8 (2021) 100075

Contents lists available at ScienceDirect

Biosensors and Bioelectronics: X

journal homepage: www.journals.elsevier.com/biosensors-and-bioelectronics-x

Recent development in electrochemical biosensors for cancer biomarkers detection

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ARTICLE INFO

Keywords:
Cancer
Biomarker
Biosensor
Electrochemistry
Nanotechnology

ABSTRACT

Cancer is the most frequent life-threatening disease which has the highest mortality rate throughout the world. Diagnosis of cancer at the early stage can plays a critical role for its effective and successful treatment. Traditional diagnostic methods for cancer screening are costly, time-consuming, and not practical for repeated screenings. However, a biomarker-based cancer diagnosis is emerging as one of the most promising strategies for early diagnosis, monitoring disease progression, and subsequent cancer treatment. This review describes the recent advances and improvements in the electrochemical biosensors designed for detecting various cancer biomarkers using different signal transduction techniques and biological recognition strategies.

1. Introduction

Cancer is one of the leading reasons for alarming global deaths with 18.1 million reported cases and 9.6 million deaths in 2018 (Bray et al., 2018). Most of the cancerous incidence and mortality are unexpectedly growing among developing countries. Cancer combines a group of diseases where body cells surprisingly start to grow and causes genetic cellular changes in solid masses of tissue (tumors) and sometimes in non-solids (leukemias) of the blood path. There are two type tumors, namely Benign and Malignant. Malignant types are mostly harmful and once they start to develop, they begin to divide and spread to other organs through blood and the lymphatic system by alternating normal cells and then metastasize causing the majority of cancer deaths. So, early stage detection of cancer is essential to ensure its diagnosis before they become incurable. To date, oncological imaging and biopsy-cytology of specimens are techniques used to detect cancers. These invasive conventional detection methods have their drawbacks such as tedious and expensive. Therefore, development of an innovative, economically feasible strategies for detecting cancer is much needed. Biological markers, sometimes called cancer biomarkers (CB) shows excellent genetic characteristics of the cancerous cells. World Health Organization (WHO) described biomarker as "any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" ("Biomarkers In Risk Assessment: Validity And Validation (EHC 222, 2001)," n. d.). CB can be used as a fundamental aspect for diagnosis or prognosis of overall monitoring of cancer status in a patient. The biomolecular origin of CB includes gene, gene products, DNA, RNA, protein, enzymes, hormones and specific cells which are most often released by genetic alteration and cell division (Chen et al., 2020). Extensive research has identified different types of cytogenic, circulating, protein, and oxidative stress biomarkers which are clinically accessible with precise measurement, can potentially ensure early detection of several cancers such as lung cancer (LC), colorectal cancer, pancreatic cancer, breast cancer, head and neck cancer, small intestine cancer, endocrine, prostate cancer, osteosarcoma, brain cancer, renal cancer, pancreatic cancer, and oral cancer (Siegel et al., 2019). Presence of these biomarkers can be confirmed by analyzing human body fluids such as blood, urine, serum, plasma, and tumor cells. Such CB can used for cancer detection/-screening, risk assessment, and monitoring of therapy due to their relevant capabilities with point of care (POC) applications (Pacheco et al., 2018b).

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https://doi.org/10.1016/j.biosx.2021.100075
Received 13 December 2020; Received in revised form 11 June 2021; Accepted 5 July 2021
Available online 10 July 2021

To name a few, Prostate-specific antigen (PSA) is both semen-based and blood-based biomarker for prostate cancer, widely used to diagnose and treatment of prostate cancer (Pinsky et al., 2017). Similarly, P53 is another promising cancer biomarker which is responsible for uncontrolled cell growth in body, so, effective for diagnosis of multiple cancers such as leukemia, breast, ovarian, bone and lung cancers (Afsharan et al., 2016). Moreover, cancer antigen 15–3 (CA 15–3) has been recently emerged as a promising serum biomarker of breast cancer recognition (Ferdinandusse et al., 2000). Additionally, CD44 is another' surface biomarker which is interesting for the diagnosis of breast cancer in stem cells (BCSCs) (Zhao et al., 2018). Apolipoprotein-A1 (Apo-A1), another prominent new biomarker for early diagnostic of bladder cancer from human urine (Kim et al., 2019). Pseudopodium-rich atypical kinase one, SGK 26699 (PEAK1) is recognized as an emerging pancreatic dental adenocarcinoma (PDAC) biomarker that regulates cell migration and cancer progression (Prasad et al., 2020). Additionally, there are different biomarkers which represent characteristics of four different lung cancer, namely AFP, CEA, CA125, and CA15-3 in blood or urine of affected patients (Tang et al., 2007). Sex-determining region Y-box 2 (SOX2) is also a potential CB, can be used for early diagnostic of various types of cancer including prostate cancer, lung cancer, skin cancer and breast cancer (Aydın and Sezgintürk, 2017). So, there can be many more CB which must be analyzed, that encouraged researchers worldwide to focus on the development a non-invasive techniques for cancer diagnosis (Ho et al., 2010).

Western blotting, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunohistochemistry (IHC), and high-performance liquid chromatography (HPLC) are the common existing techniques used for biomarker screening with technological limitations. Therefore, development of an innovative, economically feasible strategies for detecting cancer biomarkers is much needed. Last decade, various types of biosensors were reported for detecting cancer biomarkers such as electrochemical, optical, and mass-sensitive. The superior properties of those biosensors include their ease of use, high sensitivity, low detection limit, excellent specificity, and multiplexing capability. In this review, we aim to present a thorough review covering the latest design and fabrication approaches of electrochemical biosensors to detect various cancer biomarkers. Finally, a summary of future challenges and opportunities for developing such effective electrochemical biosensors are discussed.

2. Biosensors: a trend toward detecting biomarkers

Last decade, biosensors were widely used in biomarkers detection worldwide as the most reliable, fast, and precise analytical methods. To detect cancer biomarkers, biosensors utilize various biorecognition elements such as antibodies, enzymes, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), cancer cells, nucleic acid probes or other specific biomolecules which are immobilized on a transducer surface (Yang et al., 2019). The transducer transforms biological signals obtained from the interaction between target biomarker and biorecognition molecules into measurable signals either electrical or optical. Fig. 1 represents the recently reported biosensing approaches for the detection of cancer biomarkers. Depending on transducer type or according to the nature of the biological response, cancer biosensors can be divided into several types including electrochemical, mass-sensitive, and optical, as illustrated in Fig. 1. Biosensor designs differ according to the types of cancer biomarkers. For example, biorecognition molecules such as complementary nucleic acid probes, specific ligands, and specific antibodies are used to detect nucleic acid, receptors, and secretary protein biomarkers, respectively, as shown in Fig. 1.

