Emerging Biosensor Technologies for Multiplex Detection of Breast Cancer Biomarkers: From Nanomaterials to Clinical Translation

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Abstract
Breast cancer is the most prevalent type of cancer among females across the globe and early detection, and accurate diagnosis of the biomarkers are crucial to improve the prognosis of the patient. Despite their proven value, traditional immunoassay-based diagnostics platforms are not particularly sensitive, not multiplexable, and are not ideal for point of care applications. This review deals with the advanced biosensor technologies developed for the multiplex detection of breast cancer-related, important biomarkers like human epidermal growth factor receptor 2 (HER2), cancer antigen 15-3 or CA 15-3 (CA153), carcinoembryonic antigen (CEA), mutations in the BRCA1/2 genes and circulating tumor DNA (ctDNA). The electrochemical, optical, piezoelectric, and microfluidic biosensor modalities are studied with the aim of using novel nano materials such as gold nanoparticles, graphene oxide, carbon nanotubes, and metal-organic frameworks (MOFs). Signal amplification strategies, surface functionalization of chemistries, and using machine learning for data interpretation are highlighted. Clinical translation was discussed as an important topic for which biocompatibility, regulatory aspects (FDA, CE-IVD) and real time validation in patient serum are important points. Other areas of emerging interest like biosensors with CRISPR technology, wearable biosensors, and AI-assisted diagnostic pipelines also feature. The aim of this review is to provide a logical approach to designing novel multiplex biosensors for the revolution in breast cancer diagnostics.

Keyword: Biosensors, breast cancer, multiplex detection, HER2, CA 15-3, electrochemical sensors, nanomaterials, gold nanoparticles, graphene oxide, clinical translation, CRISPR diagnostics, point-of-care.

I. Introduction

Breast cancer is the leading cause of cancer-related deaths in women worldwide, as per the World Health Organization [1] 2,300,000 women are diagnosed with breast cancer and 685,000 women die of breast cancer annually. The number of 5-year survivors is >90% at Stage I, but <25% at Stage IV [2] and early detection has been shown to be the single most important way of improving 5-year survival rates. Although there are improvements in mammography and MRI screening, these technologies are not sensitive enough for dense breast tissue and do not measure the molecular level of biomarker quantification to help in the selection of targeted therapy.

Proteins and nucleic acid markers, including human epidermal growth factor receptor 2 (HER2), cancer antigen 15-3 (CA 15-3), carcinoembryonic antigen (CEA), estrogen receptor (ER), progesterone receptor (PR) and BRCA1/2 mutations, collectively, give a 'molecular fingerprint' for breast cancer subtyping, prognosis and monitoring of therapy [3]. At the same time, the idea of multiplex quantification from minimally invasive liquid biopsies (blood, saliva and urine) of such biomarkers is emerging as a big focus of the precision oncology field.

The current available detection platforms such as the enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence immunoassay (ECLIA) and the polymerase chain reaction (PCR)-based method have been clinically validated, but they suffer from long detection turnaround time, high laboratory infrastructure requirements and inability to provide true multiplex quantification in one detection process [4]. Biosensor technologies, which are analytical devices comprised of a physicochemical transducer and a biological recognition element, offer the potential to overcome these limitations.

The combination of nanotechnology, microfluidics, surface chemistry and artificial intelligence has brought about a new generation of biosensors capable of detecting femtomolar concentrations of bioanalytes and monitoring their kinetic variations in real time, as well as measuring the concentration of several different analytes in a single measurement [5]. The present review summarizes and critically evaluates the characteristics of biosensor platforms for the detection of breast cancer biomarkers during the 2018–2026 period, focusing on the technologies that rely on the use of nanomaterials for enhancing the transduction mechanism, on multiplexing architectures and on the readiness for clinical translation.

In Section II, the reader will be introduced to some of the important breast cancer biomarkers and their relevance to the clinic. In Section III, the emphasis is placed on review of the types of transductions of biosensors. Section IV looks at the engineering strategies of nanomaterials. The concept of multiplexing architectures is discussed in Section V. In Section VI, clinical

translation and regulatory challenges are discussed. Emerging technologies are discussed in Section VII, and projections and conclusions are discussed in Section VIII.

