dimensional frameworks are also used as promising nanomaterials. The MOFs served as scaffolds for adsorption of various metallic nanoparticles and biomolecules such as aptamers due to their high surface area and excellent adsorbability [93, 94]. Gu et al. reported development of a bimetallic zirconium- hafnium MOF coupled to carbon dots,denoted as CDs@ZrHf-MOF) aptasensor, for simultaneous detection of HER2 proteins and breast cancer cells [59]. The use of metal nanoparticles and MOF endows the aptasensor with high sensitivity, improved stability, and acceptable applicability. Similarly, an HER2 aptasensor based on bimetallic Mn-Fe Prussian blue analogue coupled to gold nanoparticles (denoted as MnFePBA@AuNP) scafffold that provide ample sites for aptamer immobilization with optimum electrochemical activity has been reported [52].

Also, functionalized magnetic nanoparticles such as iron oxide nanoparticles (Fe_3O_4 NPs) have attracted much attention in the fabrication of aptamer, and antibody-based sensing platforms for HER2 detection. These nanoparticles benefit from their unique properties such as biocompatibility, signal amplification, ability to bind covalently to antibodies, and high

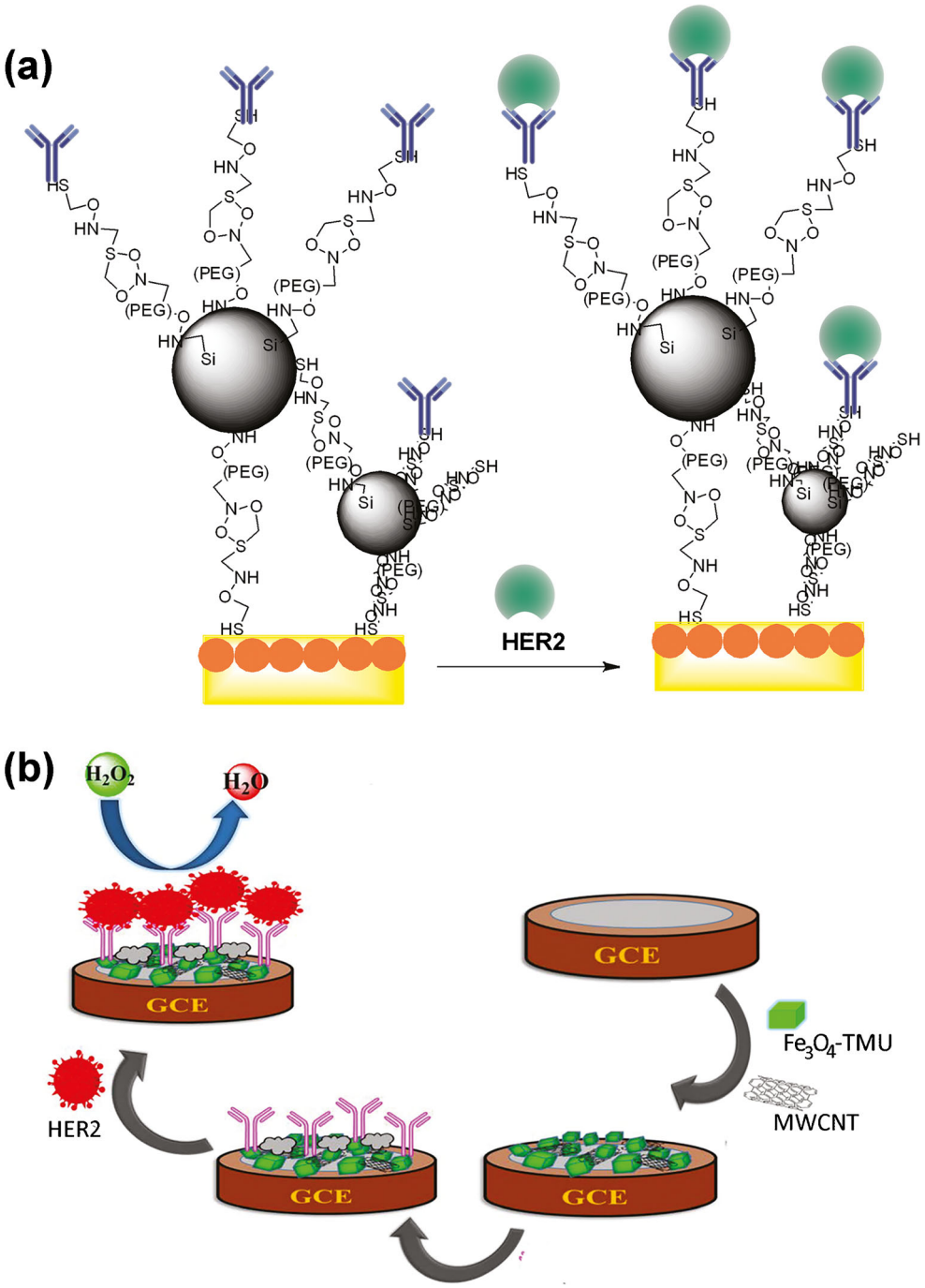

Fig. 5 Schematic illustration of fabrication of iron oxide nanoparticles (Fe_3O_4 NPs) based electrochemical immunosensors for HER2. Immunosensor in (**a**) is fabricated by layering anti-HER2 antibodies coupled iron oxide nanoparticles (Fe_3O_4 NPs) that provided long terminals (most possible space) for the immunoreaction onto the gold electrode surface. Immunosensor in (**b**) is constructed by modifying the glassy carbon electrode surface with HER2 antibody coated magnetic framework Fe_3O_4@TMU-21 and MWCNT. H_2O_2 reduction by Fe_3O_4@TMU-21 allowed achieving increased catalytic efficiency. Images adopted/ reproduced from references [95, 96] with permission from publisher

magnetization. The iron oxide nanoparticles used in biosensing applications, in general, have a magnetite core surrounded by a non-magnetic coating (e.g. aminopropyltrimethoxysilane) for easy and efficient attachment of antibody molecules, and to prevent aggregation of bare nanoparticles [95]. Encapsulation of Fe_3O_4 in the MOFs has been shown to enhance the electrocatalytic activity of the composite towards reduction of H_2O_2, increase electrode surface area, porosity, and the chemical tenability of magnetic framework composite (Fe_3O_4@TMU-21) that lead to increased catalytic efficiency. Three studies included in this review reported use of Fe_3O_4 NPs and MOF encapsulated Fe_3O_4 NPs in modifying the electrode surfaces for immobilization of anti-HER2 antibodies, and thus provide the most possible space for the biorecognition event to take place (Fig. 5a, b) [95, 96].