3. Electrochemical biosensor for biomarker detection

Biomarkers detection processes are mainly focused on the tracing of proteins on the membrane surface of tumor cells and/or cancer

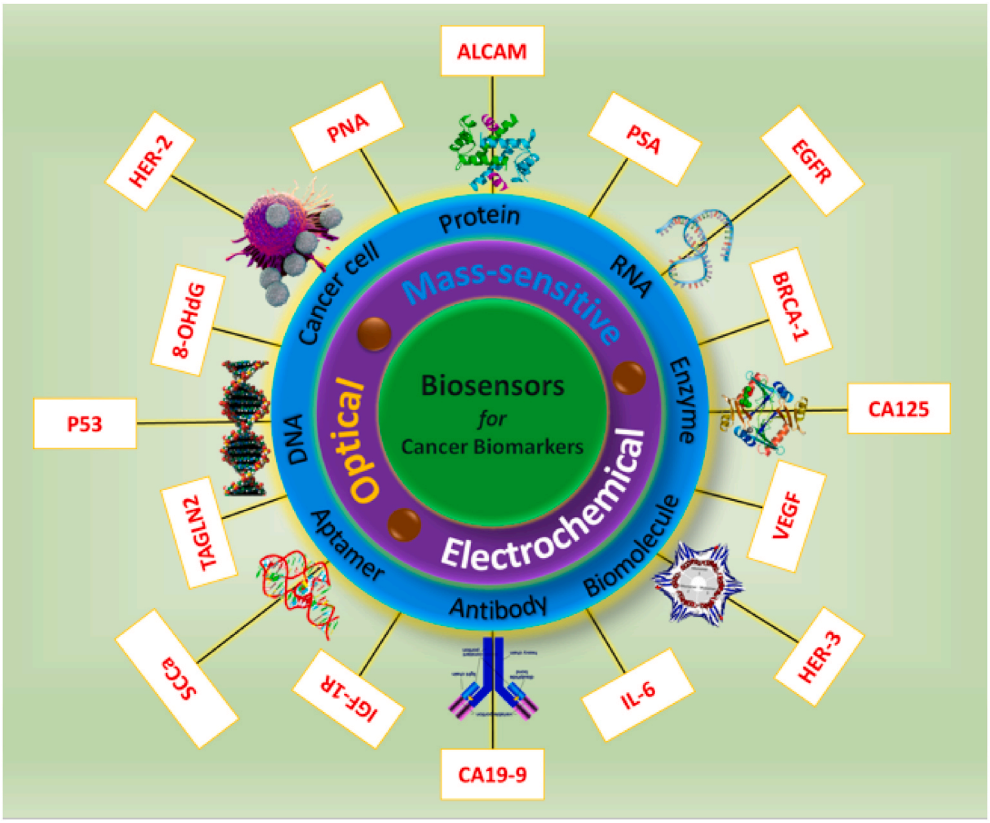

Fig. 1. Schematic representation of the working principle of biosensors for the detection of cancer biomarkers.

associated microRNA. Although several alternative methods were reported for such biomarker diagnosis, an electrochemical method is preferred because of its moderate cost, rapid response, ease of operation, readily quantifiable, possibility of miniaturization as well as high sensitivity and selectivity with lower detection limit (Chang et al., 2019; Zhang et al, 2019, 2020). Electrochemical biosensors are comprising of three components, namely biorecognition element, signal transducer, and three electrodes based electrochemical systems. In such biosensors, change in electrical signal causes electrochemical reactions with target components at the electrode surface that are then monitored and recorded. There is a set of biorecognition elements to detect cancer biomarkers such as antibodies, enzymes, and synthetic molecules (such as - aptamers, DNA fragments, peptides, etc.) (Khanmohammadi et al., 2020) as can be seen in Fig. 1. Depending on the specific biorecognition elements being used, biosensors are classified into immunosensors, aptasensors, enzymatic biosensors, and genobiosensors (nucleic acid biosensors). Here, we will discuss an overview of different cancerous biomarker detection with electrochemical methods.

3.1. Nucleic acid-based biomarker detection

Signature of the normal cells is altered by multiple cancer-causing anomalies, such as inactivation of the anti-tumor gene, chromosomal deterioration, and hypermethylation of gene. These cancer-causing anomalies are all considered as nucleic acid-based cancer biomarkers, such as micro RNAs (miR) and p53 gene mutation. These biomarkers enable cancer diagnosis even if there are no physical symptoms shown by the patients. Wang et al. demonstrated a highly sensitive POC adoptable magnetic-controllable electrochemical based biosensor (Wang et al., 2013). It presents oral cancer biomarker (miR) diagnosis in early stage that showed limit of detection (LOD) down to 0.22 aM (2.2×10^{-19} M) with a recovery rate of 93% and relative standard deviation (RSD) < 6 (n = 5). Additionally, Boriachek et al. reported an efficient biosensor for miR diagnosis from human serum where they immobilized biotinylated complementary probes on commercial streptavidin-coated magnetic beads (MBs) (Boriachek et al., 2018). The level of adsorbed miRNA was detected electrochemically in presence of the $[Fe(CN)_6]^{4-/3-}$ in redox system. The developed biosensor has a significant detection limit as low as 1.0 pmol/L with RSD (less 5.5%) that were satisfactorily observed with differential pulse voltammetry (DPV). The proposed method offers several advantages such as enhanced capture, low manufacturing cost, reduced assay time and matrix effect. Moreover, Luo et al. revealed a locked nucleic acid (LNA) assisted strand displacement reaction-based ratiometric electrochemical biosensor having higher reproducibility to detect exosomal miR-21 derived from cancer with LOD of 2.3 fM (Luo et al., 2020). They used Y-like structure facilitated by LNA that gets activated in the presence of miR-21 as a target biomarker, and detection was confirmed by electrochemical impedance spectroscopy (EIS) and DPV. Sabahi et al. recently fabricated single-wall carbon nanotubes (SWCNTs)-grafted dendritic Au nanostructure modified fluorine-doped tin oxide (FTO) electrode-based biosensor to detect miR-21 (Sabahi et al., 2020). miR-12 was used as a specific biomarker for several cancers in the range of 0.01 fmol L^{-1} - 1 μmol L^{-1} with a detection limit down to 0.01 fmol L^{-1}. Furthermore, the proposed biosensor revealed an acceptable performance in human serum and also good selectivity. The fabricated biosensor could potentially be used in clinical processes as a point of care device for early diagnosis of Pca. Additionally, Hong et al. fabricated an ultrasensitive biosensor that can directly detect miR from human serum without prior treatment (Hong et al., 2013). The developed electrochemical biosensor showed a high sensitivity for target miRNA-21 in a concentrating range from 100 aM to 100 pM with a detection limit of 100 aM. The self-assembled DNA concatemers can carry numerous RuHex that results in the significantly enhanced electrochemical signals. In another work, Topkaya*et* et al. formulated a single-use carbon graphite working electrode-based biosensor to detect specific biomarker (hypermethylation of the glutathione S-transferase P1 (GSTP1) gene) for prostate cancer (Topkaya et al., 2012). The detection of the biomarker was as low as 2.92 pM of target sequence in a 100 μl reaction volume and was confirmed by EIS and DPV. It was a less invasive diagnostic method than biopsy and may be useful as a screening tool for people at high risk for developing prostate cancer. Similarly, Peng et al. developed an immunosensor based on Au nanoparticles/toluidine blue–graphene oxide (Au NPs/TB–GO) modified electrodethat was able to detect multidrug resistance (MDR) gene down to 2.95×10^{-12} Mand was monitored by EIS and DPV (Peng et al., 2015). It was observed that the decreased currents were proportional to the logarithm of the concentration of the target DNA in the range of 1.0×10^{-11} - 1.0×10^{-9} g mL^{-1} with the above detection limit. Additionally, Zeng et al. found that a sandwich-type electrochemical immunosensor coupled with a signal enlargement approach can detect Cytokeratin 19 fragment 21–1 (CYFRA21-1), a biomarker for non-small cell lung cancer (NSCLC) down to 43 pg mL^{-1} (Zeng et al., 2018). The proposed immunosensor was prepared by chitosan (CS), three-dimensional graphene (3D-G), and glutaraldehyde (GA) composite on the GCE containing large surface area, which provides excellent conductivity. It was reported that the developed sensor showed an excellent analytical performance in the range of 0.1–150 ng mL^{-1} with low detection limit. Azmi et al. found that a silicon nanowire (SiNW) based biosensor showed good selectively and efficiently diagnoseprostate cancer using biomarker,- 8-hydroxydeoxyguanosine (8-OHdG) with LOD of1 ng/ml (3.5 nM) (Mohd Azmi et al., 2014). They claimed that the described SiNW biosensor is very fast andcan be considered as a potential candidate for POC application because of its ultra-sensitivity and ease of operation.