II. BREAST CANCER BIOMARKERS: CLINICAL SIGNIFICANCE AND DETECTION REQUIREMENTS

A. Protein Biomarkers

The ERBB2 proto-oncogene encodes HER2, which is over-expressed in approximately 15-20% of all breast cancers and a prognostic factor and therapeutic target for trastuzumab (Herceptin) therapy [6]. To quantify HER2, the molecule needs to be detectable in the clinically relevant concentration range (0.1-100 ng/mL) in serum, and the concentration of shed extracellular domain (ECD-HER2) needs to be correlated with the tumour burden and treatment outcome. The mucin-like glycoprotein CA 15-3 is shed and has a clinical cutoff value of 30 U/mL and is sensitive and specific at about 75% and 90%, respectively, in detecting metastatic disease [7].

CEA is not a breast cancer-specific marker but may be used with CA 15-3 in a multi-marker panel to provide additional prognostic information. For non-smoker patients, the cut-off of CEA in the serum is 5 ng/mL and the greater the level, the more likely the patient is to have hepatic metastasis [8]. Protein markers such as interleukin-6 (IL-6), vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) have emerged as potential biomarkers of the activity of the tumor microenvironment and its angiogenic potential [9].

B. Nucleic Acid Biomarkers

The mutation rate in BRCA1 and BRCA2 is 57-65% and 45-55% respectively for the acquisition of breast cancer in lifetime and it is based on these mutations that decisions are made for preventive intervention and familial screening [10]. Plasma contains circulating tumor DNA (ctDNA) and cell-free DNA (cfDNA) from tumor apoptosis and necrosis, which are found in the plasma at femtomolar levels and contain somatic mutations such as PIK3CA mutations, TP53 mutations, and mutations in ESR1 that have a role in therapeutic decision making in metastatic disease [11]. Another class of nucleic acid markers of tumor activity that can be identified in exosomes of serum have been identified as microRNAs (miR-21, miR-155, miR-210) that are very specific to the tumor state [12].

TABLE I. Key Breast Cancer Biomarkers and Clinical Diagnostic Parameters

Biomarker	Biomarker Type	Clinical Cutoff Value	Associated Breast Cancer Subtype	Required Assay Sensitivity
HER2/ECD	Protein	15 ng/mL	HER2-positive (~20% of breast cancer cases)	0.01–100 ng/mL
CA 15-3	Glycoprotein	30 U/mL	Metastatic Breast Cancer	1–500 U/mL
CEA	Protein	5 ng/mL	Advanced/Metastatic Breast Cancer	0.5–100 ng/mL
BRCA1/2	DNA Mutation	Mutation Present/Absent	Hereditary Breast Cancer	Allele frequency <0.1%
ctDNA (PIK3CA)	Cell-free DNA (cfDNA)	~1–10 ng/mL	Hormone Receptor-Positive (HR+), Metastatic Breast Cancer	Femtomolar Range
miR-21	microRNA (miRNA)	Relative Expression Level	Triple-Negative Breast Cancer (TNBC)	Sub-fmol/L
VEGF	Protein	200 pg/mL	Angiogenic Breast Cancer	10–5000 pg/mL

III. BIOSENSOR TRANSDUCTION MODALITIES

A. Electrochemical Biosensors

Biosensors, which are inherently suited for miniaturization and low-cost production and direct electrical readout, are the most advanced type of electrochemical biosensors that are available for breast cancer biomarkers detection [13]. Immunoassays for HER2 and CA 15-3 have been successfully applied by the three major electrochemical transduction techniques: amperometric, impedimetric (EIS), and voltammetric (DPV/SWV). Amperometric biosensors have been employed to determine the presence of HER2 in the range of 1–10 pg/mL by using signal amplification, following the binding of the antigen to an enzyme label (horseradish peroxidase or alkaline phosphatase) [14].

The changes in electron transfer resistance (Rct) of the electrode surface when the antigen is bound to the antibody are measured by electrochemical impedance spectroscopy (EIS). A gold electrode with anti-HER2 aptamers functionalized was used in a landmark study by Ribaut et al. to develop a HER2 aptasensor with a limit of detection (LOD) of 0.3 ng/mL in undiluted serum

[15]. The LODs for CA 15-3 obtained by DPV on graphene modified electrodes were two orders of magnitude lower than the sensitivity of clinical ELISA, ranging from 0.2pg/mL using the sandwich immunoassay formats [16].