Carbon-based nanostructures such as carbon nanotubes (single/multiwall), carbon dots, and graphene have been extensively used in HER2 biosensors. Carbon nanotubes (CNT) possess an outstanding combination of mechanical flexibility, high surface-to-volume ratio, nanowire morphology, biocompatibility, high charge transfer efficiency, thermal conductivity, and exceptional electronic properties [97]. However, the use of CNT requires functionalization with anchor molecules and/or redox-active species to provide favouring conditions for efficient electron transfers via electron shuttle. Nanocomposites prepared by combining single/multiwall carbon nanotubes (SWCNT/MWCNT) with metal nanoparticles (e.g. AuNPs, Fe_3O_4NPs, and rGO), polymers (e.g. chitosan) or ionic liquids having improved electrocatalytic properties and ability to immobilize high load of biorecognition elements have been reported in the development of both immunosensors and aptasensors for HER2-ECD [51, 78, 96, 98]. Reduced graphene oxide-chitosan film is also reported as suitable electrode material in multiple studies owing to its high homogeneity, good stability, large surface area and a high fraction of amine groups for binding to aptamers [58].

DNA nanostructures such as tetrahedral DNA nanostructures (TDN) constructed from complementary base-pairing reactions of single-stranded nucleic acids are also reported as a versatile nanomaterials for biosensing application. Their stable and easy synthesis, with precise control on shape and size, makes TDN excellently biocompatible for labelling with biorecognition elements. A few studies reported the development of sandwich assay-based immunosensors by decorating the gold electrode interface with TDN having expanded aptamers as biorecognition probe for HER2 analysis (Fig. 2a) [43, 77].

Nanomaterials as a signal carrier or detection probe

While modifying the electrode surface with nanomaterials allows enhanced and stable coating of aptamers and antibodies, achieving increased sensitivity to be able to detect low limits of HER2 required adopting a variety of signal amplification strategies. Functionalized signal nanoprobes and nucleic acid amplification techniques represent two most widely applied signal enhancement methods. Nanoprobes such as nanozymes (e.g. noble metals, metal oxide, bimetallic nanostructures, and metal-organic frameworks), owing to their easy surface modification and comparably better stability over natural enzymes, have been used to enhance biosensor performance and enable HER2 detection at substantially low concentrations [99]. One study reported the use of aptamer functionalized MnO_2 nanosheets as a probe for dual signal amplification from adsorbed phosphate ions and phosphate groups on aptamer backbone that reacted with molybdate to form electroactive molybdophosphate, which significantly increased the current intensity of the electrode [56]. Another study reported the use of gold nanorods bioconjugated to aptamer and horseradish peroxidase enzyme as the signal probe in the sandwich-like biosensor platform for HER2 analysis [77]. Also, the use of AuNPs modified with glucose oxidase (GOx) was reported as a dual signal amplification probe, where the GOx catalyse the oxidation of glucose to produce H_2O_2 which amplifies the photoelectric current signal along with the localized surface plasmon resonance of AuNPs that enhance photoelectric transfer efficiency and increase the photocurrent response as well [47].