3.2. Protein-based biomarker detection

Protein molecular biomarkers are particularly popular due to the availability of a large range of analytical instrumentation, which can identify and quantify proteins in complex biological samples (Michalski et al., 2011). Proteins are key compounds in a biological cell, tissue and different organs. Elshafey and his team formulated a biosensor that can sense cancer biomarker, epidermal growth factor receptor (EGFR) in both phosphate buffer (PBS) and human plasma with detection limits of 0.34 pg/mL and 0.88 pg/mL, respectively (Elshafey et al., 2013). The active surface area of the Au electrode was increased by electrodeposition of AuNPs on which protein G was immobilized to detect EGFR by using the electrochemical impedance spectroscopy (EIS) method which was successful in a dynamic range of 1 pg/mL ¯ 1 μg/mL. Additionally, Ilkhani et al. fabricated an AuNPs modified aptamer (Apt) sandwich biosensing platform by immobilizing a biotinylated EGFR Apt to detect EGFR biomarker. Which evaluating the complexation by DPV method measured from 1 to 40 ng/mL, with a LOD down to 50 pg/mL (Ilkhani et al., 2015). Zhang et al. recently fabricated a nanocomposite based ultrasensitive electrosensitive platform by immobilizing carcinoembryonic antigen (CEA) antibody (anti-CEA) for the detection of CEA (Zhang et al., 2020). The described immunosensor confirmed the detection of CEA in the range of 0.002–50 ng mL^{-1} in human serum with LOD down to 1×10^{-4} ng mL^{-1}. Cyclic voltammetry (CV), linear sweep voltammetry (LSV), and EIS were used to investigate the performance of the biosensor that was proved to be stable, generate reproducible results, and highly selective to diagnosis tools. In another proposition, Luo et al. announced an enzyme-free electrochemical immunosensor based on SWCNTs and peroxidase-like graphene quantum dots (GQDs) (SWCNTs@GQDs) composite platform modified with reduced graphene oxide-AuNPs (rGO - AuNPs) to trace CEA (Luo et al., 2018). Square wave voltammetry (SWV), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to investigate the interface characteristics of modified electrodes. The proposed immunosensor leads to a dual-signal amplification results in a linear range of 50–650 pg/mL with a lower detection limit down to 5.3 pg/mL.

A well-recognized breast cancer-specific biomarker, human

epidermal growth factor receptor 2 (+) BT 474 (HER 2 (+) BT 474) was efficiently detected from the initially captured population using its complementary HER 2 antibody with a detection limit as low as 4.7 × 10^5 exosomes/μL (Xie et al., 2020). Canbaz and his group covalently immobilized anti-HER3 antibody on the modified Au electrode to sense cancer-specific biomarker HER3 (Canbaz et al., 2014). The described sensors characterization was performed by voltammetry and EIS which showed a linear detection in the range of 0.2–1.4 pg/mL. Another group reported an early-stage detection of breast cancer-specific biomarker HER2-ECD in human serum within a detection range of 10–150 ng/ml and a detection limit down to 2.1 ng/mL using a disposable electrochemical biosensor. The authenticity of the described sensor was carried out by screening some human proteins and other cancer biomarker (CA15-3) (Freitas et al., 2020a). Moreover, Yang et al. reported an efficient Au nanoparticles modified Au electrode-based biosensor to trace HER2 in human serum by DPV within the range of 10–150 ng ml^{-1} and the LOD down to 4.9 ng/mL (Yang et al., 2018). Similarly, Pacheco et al. reported a molecularly imprinted polymer-based electrochemical biosensor that can detect breast cancer biomarker HER2-ECD in the range of 10–70 ng/mL as low as 1.6 ng/mL. A screen printed Au electrode was electropolymerized by using cyclic voltammetry with a solution which containing HER2-ECD and phenol for the fabrication of proposed sensor. (Pacheco et al., 2018a). Additionally, Freitas et al. fabricated an immunosensor based on carboxylic acid-functionalized MBs (COOH-MBs) to detect HER2 in human serum within a wide range of 5–100 ng/mL with a LOD down to 2.8 ng/mL by using linear swap voltammetry (LSV). (Freitas et al., 2020b). Moreover, Carvajal et al. demonstrated POC adoptable biosensor comprise of a disposable inject-printed electrochemical platform that can detect breast cancer biomarker, HER2 in faint concentration as minimal as 12 pg/mL within 15 min (Carvajal et al., 2018). Another group reported rapid HER2 biomarker detection using cerium oxide-monoclonal antibody-based immunosensor that showed a detection range of 0.001–20 ng/mL with an acceptable LOD down to 34.9 pg/mL (Hartati et al., 2020).

Lin and his colleagues modified an Au electrode with magnetic graphene oxide (MGO) on which Avastin was immobilized as a biorecognition element to detect cancerous vascular endothelial growth factor (VEGF) (Lin et al., 2015). The proposed biosensor was performed in a wide range of 31–2000 pg/mL in human plasma. Additionally, Prabhulkar et al. introduced an amperometric micro-immunosensor to detect VEGF with LOD down to 38 pg/mL (Prabhulkar et al., 2009). They used a disc-shaped carbon fiber microelectrode platform on which ferrocene monocarboxylic acid labeled anti-VEGF was covalently immobilized to diagnose target biomarkers. It was found that extracellular vehicles (EVs) including exosomes are nanoscale membrane particles that carry molecular information and are considered as biomarkers