B. Optical Biosensors

The binding event can be detected by a surface plasmon resonance (SPR) biosensor, that will give real time kinetic analysis of the binding event. Localized SPR (LSPR) of gold or silver nanoparticles can be used to increase the sensitivity because of the near field plasmonic effects [17]. Acceptable matrix performance (LODs) of 0.5 ng/mL in PBS and 1.2 ng/mL in human serum have been obtained with LSPR chips functionalized with anti-HER2 antibodies. For multiplexing, surface-enhanced Raman spectroscopy (SERS) can be applied with spectrally distinct Raman reporter molecules, which can be applied to simultaneous detection of HER2, CEA and CA 15-3 in a single droplet using SERS-encoded nanoparticle probes [18].

Fluorescence technology using with quantum dots (QDs) and upconversion nanoparticles (UCNPs) has made the detection of miRNAs and ctDNA at sub-picomolar concentrations possible by Förster resonance energy transfer (FRET). Zhang et al. developed a QD-FRET biosensor which detected miR-21 at 0.3 fmol/L in clinical serum samples with a dynamic range of 3.5 Logs [19]. The new modality of ultrahigh sensitive SPR sensors based on photonic crystal fiber (PCF) has ultrahigh sensitivity (>4000 nm/RIU) and is suitable for the detection of HER2 at sub-pg/mL level [20].

C. Piezoelectric and Acoustic Biosensors

Mass binding events are measured by the frequency shift (Δf) of quartz crystal microbalance (QCM) biosensors, which allows the measurement of mass binding events to be done by simply measuring the frequency shift. Molecularly imprinted polymers (MIPs) and aptamers have been used to functionalize QCM in order to attain LODs as low as 0.1 ng/mL for HER2 and 0.5 U/mL for CA 15-3, respectively [21]. In an innovative method, bulk acoustic wave (BAW) and surface acoustic wave (SAW) devices are coupled to microfluidic channels to detect markers for breast cancer in a flow-through manner with real-time readout [22].

D. Microfluidic and Lab-on-Chip Integration

Integrating microfluidic devices into single biosensor elements is the integration of the diagnostic platform under a single envelope, and thus sample-to-answer operation. Recently, microfluidic chips based on polydimimethylsiloxane (PDMS) having electrochemical detection arrays have been developed to quantify concurrently three cancer markers, HER2, CA 15-3 and CEA, from 50 μL of blood in 45 min [23]. The use of digital microfluidic (DMF) platforms with electro-wetting-on-dielectric (EWOD) actuation enables automated droplet based-Immunoassays (IDA) with programmable washing step and signal readout step, reducing the human error and increasing the assay reproducibility [24].

IV. NANOMATERIAL ENGINEERING FOR ENHANCED BIOSENSING

A. Gold Nanoparticles (AuNPs)

The exceptional biocompatibility, tunable LSPR, easy surface chemistry and ability to be thiol/amine conjugated, and their high surface-area-to-volume ratio allowing for high antibody loading densities make gold nanoparticles an important component of biosensor signal amplification [25]. AuNPs can be used in the secondary antibody to amplify the electrochemical signal by a factor of 1000 in sandwich immunonassays. The gold nanospheres and gold nanorods are named as hollow gold nanospheres (HGNs) and gold nanorods (GNRs), respectively, and provide SERS enhancement factors of 108–1010, enabling single molecule sensitivity for the detection of mutations in the gene BRCA1 by SERS spectroscopy [26].

B. Graphene and Carbon-Based Nanomaterials

Graphene oxide (GO) and reduced graphene oxide (rGO) have a large theoretical specific surface area (>2600 m2/g) with exceptional electron transfer kinetics and functional groups that are suitable for conjugating biomolecules. The rate constants (k^0) of rGO modified glassy carbon electrodes are as high as 0.01–0.1 cm/s, significantly higher than those of the redox reaction of the electrode in electrochemical immunoassays [27]. Multi-walled carbon nanotube (MWCNT)/chitosan nanocomposite modified electrodes have been shown to be able to detect HER2 at 0.01 pg/mL, which is 300-fold lower than the known clinical cutoff, thus offering a large analytical safety margin for early detection [28].

The photoluminescent property of the biowaste derived carbon quantum dots (CQDs) can be tuned between 400-700 nm, which enables multiplexed optical detection by separation of the different wavelengths. The quantum yields of N-doped CQDs are enhanced to over 60%, and such CQDs are used to detect both miR-21 and miR-155 in the serum of TNBC patients [29].