Another prominent nanomaterial reported for HER2 biosensing is the luminescent semiconducting nanocrystals, commonly known as QDs. They are promising nanomaterials for labelling as they can be conjugated to aptamers or antibodies, and can be synthesized with different compositions or with distinct core-shell structures [100]. The commonly used QDs are composed of cadmium chalcogenides (S, Se, Te) core and an external shell having functional moiety for biomolecular immobilization. The use of QDs in biosensing applications contributes to reduced analysis time as it requires no additional steps of substrate addition [79]. A study by Freitas et al. reported the use of QDs in magnetic immunosensors. QDs bioconjugated to streptavidin were used to bind with biotinylated detection antibodies and produce electrochemical signal from the deposition of metal ions (Cd^{2+}) released from QDs in an acidic medium on electrode surface [79, 101]. The obtained electrochemical signals were then related to the analyte concentration.

Analytical figures of merit

Characterising the relative performance of analytical methods or devices in comparison to other available methods is an essential exercise to determine detection capabilities of the newly developed method. Limit of detection (LOD) is an important figure of merit that not only allows comparing methods but also establishes whether a method can be applied to detect low levels of analyte (i.e. HER2) in complex matrices. LOD is defined as "the lowest concentration of an analyte that can be reliably detected by the method" [102]. A similar term, linearity "the concentration range between a minimum and a maximum value where the signals are directly proportional to the concentration of the analyte in the sample" is also used as an indicator of the dynamic range of the analyte (levels) that a given method can determine with stated certainty.

For the HER2-ECD having the serum levels varying between 2 and 15 $ngmL^{-1}$ in healthy individuals, and 15–75 $ngmL^{-1}$ in breast cancer patients, the included HER2 biosensors may allow easy detection of both baseline as well as elevated levels of HER2-ECD in the appropriately diluted serum samples (Table 3 and Table 4) [103]. For example, the achieved LOD of 2.1 $ngmL^{-1}$ allows detecting HER2

levels of 50 ngmL^{-1} after 1:25 dilution (Table 3). A detailed list of the yielded detection limits and achieved linearity in various immunosensors and aptasensor studies are provided in Table 3 and Table 4.

Selectivity, yet another crucial parameter of the analytical performance of biosensors refers to the ability of a method to selectively report a defined target among a complex mixture of substances. IUPAC defines selectivity as "the extent to which other substances interfere with the determination of a substance according to given procedure", wherein a larger interference is regarded as lesser selectivity, and at 100% selectivity, the method is considered to be specific. Selectivity of the immunosensors and aptasensors towards HER2 in the reviewed studies was determined by analysing a fixed concentration of HER2 in combination with various interfering species such as other proteins/biomarkers undergoing deregulated expression in carcinogenic breast epithelial cells (e.g. CEA, CA15.3, AFP, p53, PKA etc.) or cells differing in the expression pattern of surface proteins (e.g. MCF-7, MDA-MB-231, SK-OV-3, HMEC etc).

Rapid diagnostics, a test that is quick and easy to perform, is of high importance in assessing both communicable and non-communicable diseases. Biosensors that provide quick assay outcome could be used at the point-of-care, setting the bases for the eventual point-of-care (POC) monitoring of this disease. For example, the aptasensor and immunosensor yielding assay time of 4 min and 17 min respectively may be the good candidates for POC applications. Ease of use as well as ease of preparing electrodes and conducting electrochemical measurements is also equally important. For this, the sensor should be, not only fast, also extremely easy to use. Biosensor stability is another parameter that determines how long a modified transducer can remain active to produce desirable outcomes. While the storage stability has no role for single-use disposable biosensors (e.g. screen-printed electrode-based biosensors), it may depend on a number of factors, including thermal and electrical stability of transducers and electronic components, the nature of membranes and/or coating on the electrode surface, and biosensor fabrication and analysis method for reusable biosensors.