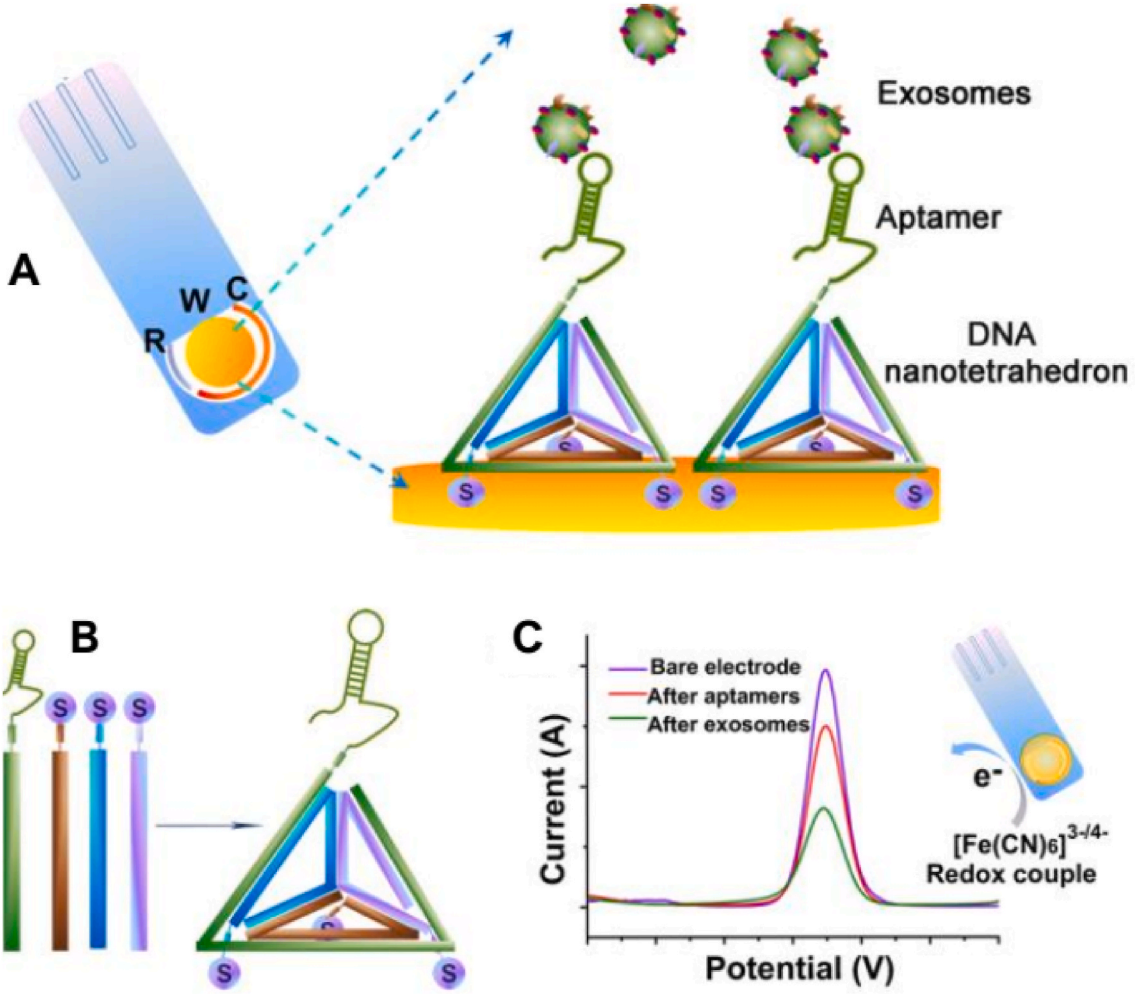

Fig. 2. Illustration of NTH-assisted aptasensor: (A) Aptamer-containing NTHs were immobilized via three thiol groups onto the gold electrodes for direct capture of exosomes in suspension. R: reference electrode area; W: working electrode area, with a diameter of 4 mm; C: counter electrode area. (B) Facile self-assembly of DNA nanotetrahedra from four single-stranded DNA sequences. (C) Redox signal changes after aptamer immobilization and after incubation with exosomes. Adopted from (Wang et al., 2017) with permission; copyright @ 2017 American Chemical Society. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of cancers (Pang et al., 2020). In another work, an Au electrode modified with a rabbit antih-uman CD9 antibody was used to capture exosomes from human serum (Doldán et al., 2016). Afterward, a surface protein of the captured exosomes was diagnosed with sandwich electrochemical immunosensor using biorecognition element - horseradish peroxidase (HRP)-conjugated anti-IgG antibodies. The surface marker-mediated amplification-based sandwich immunosensor showed a sensitivity of detection as low as 200 exosomes/μL. Moreover, Jeong and his group fabricated a portable biosensor (integrated magnetic-electrochemical exosomes (iMEX)) that can detect multiple exosomal proteins having LOD of 3×10^4 exosomes from plasma that is 100 times higher than ELISA (Jeong et al., 2016). Wherein, immunomagnetic method initially captured exosomes from collected samples and then electrochemical detection enabled ultimate detection. In another work, exosomes derived from liver cancer cells were directly captured and detected by using fast and ultrasensitive nanotetrahedron (NTH)-assisted aptasensors as schematically represented in Fig. 2 and that provided satisfying detection limit of 2.09×10^4 exosomes/mL (Wang et al., 2017). In the experimental process, the Au electrode was functionalized with NTH on which specific aptamer, LZH8 was immobilized and able to directly capture target exosome under incubation. Another ultrasensitive aptasensors to detect tumor exosomes and work in the principle of signal-amplified DNA hybridization chain reaction (HCR) was reported (An et al., 2019). A glassy carbon electrode (GCE) functionalized with specific aptamer CD63 was used to capture exosomes. The addition of azide-labeled DNA probe enabled HCR to form a self-assembled DNA linker that helped signal amplification. The described aptasensors can detect exosomes of the tumor in the range of 1.12×10^2–1.12×10^8 particles/μL with the LOD as low as 96 particles/μL under optimum condition. Additionally, it does not interfere with human serum protein, so, it can be adopted for clinical applications. Moreover, Ho et al. reported an electrochemical disposable biosensor to detect lung cancer-specific biomarker, alpha-enolase 1 (ENO1) that was found efficient in a linear range of 10^{-8} – 10^{-12} g mL^{-1} with LOD of 11.9 fg/mL (Ho et al., 2010). Another group revealed a highly sensitive immunochemical aptasensor to detect gastric cancer-derived exosomes (Huang et al., 2019). Initially, exosomes of human plasma were captured using anti-CD63 mediated exosome capture probe among which only gastric cancer exosomes can interact with RCA resulting the G-quadruplex illustrated in Fig. 3. An incubation resulted in forming a heme-G-quadruplex structure that exhibited electrochemical signal confirming detection of gastric cancer biomarker with a detection limit of 9.54×10^2 exosomes/mL and a linear range from 4.8×10^3–4.8×10^6 exosomes/mL.

3.3. Immunoassay based electrochemical sensor for cancerous biomarker

Immunosensor is one of the successful biosensors that involves immunochemical reaction between antigens and antibodies to detect and quantify different concentrations of target analytes. Generally, biological recognition elements (antigens or antibodies) are used as receptors and the immune response of these biological recognition elements is converted into a desired analytical signal by different types of transducer such as electrochemical, magnetic, thermometric, and optical (Derkus et al., 2015). Based on the type of transducer, numerous immunosensors such as electrochemical, microfluidic, electrochemiluminescence (ECL), and surface plasmon resonance (SPR) were developed for the successful detection of cancer biomarkers. Electrochemical immunosensor that is used for the detection of protein biomarkers provides simple, sensitive, rapid response, and cost-effective operation. The outstanding performance of an electrochemical immunosensor depends on the high affinity of immunocomplex (antigen-antibody complex) formation.

Moon et al. fabricated an immunosensor by immobilizing complementary antibody on an Au electrode followed by proper pre-treatment to detect prostate-specific antigen (PSA) (Moon et al., 2014). In this method, conducting polypyrrole film on Au nanowire (NW) enhanced the anti-PSA immobilization capacity and sensitivity of the sensor. The developed immunosensor selectively performed in the linear range of 10 fg/mL - 10 ng/mL with a detection limit of 0.3 fg/mL. In another study, Yang et al. reported QDs modified GO-based sandwich-type electrochemical biosensor to detect PSA in human serum (Yang et al., 2011). Here, graphene sheets (GS) were introduced as carriers for primary anti-PSA antibody (Ab_1) and QD-functionalized GS were used as labels for secondary anti-PSA antibody (Ab_2) which enhanced electrochemical signal of the sensor. The fabricated immunosensor showed a linear detection range of 0.005–10 ng/mL with LOD of 3 pg/mL. Additionally, a GCE based sensor to detect and quantify p53 cancer biomarkers was reported by Heidari *el al.* (Heidari et al., 2019). This sandwich assay utilized GCE/CdS/p53-Ab1 and p53-Ab2-tGO-AuNPs for the detection of p53 cancer biomarkers. The detailed fabrication procedure of the immunosensor is presented in Fig. 4 (A). As represented in Fig. 4 B, fabrication of the sensor using gold nanoparticles and graphene oxide considerably promoted the intensity of the immunosensor. To