C. Metal-Organic Frameworks (MOFs)

Metal-organic frameworks (MOFs) are porous, crystalline materials constructed from metal nodes and organic linkers and have become highly attractive scaffolds to host signal reporters and to build biosensors because of their extremely porous nature (BET surface area up to 7000 m2/g), tunable pore chemistry and capacity to host signal reporters. Zeolitic imidazolate framework-8 (ZIF-8) was designed to be used either as an encapsulant for antibody-enzyme conjugates or as an electroactive label that, when dissolved at the electrode surface, releases electrochemically active zinc ions. Finally, the LOD of 0.001 U/mL achieved with the CA 15-3 immunosensor using MOF as the signal amplifier was 30,000 times lower than that of clinical ELISA [31].

D. Magnetic Nanoparticles and Immunomagnetic Enrichment

Immunomagnetic enrichment (IOZ) is performed by using capture antibody functionalized superparamagnetic iron oxide nanoparticles (SPIONs, Fe_3O_4 or γ-Fe_2O_3) which allow to pre-concentrate analytes by 100- to 1000-fold before they are transduced from complex biological matrix [32]. Microfluidic devices have also been constructed to capture and electrochemically detect circulating tumor cells (CTCs) expressing EpCAM, HER2 and N-cadherin in patient blood with a sensitivity of 1–5 CTCs/mL [33].

TABLE II. Performance Comparison of Representative Biosensor Platforms for Breast Cancer Biomarker Detection

Sensor Type	Target Biomarker	Nanomaterial	Limit of Detection (LOD)	Linear Detection Range	Sample Matrix
Electrochemical (DPV)	HER2	Gold nanoparticles/reduced graphene oxide (AuNPs/rGO)	0.01 pg/mL	0.1 pg/mL–100 ng/mL	Serum
Electrochemical Impedance Spectroscopy (EIS) Aptasensor	HER2	Gold electrode	0.3 ng/mL	1–500 ng/mL	Serum
Localized Surface Plasmon Resonance (LSPR)	CA 15-3	Gold nanorods	0.5 U/mL	1–200 U/mL	PBS/Serum
Surface-Enhanced Raman Scattering (SERS)	CEA + CA 15-3	Au@Ag core–shell nanoparticles	0.1 pg/mL	1 pg/mL–1 μg/mL	Blood
Quartz Crystal Microbalance–Molecularly Imprinted Polymer (QCM-MIP)	HER2	Silica nanoparticles (SiO_2 NPs)	0.1 ng/mL	0.5–500 ng/mL	Serum
Fluorescence Resonance Energy Transfer (FRET)	miR-21	Quantum dots/Carbon quantum dots (QDs/CQDs)	0.3 fmol/L	1 fM–10 nM	Serum exosomes
MOF-Based Electrochemical Sensor	CA 15-3	Zeolitic imidazolate framework-67 (ZIF-67)	0.001 U/mL	0.005–100 U/mL	Serum
Microfluidic Electrochemical Assay (ECA)	HER2 + CEA + CA 15-3	Multi-walled carbon nanotubes/Gold nanoparticles (MWCNTs/AuNPs)	0.05 pg/mL	0.1 pg/mL–1 μg/mL	Whole blood

V. MULTIPLEXING ARCHITECTURES AND SIGNAL PROCESSING

A. Spatial Multiplexing

Spatial multiplexing involves the use of multiple sensing elements (biorecognition elements) on a single substrate. The individual working electrodes can be functionalized with different antibodies for the measurement of three different biomarkers simultaneously, specifically anti-HER2, anti-CEA and anti-CA 15-3, from a single 5 μL serum sample [34]. Breast cancer patients have been profiled on array surfaces with up to 100 functionalized spots that achieved a high classification accuracy of more than 92% of molecular subtypes (luminal A, luminal B, HER2-enriched and triple-negative) [35].

B. Spectral and Electrochemical Multiplexing

The spectral multiplexing of nanoparticles that are encoded with the spectral features of the Raman spectra (SERS) is achieved without spatial separation in the libraries. An immunoassay platform that utilized a six-plex SERS AuNPs encoded with 4-aminothiophenol, 4-nitrothiophenol, 2-naphtalenethiol, 4-mercaptobenzoic acid, thiophenol and 4-fluorothiophenol was developed to quantify HER2, CEA, CA 15-3, AFP, PSA, and IL-6 from a single 20 μL sample without cross-reactivity [36]. The principle of M multiplexing is the use of the different formal potentials of the various metal ions or/or organic redox mediators as electrochemical barcodes to enable parallel reading of biomarkers on a single electrode (working electrode) [37].