Conclusions and future perspectives

Overexpression and/or amplification of HER2 in approximately 15–20% of all breast cancer patients is associated with aggressive disease, poor prognosis, shorter disease-free survival, and high a propensity of metastasis [10, 11, 15]. Structurally, HER2 is composed of an extracellular ligand binding domain (ECD) along with a lipophilic transmembrane, and an intracellular tyrosine kinase domain. The ECD can be released into the blood via proteolytic cleavage by ADAM proteases, making it accessible in serum of approximately 15–40% of breast cancer patients [113–115]. The possibility of assessing HER2-ECD in human serum has attracted great attention in the past few decades. HER2 status of breast cancer patients is required for their eligibility for HER2-targeted therapy. It is conventionally determined using slide-based tests such as IHC, FISH or CISH. Dependent on invasively collected primary tumours for histological assessment of HER2, these techniques cannot be used in the follow-up to monitor the disease and treatment response [23]. This makes the circulating HER2 ECD a surrogate marker for minimally invasive analysis of HER2 status in tissue. Multiple commercial ELISA kits are available for sensitive detection of HER2-ECD (Table 1). In the past decade, several strategies have been employed to make HER2 diagnosis in serum samples more economic, sensitive, and easily available through the use of antibodies and aptamers in biosensors. Rapid emergence of HER2 immunosensors and aptasensors stems from (i) the emerging need for alternative modalities to the widely used invasive IHC for clinical determination of HER2 expression levels in newly diagnosed breast cancer patients, (ii) the need for an alternative method to ELISA for detection of circulating HER2 in follow-up patients, (iii) reliance of the IHC or ELISA methods on sophisticated and laboratory-ridden instruments and time-taking protocols, and (iv) to harness the relative simplicity, low cost, high sensitivity of biosensor in HER2 diagnostics. This review is aimed to provide recent advancements in biosensor development for HER2-ECD, in particular the various tools (nanomaterials) and techniques (electrochemical techniques and detection approaches) used in developing HER2 biosensors, and their yielded analytical parameters for comparative evaluation of analytical merits. All recent studies published in the last decade that reported the development of electrochemical biosensors for HER2 are included in the review. The key electrochemical techniques used for signal measurement in reviewed HER2 biosensors are cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), square-wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS), and capacitance measurement. Both label-free as well as label-based methods that employed physical properties of analytes (e.g. electric impedance and capacitance) or the enzyme/nanoparticles-based tags for signal amplification are mostly reported. Nanomaterials were used as transducer materials (substrate for immobilization of aptamers/ antibodies) and labels to amplify electrochemical signal. Immobilization of biorecognition elements (aptamers and antibodies) onto working electrode (gold, screen-printed carbon/graphene, glassy carbon) or onto various nanoparticles has been reported using physical adsorption as well as via chemical modifiers and linkers, such as poly-L-lysine, glutaraldehyde, 3-aminopropyl trimethoxisylane (APTMS), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), n-hydroxide succinimide (NHS), polyethylene glycol-α-maleimide-ω-NHS, and gold-

thiol chemistry [46, 58, 78, 91, 95]. Aptamers used in developing various HER2 immunosensors are summarized in Table 2. Finally, the most widely reported analytical parameters viz. detection limits, linearity, selectivity, precision, and biosensor response/ assay times are compared.

While the recent developments in nanomaterials-based HER2 biosensors may proclaim high sensitivity, robustness, and other technical benefits over conventional techniques of HER2 analysis, their true utility in clinical analysis or as an alternative to ELISA-based methods will depend on multiple factors, viz. better relative performance on a sufficiently large number of clinical samples, ability to transform into portable tools, automation in assay protocol, and relative ease of sampling and analysis (e.g. finger prick assay). Sterilization, cost and stability and robustness that have been the bottleneck in translating many biosensors from laboratories to clinics will need to be overcome. Effective design that can be materialized to portable systems will be needed to be worked upon. Lack of robust validation has been the prime drawback of recently developed HER2 biosensors. Evaluating these biosensors for analytical performance on clinical samples in collaboration with tertiary health care facilities may provide at least a few biosensors that could be commercialized. Also, while evaluating the performance of newly developed biosensors, care must be taken to compare only methods that are based on the same units for measured signals. For instance, sensitivity derived from spectral and electrochemical measurements cannot be truly compared. Also, adopting updated methods and norms of biosensor development, for instance, the use of the error propagation approaches to determine the limit of detection rather than deriving it using the classical '3 sigma' concept may assist in developing truly novel biosensing methods.

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Code availability Not applicable.

Author contribution RA conceptualized the work, carried out literature survey, extracted data, and wrote the manuscript.

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Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The author declares no competing interests.

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