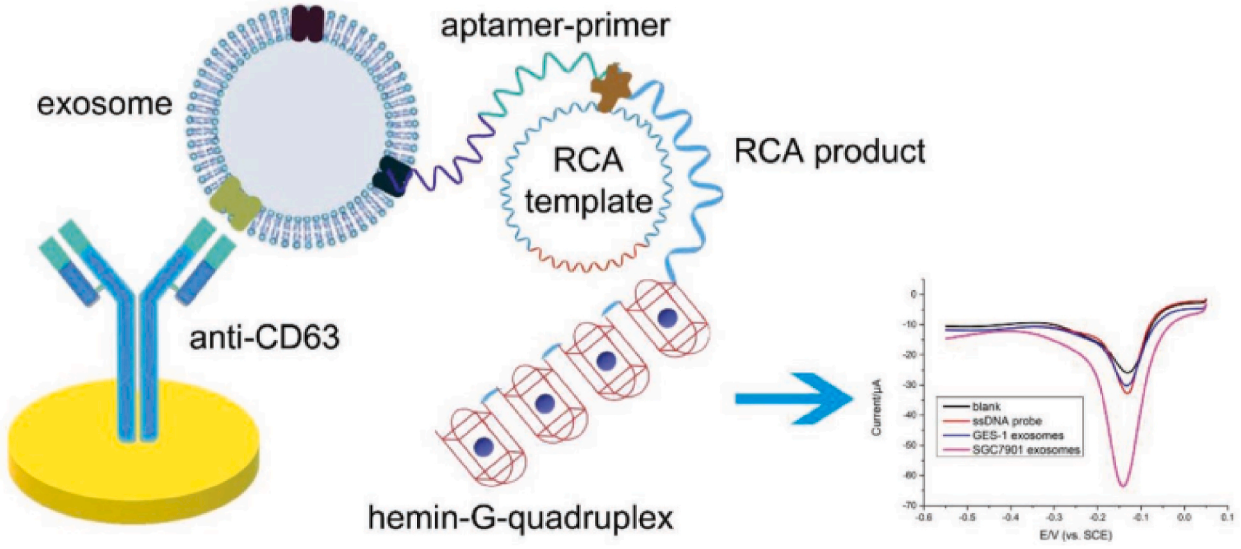

Fig. 3. Illustration of the label-free electrochemical aptasensor for highly sensitive detection of exosomes. Adopted from (Huang et al., 2019) with permission; copyright @ Wiley -VCH Verlag GmbH & Co. KGaA, Weinheim.

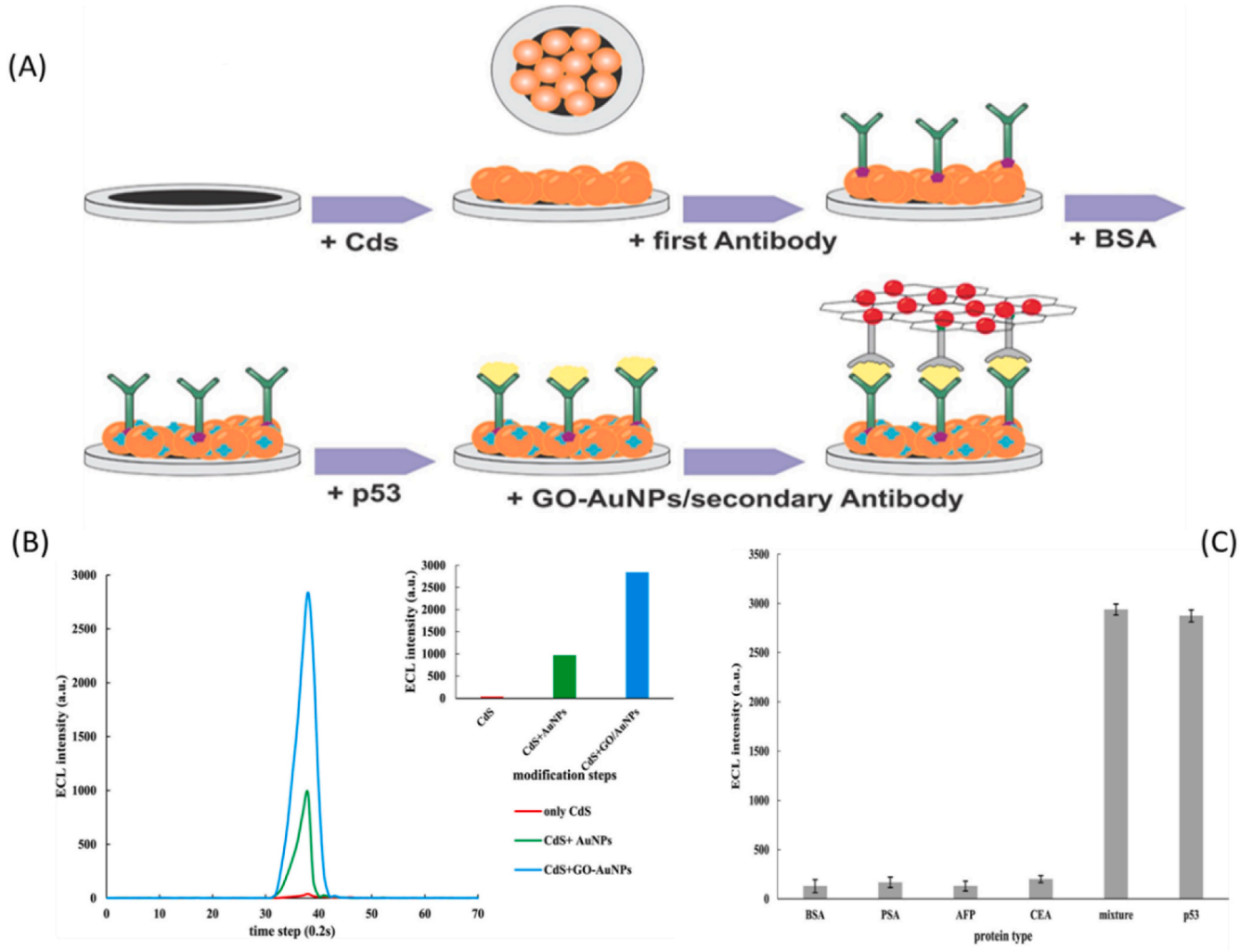

Fig. 4. (A) Schematic representation of the ECL immunosensor preparation steps for p53 cancer biomarker. (B) The ECL immunosensor responses of the modified GCE/CdS/p53-Ab1/p53/p53-Ab2 electrode in the absence of tGO and AuNPs (red), in the presence of only AuNPs (green) and in the presence of tGO-AuNPs (blue) in 0.1 M PBS (pH = 7.5) containing 20 mM of H_2O_2 and with a scan rate of 0.1 V/s. (C) The interference investigation in the mixture of BSA, PSA, CEA, AFP, and p53 proteins using the modified electrode. Adopted from (Heidari et al., 2019) with permission; copyright @ 2018 Elsevier B.V. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ensure the reliability of the sensor, selectivity and specificity tests were also investigated as shown in Fig. 4 C. This sensor showed a linear range of 20–1000 fg/mL with a detection limit of 4 fg/mL for p53.