C. Machine Learning-Assisted Data Interpretation

Pattern recognition and diagnostic classification of the multidimensional data obtained from multiplex biosensor arrays can be achieved using machine learning (ML) algorithms. A set of multiplexed electrochemical biosensor outputs were then used to train SVM and random forest classifiers to discriminate between breast cancer patients and healthy controls, achieving receiver operating characteristic area under curve (ROC-AUC) of 0.97–0.99 [38]. A different method is based on convolutional neural network (CNN) to classify multiplexed SERS spectra of patient's serum with sensitivity and specificity values of 95.3% and 97.1%, respectively, for early-stage (Stage I-II) breast cancer [39]. The use of deep learning frameworks that combine the

results of biosensors with clinical data (age, family history, imaging results) also enhanced the accuracy of the diagnosis, underscoring the potential of the hybrid human-machine diagnostic systems [40].

VI. CLINICAL TRANSLATION: CHALLENGES AND PATHWAYS

A. Biological Matrix Effects and Assay Validation

Biofouling, interferent species (bilirubin, lipids and hemoglobin) and variations in pH/ionic strength of the sample are examples of biological matrix effects that must be evaluated when moving a proof-of-concept (PoC) test to clinically validated diagnostic testing. Preserving analytical specificity in undiluted serum specifically has been important for polyethylene glycol (PEG) blocking, zwitterionic polymers (carboxybetaine) or bovine serum albumin (BSA) blocking coatings. Clinical validation to be carried out based on CLSI EP-series requirements: precision (CV < 10%), comparison of methods (Spearmen r > 0.95) and interference (CLSI EP7 protocols) testing per protocol [42].

B. Regulatory Frameworks: FDA and CE-IVD

In-vitro diagnostic (IVD) devices to detect breast cancer biomarkers are classified by US FDA as Class II (substantial equivalence 510(k) pathway) or Class III (premarket approval PMA) depending on the intended clinical application and risk profile [43]. Already, the liquid biopsy devices for the detection of ctDNA have been enjoying the support of the Breakthrough Device Designation from the FDA; the designation could be used as soon as the diagnostics are able to offer more effective early detection of life-threatening diseases. The new In Vitro Diagnostic Regulation (IVDR EU 2017/746) has stricter requirements than the previous IVD Directive for clinical evidence such as clinical performance studies to demonstrate the analytical sensitivity, specificity and positive and negative predictive values for the intended patient population [44].

C. Point-of-Care Implementation

Point-of-care (POC) platforms need to satisfy the WHO ASSURED criteria: Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free and Deliverable to the end-users for deployment in resource limited settings [45]. Community clinic usage of a lateral flow assay (LFA) format is possible because of the limit of detection of 5 ng/mL for detection of HER2 using colloidal gold or fluorescent nanoparticle labels within 15 minutes. Paper-based microfluidic devices with the optical readers connected by smartphone are able to provide quantitative measurement of HER2 with LODs as low as 1–5 ng/mL, without the need for any laboratory infrastructure [46].

Electrochemical readout of screen-printed electrode immunosensors is possible on portable potentiostat modules like PalmSens4, EmStat Pico, which are miniaturised versions of potentiostats and can be connected to mobile applications for real-time data analysis and cloud reporting of the results. POC methods using biosensors may reduce the cost of each test to $5-15, significantly cheaper than the $50-200 of ELISA-based biomarker testing in the laboratory, allowing the possibility of breast cancer screening at a population level in low and middle income countries [47].

VII. EMERGING TECHNOLOGIES AND FUTURE DIRECTIONS

A. CRISPR-Based Nucleic Acid Biosensors

platforms have been engineered by engineering the activity of collateral cleavage of Cas12a and Cas13a, which is activated upon recognition of the target, as a nucleic acid detection technology [48]. In addition, attomolar single molecule detection (10^{-18} mol/L) (SHERLOCK) and DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) with rolling circle amplification (RCA) pre-amplification can detect the mutations of ctDNA in the absence of PCR amplification and with attomolar sensitivity [49]. The CRISPR-Cas12a was also linked up to electrochemical biosensors, which provides a single nucleotide-specific detection of PIK3CA H1047R ctDNA with a limit of detection of 100 aM in 30 minutes from patient plasma [50].