Lin et al. reported similar biosensors to detect another potential prostate cancer biomarker, alpha-methyl-acyl-CoA racemase (AMACR) from a human blood sample (Lin et al., 2012). It was reported that a disposable electrochemical sensor was fabricated with a screen-printed carbon electrode (SPCE) that was modified with carboxylic acid itched GO (GO–COOH–SPCE) (Rauf et al., 2018). The reported sensor had the ability to detect cancerous biomarker Mucin1 (MUC1) from human serum with a detection limit as low as 0.04 U/mL. Bravo et al. fabricated an AgNPs covered with polyvinyl alcohol (AgNPs- PVA) based immunosensor to detect epithelial cell adhesion molecule (EpCAM) that is known as an epithelial cancer biomarker (Bravo et al., 2017). The authors presented that the proposed AgNPs-PVA based immunosensor can be employed in medical application with a detection limit down to 0.8 pg/L. The described AgNPs-PVA based immunosensor produced a detection limit down to 0.8 pg/L. In another work, a novel strategy for the detection of cancer stem cells biomarker (CSCs), CD44 specific for breast cancer was proposed and reported being highly sensitive to the electrochemical detection of CD44 protein and CD44-positive CSC with a detection limit of 2.17 pg/mL and 8 cells/mL, respectively (Zhao et al., 2018). Sex-determining region Y-box 2 (SOX2) is a cancerous biomarker specific for a set of cancers such as small cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, skin cancer, prostate cancer, and breast cancer. SOX2 can be detected using disposable ITO-based electrochemical immunosensor with a detection limit down to 7 fg/mL that is linearly performed in the range of 25 fg/mL - 2 pg/mL (Aydın and Sezgintürk, 2017). Epithelial ovarian cancer has an especial biomarker, human epididymis protein 4 (HE4) that can also be traced in human serum down to 2.8 pM (Mattarozzi et al., 2020). Pacheco et al. demonstrated an Au-SPE based biosensor to diagnose carbohydrate antigen 15–3 (CA 15–3) in human serum which can be detected with a concentration as low as 1.5 U/mL (Pacheco et al., 2018b). This sensor utilized a molecularly imprinted polymer (MIP) around the target analyte and detected CA 15-3 by occupying cavities on the polymeric film with a detection range between 5 and 50 U/mL. Aydin et al. recently reported another immunosensor based on a label-free epoxy-substituted-polypyrrole P modified ITO platform that can detect neuron-specific enolase antigen (NSE) with a linear range of 0.02–7.5 pg/mL and a low detection limit of 6.1 fg/mL (Aydın et al., 2020). Kim et al. introduced an efficient electrochemical immunosensor to detect apolipoprotein-A1 (Apo-A1) proteins (Kim et al., 2019). The described POC adoptable sensor was fabricated using an enzyme-linked

immunosorbent assay on an ITO electrode that performed with a detection limit of as low as 1 pM in both PBS and urine. Rajaji et al. reported an iron nitride NPs (Fe_2N NPs) decorated reduced graphene oxide sheets (rGOS) nanocomposite (Fe_2N NPs@rGOS) based biosensor to detect cancerous biomarker - 4-nitroquinoline N-oxide (4-NQO) with a LOD as low as 9.24 nM along with a wide linear range of 0.05–574.2 μM (Rajaji et al., 2019). Here, introducing of nanocomposite making the sensor more conductive to a LOD as low as 9.24 nM along with a wide linear range of 0.05–574.2 μM. Additionally, Prasad et al. recently fabricated a low cost disposable paper-based electrode modified with GO-based electrochemical biosensor to detect pancreatic cancer-specific biomarkers pseudopodium-enriched atypical kinase 1, SGK269 (PEAK1) (Prasad et al., 2020). The described immunosensor efficiently performed with a wide linear range of 10 pg/mL - 10^6 pg mL^{-1} with a LOD of 10 pg/mL. Martin et al. lately reported an MBs-based microfluidic electrochemical biosensor that was capable of detecting tumoral hypoxia biomarker-hypoxia-inducible factor-1 alpha (HIF-1α) down to 76 pg/mL (Muñoz-San Martín et al., 2020). In another report, Mathew and his colleagues formulated an electrochemical immunosensor based on the principle of redox reaction on nano-finger electrodes (Mathew et al., 2020). It comprised of sandwich immunoassay and enzyme-linked assays, efficiently diagnosed prostate tumor-derived extracellular vesicles (tdEVs) with detection limit of 5 tdEVs/mL. Similarly, Munge and his colleagues reported SWCNT based sandwich immunosensor to detect cancerous biomarker - protein matrix metalloproteinase-3 (MMP-3), where they introduced two different labels for antibody conjugation, namely HRP (14–16) and polymer bead loaded multi-enzyme for amplification and their performance were compared in terms of detection limit of 0.4 ng/mL (7.7 pM) and 4 pg/mL (77 fM), respectively (Munge et al., 2010). Electrochemical biosensors and their performance for cancer biomarker detection are shortlisted in Table 1.

3.4. Biosensors for diagnosis multiple biomarkers

Since there are no single biomarkers for specific cancer, research interest intensified to fabricate a multi-biomarker detection device and was expressed in few literature surveys. Chen et al. fabricated a biofunctional carboxyl graphene nanosheets (CGS) based sandwich electrochemical biosensing platform by immobilizing anti-CEA and anti-AFP sequentially to detect multiple biomarker CEA and alpha-fetoprotein (AFP) that performed with LOD of 0.1 ng/mL for CEA and 0.05 ng/mL for AFP, respectively and the linear detection range of 0.5–60 ng/mL for both biomarkers (Chen et al., 2013). Atlintas and his group demonstrated a biosensor that can trace CEA and EGFR with a wide linear range of 20–1000 pg/mL while detecting CA15-3 in the range of 10–200 U/mL. The signal enhancement of interdigitated electrode (IDE) was increased by AuNPs layer deposition on which antibody were immobilized to detect CEA and EGFR biomarker (Altintas et al., 2014). Chikkaveeraiah et al. announced an immunosensor that can detect a couple of prostate cancer-specific biomarkers, namely PSA and IL-6 with LOD of 0.23 pg/mL and 0.30 pg/mL,respectively (Chikkaveeraiah et al., 2011). Wan et al. found similar biosensor that can detect another pair of prostate cancer-specific biomarkers, PSA and IL-8 with detection limit down to 5 pg/mL and 8 pg/mL, respectively. In this described work a screen-printed carbon electrode (SPCE) was modified by multiwall carbon nanotube (MWNT) on which anti-rabbit LgG and HRP antibody were immobilized to detect PSA and IL-8 biomarkers. (Wan et al., 2011). Hong and his co-authors reported an Au decorated ITO electrode modified with a temperature-sensitive polymer-based reusable sensor that can detect three different biomarkers, CA125, CEA and PSF with detection limit of 0.007 U/mL, 0.7 pg/mL, and 0.9 pg/mL, respectively (Hong et al., 2016b). In another work, they demonstrated a nano-roughened biotin-doped polypyrrole immunosensor to detect CA125, CEA, and PSF tumor biomarkers with a LOD of 0.005 U/mL, 0.8 pg/mL, and 0.7 pg/mL, respectively (Hong et al., 2016a). Wang et al. fabricated an efficient paper-based electrochemical aptasensor that can

Table 1
List of reported electrochemical biosensors with their performance to detect cancerous biomarkers.

Electrode	Target biomarker	Detection limit	Linear range	Reference
anti-CEA/PEDOT/Ag@BSA/rGO/CNTs-COOH/Au	CEA	1.0×10^{-4} ng/mL	0.002–50 ng/mL	Zeng et al. (2018)
FTO/SWCNTs/den-Au/prob	miR-21	0.01 fM L^{-1}	0.01 fM L^{-1} - 1 μM L^{-1}	Sabahi et al. (2020)
Au NPs/TB–GO/prob	MDR1	2.95×10^{-12} M	0.01–1.0 nM	Peng et al. (2015)
SOI/SiNW/$PhNO_2$/Ab	8-OHdG	1 ng/mL	–	Mohd Azmi et al. (2014)
Ab/PG/PDITC/Cys/AuNPs/Au	EGFR	0.34 pg/mL	1 pg/mL – 1 μgmL^{-1}	Elshafey et al. (2013)
Apt-EGFR-Ab/MB	EGFR	50 pg/mL	1–40 ng/mL	Ilkhani et al. (2015)
Ab1/rGO-AuNPs/GCE	CEA	5.3 pg/mL	50–650 pg/mL	Luo et al. (2018)
ITO/Au NWs/Ab	PSA	0.3 fg/mL	10 fg/mL - 10 ng/mL	Moon et al. (2014)
p53-Ab2-tGO-AuNPs	p53	4 fg/ml	20 - 1000 fg/ml	Heidari et al. (2019)
ITO-PET/EDC-NHS/Ab	SOX2	7 fg/mL	25 fg/mL – 2 pg/mL	Aydın and Sezgintürk (2017)
Fe_2N NPs@rGOS/prob	4-NQO	9.24 nM	0.05–574.2 μM	Rajaji et al. (2019)
GCE/AuNPs/Ab1/Ag/PBG-AuNPs	CEA	0.2 ng/mL	1.0–150 ng/mL	Shan and Ma (2016)
Ab2 GCE/AuNPs/Ab1/Ag/PPP-AuNPs-Ab2	NSE	0.9 ng/mL	1.0–150 ng/mL	
GCE/AuNPs/Ab1/Ag/PTB-AuNPs-Ab2 GCE/AuNPs/Ab1/Ag/PCP-AuNPs-	CA125	0.4 ng/mL	1.0–150 U/mL	
Ab2	CYFRA21-1	0.9 U/mL	1.0–150 ng/mL	
GCE/AuNP/Ab1/Ag/CdNC–AuNP-Ab2		30 pg/mL	0.1–100 ng/mL	
GCE/G2Fc/Ab	IgG	2.0 ng/mL	5.0–50 ng/mL	Khanmohammadi et al. (2020)
SPCE/PEG/anti-ENO1	ENO1	11.9 fg/mL	10^{-8} – 10^{-12} g mL^{-1}	Ho et al. (2010)
GCE/r-GO/AuNPs/PMO-AuPd/Ab	CEA	8.1 pg/mL	0.01–100 ng/mL	(L. Wang et al., 2015)
GCE/r-GO/AuNPs/PPO-AuPd/Ab	CA199	0.0076 U/mL	0.01–100 U/mL	
GCE/r-GO/AuNPs/PPP-AuPd/Ab	CA724	0.0069 U/mL	0.01–100 U/mL	
GCE/r-GO/AuNPs/PTMB-AuPd/Ab	AFP	6.3 pg/mL	0.01–100 ng/mL	
ssDNA modified prob	CYFRA21-1	1.0×10^{-14} M	10 fM – 100 nM	Chen et al. (2018)
ssDNA λ-exo modified prob	EGFR exon 21	120 nM	0.1 μM–3 μM	Shoja et al. (2018)
Primer probes	MEG3	0.25 fM	1 fM - 100 pM	Li et al. (2018)
Dye labeled DNA probe	CA15-3	0.0039 U/mL	0.01–1 U/mL	Zhao et al. (2020)
Single-strand 19-mer oligonucleotides modified prob	BRCA1	1.72 fM	50.0 fM - 1.0 nM	(W. Wang et al., 2015)
ERBB2c modified prod	HER2	0.16 nM	0.37–10 nM	Saeed et al. (2017)
CD24c DNA modified prob		0.23 nM		
Self-assembled ferrocenecored poly (amidoamine) dendrimers	BRCA1	0.38 nM	1.3–20 nM	Senel et al. (2019)
Probe ssDNA (26-mer) modified with eSH	TP53	0.01 fM	1 fM – 100 nM	Shin et al. (2013)

simultaneously detect cancerous biomarkers, CEA and NSE while having a linear response of 0.01–500 ng/mL and 0.05–500 ng/mL along with detection limit down to 2 pg/mL and 10 pg/mL,respectively (Wang et al., 2019). Additionally Tang et al. fabricated a biosensor that was capable of detecting four different lung cancer biomarkers, namely AFP, CEA, CA125, and CA15-3 with a limit of detection <0.5 μg/L from blood or urine of affected patients (Tang et al., 2007). Moreover, Wilson and Nil demonstrated another effective biosensor that can detect seven cancerous biomarkers: CEA, human chorionic gonadotropin (hCG), CA15-3, AFP, Ferritin, CA19-9, and CA125 that are associated biomarkers for different types of cancers (Wilson and Nie, 2006).

3.5. DNA based electrochemical sensing

Due to the growth of tumoral phenotypes with an uncontrolled and unregulated manner, DNA based electrochemical biosensors are one of the most efficient approaches for early detection of cancer biomarkers. DNA based biosensors obtain the sequential information of target-DNA based on the hybridization of ssDNA and the immobilization of complementary DNA/RNA molecules (probe) on the modified electrode surface for further producing an electrical signal of that specific analyte to be analyzed. Depending on the properties of the transducer, different types of immobilization techniques are introduced to get fast accurate detection stability. Electrode surface modification with different nanoparticles influence the immobilization and hybridization process. DNA biosensors are capable of fast detection of cancer biomarkers generated from the cancerous cell due to the recognition of low concentrated oligonucleotides, single-base mismatch, and easy structural assembly (Abu-Salah et al., 2015). Many research works on this topic were undertaken in the last few years and many methods were developed to make it better and faster and that played an important role in identifying different types of cancer. In this part, various designed DNA based biosensors for selective detection of different cancer biomarkers are discussed on a functional basis.

CYFRA 21–1 is considered as an effective biomarker for NSCLC detection. Blotting, immunocytochemistry and PCR-based methods are frequently applied to detect these biomarkers, but they have some disadvantages such as high cost and low precision. Chen et al. developed a DNA based biosensor for the recognition of CYFRA21-1 where they used functionalized three-dimensional graphene and AgNPs (3D GF/AgNPs), as illustrated in Fig. 5 (A-D) (Chen et al., 2018). 5′–SH–GAAGGGAGGAATGGTGTCAGGGGCG-3′ was used as a probe DNA to trace its complementary target DNA 5′-CGCCCCTGACACCATTCCTCC-CTTC-3′. The extracted genomic DNA of a clinical sample obtained from LC tissue electrophoresed after PCR amplification is shown in Fig. 5 (B, C). λ-Exo digestion confirms the target DNA generation without any binding. As shown in Fig. 5D, CYFRA21-1 biomarker develops higher signal and peak current in the presence of MB indicator with a linear relation in different concentrations (of normal tissue) from 1.0×10^{-14} to 1.0×10^{-7} M to confirm the detection of LC. Focusing on the EGFR mutations as a biomarker for NSCLC, Shoja et al. reported a cancer biosensor based on rGO/-functionalized ordered mesoporous carbon (rGO/f-OMC) coupled electropolymerization of Ni(II)-oxytetracycline conducting metallopolymer nanoparticles (Ni-OTC NPs) on PGE surface to identify the point mutation L858R, of EGFR exon2 (Shoja et al., 2018). Use of Ni-OTC NPs as electroactive media in sensing platform in immobilization of ssDNA capture probe exhibited high sensitivity (0.0188 mA/μM) with LOD of