B. Wearable Biosensing Platforms

Patch or arrays of flexible and wearable biosensors worn on the breast offers the potential for continuous, non-invasive monitoring of biomarkers [51]. To be used as a wearable, the flexural durability of flexible electrodes (carbon nanotubes printed on polyimide substrates) was demonstrated to be able to withstand 1000 flexural cycles (radius = 5mm) without any loss of electrochemical performance. Here, the HER2 detection method involves embedding anti-HER2 aptamer in a carbon paste electrode onto a textile patch and using sweat to detect HER2, which showed a range of detection of 1–100 ng/mL, but needs to be further validated in clinical samples for correlation with serum HER2 levels [52].

C. Exosome-Based Liquid Biopsy Biosensors

Tumor-derived exosomes (30-150 nm extracellular vesicles) are also a window into the tumor biology, and contain surface proteins, miRNAs and DNA with the same molecular profile as the parent tumor [53] and represent a minimally invasive approach into tumor biology. For anti-CD63 and anti-HER2, the nPES sensors have been able to detect the HER2-positive exosomes in 1 μL serum as low as 36 exosomes/ μL, which has enabled non-invasive subtyping of HER2 without a tumor biopsy [54]. An immunoaffinity capture process of vesicles followed by surface protein detection by SERS has been realized by a microfluidic herringbone chip (HB-Chip) system [55].

D. Artificial Intelligence Integration

Foundation models trained on multi-omics data are now being fine-tuned to be able to interpret multiplex biosensor outputs, on large-scale cohorts of breast cancer patients (TCGA, METABRIC, BeatBC) [56]. The use of graph neural networks (GNNs) for modelling interaction networks between biomarkers has been shown to achieve comparable accuracy with 98.4% when using multiplexed biosensor data to classify breast cancer subtypes [57] compared to linear classifiers. For clinical adoption of AI empowered biosensor platforms, privacy concerns are also addressed by federated learning algorithms that do not require

sharing the raw information of patients to train multi-institutional models, allowing for the creation of powerful and generalizable diagnostic algorithms [58].

VIII. Conclusions And Future Perspectives

This review has systematically discussed the fast development of the biosensor technologies used for multiplex detection of the breast cancer biomarkers such as electrochemical, optical, piezoelectric and microfluidic transduction modalities using a variety of nanomaterials. This is a comprehensive study and the following are some important conclusions that can be drawn. First, analytical sensitivity, which has been enhanced by 2-4 orders of magnitude compared to traditional ELISA through nanomaterial engineering including AuNPs, graphene derivatives, MOFs and carbon quantum dots, is suitable for the early detection of disease and the monitoring of ctDNA, with detection limits at the femtomolar or attomolar level.

multiplex architectures that can detect spatial, spectral and electrochemical encoding can detect 3-6 breast cancer biomarkers on a micro-volume sample, producing a superior clinically relevant multi-marker panel than single analytes based assays. The synergistic results of combining several biosensors and machine learning algorithms have been demonstrated to deliver a diagnostic accuracy that equals or exceeds that of a specialist clinician, suggesting the potential of AI-powered biosensors to transform diagnostics. Third, as for nucleic acid biosensors, the development of ctDNA and miRNA detection using CRISPR technology is a paradigm-shifting technology that can achieve PCR free sensitivity and single-nucleotide specificity, which could turn liquid biopsy testing into a routine clinical test [59].

Noticeable progress has been made, but there are also a number of translation problems. The majority of the reported sensors have only been tested in a buffer or spiked serum under optimal laboratory conditions and are therefore at Technology Readiness Level (TRL) 3-5. There is a great need for clinical validation in large, demographically wide patient populations with standardization of pre-analytical conditions [60]. The harmonisation of FDA and EU-IVDR, development of certified reference materials (CRMs) for calibration of biosensors, and the establishment of standards for interlaboratory reproducibility are important prerequisites for clinical deployment.

The next steps for future research include: (i) development of surface chemistries suitable for complex biological matrices (biosensors); (ii) sample to answer operation involving on-chip preparation of cell lysis, nucleic acid extraction, plasma separation; (iii) prospective clinical applications exploring the clinical applicability of biosensor-guided treatment monitoring in HER2-positive and triple-negative breast cancer; and (iv) economic modelling to support reimbursement pathways for liquid biopsy in national breast cancer screening programmes. Biosensor-based multiplex diagnostics is therefore considered as a cornerstone technology for the coming era of precision oncology in breast cancer treatment when combined with other technologies, such as nanotechnology, microfluidics, synthetic biology and artificial intelligence.

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