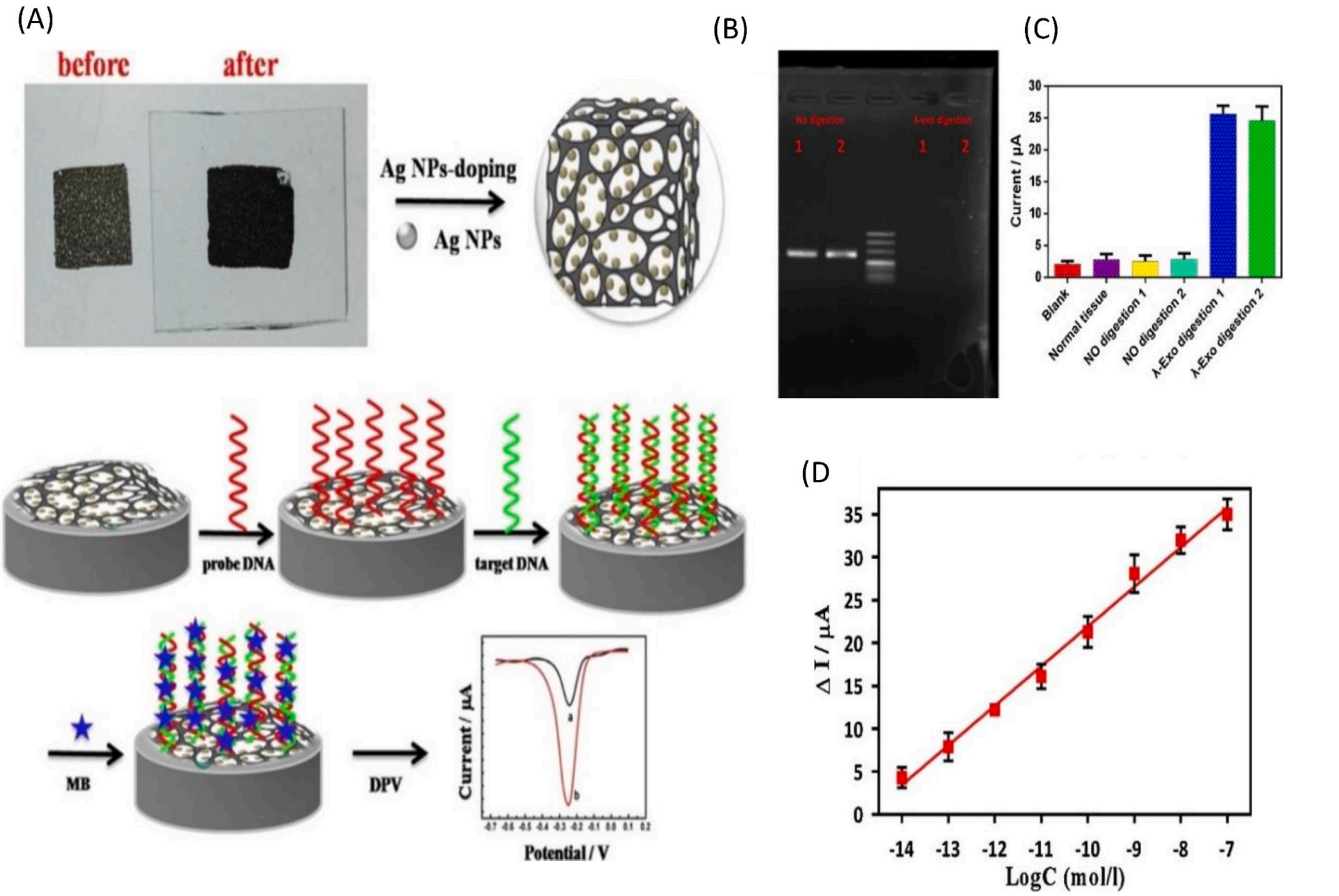

Fig. 5. (A) Schematic representation of the preparation of 3D/AgNPs & detection process of target DNA. (B) PCR product via gel electrophoresis. (C) Amperometric response compared to λ-exo digestion to capture PCR product. (D) Peak current vs. log of the concentration of target ssDNA by linear regression equation: ΔI (μA) = 68.15 + 4.623 log C (M). Adopted from (Chen et al., 2018) with permission; copyright @ 2018 Elsevier B.V.

120 nm and long stability up to 21days. Li et al. fabricated a genosensor to detect multiple specific sequences of maternally expressed gene3 (MEG3) responsible for LC cells (Li et al., 2018). Use of cascade signal amplification strategy and Fe_3O_4@C nanospheres with dendritic gold nanostructures/tungsten disulfide (DGN/WS_2) film improved the bio-recognition ability of target specific DNA strands and ferrocene (Fc) along with methylene blue (MB) help to read out the signal of individual sequence. This biosensor shows a high potential for detection of targets in serum samples.

Several DNA based biosensors were also found to detect breast cancer biomarkers. Recently, Zhaoa et al. reported a DNA based apta-sensors for the detection of breast cancer biomarker, carbohydrate antigen 15–3 (CA15-3) (Zhao et al., 2020). The DNA labeled with MoS2 nanosheets (NSs) was used as a detection probe for the measurement of CA15-3. The use of MoS2 NSs on fluorescence sensing platform showed excellent quenching competency and variant adsorption affinity for aptamer and aptamer - CA15-3 protein. The fabricated biosensor showed a good performance with an improved detection limit of 0.0039 U/mL. In another report, AuNPs based DNA hybridization biosensor is developed for BRCA1 gene detection in patient samples (W. Wang et al., 2015). The antifouling property of polyethylene glycol/AuNPs (PEG/AuNPs) composite successfully exploited the GCE surface for the immobilization of DNA probe and the EIS techniques assisted in higher detection performance such as facile fabrication, high sensitivity, and selectivity. The linear detection range of the biosensor is 50.0 fM to 1.0 nM with a detection limit of 1.72 fM. Cui *et at.* have also developed a label-free electrochemical biosensor for BRCA1 using pretreated bare gold electrode surface as the sensing platform on which self-assembled monolayer (SAM) of zwitterionic peptide immobilized (Cui et al., 2017). In this biosensor, the carboxyl functionalized capture probe of sequence-specific nucleic acid attached with amino groups of peptide sequence through covalent binding. The remaining part of peptides with-out immobilized probe utilized for antifouling capacity for the nonspecific adsorption onto sensing surface. The detection limit of this biosensor is found to be 0.3 fM with favorable reproducibility. Recently, Senel et al. mentioned a self-assembled ferrocene-cored poly (amido-amine) derived DNA biosensor for the detection of BRAC1 gene associated with breast cancer (Senel et al., 2019). Here, the authors introduced a ferrocene redox marker to increase the electron-transfer towards the electrode surface which enabled the biosensor sensitive enough to detect BRAC1 biomarker with a LOD of 0.13 μA/9 (ng/mL). In another study, Saeed et al. used AuNPs and GO modified DNA based biosensors for detecting HER2, which is a recognized marker for breast cancer (Saeed et al., 2017). They employed immobilization of ERBB2c and CD24c DNA on the modified electrode using a sandwich hybridization process to detect ERBB2 and CD24. High sensitivity of the sensor was achieved by utilizing the AuNPs-GO nanocomposite fabrication and chro-noamperometry study was performed that confirmed LOD for ERBB2 and CD24 detection as low as 0.16 nM and 0.23 nM, respectively. Additionally, Topkaya et al. demonstrated a DNA based electrochemical biosensor for the detection of PSA as hypermethylation of the gluta-thione S-transferase P1 (GSTP1) gene (Topkaya et al., 2012). In this research, sequences of methylation-specific and unmethylated GSTP1 were directly immobilized on the electrode surface and DNA hybridi-zation was characterized by the guanine oxidation under the examina-tion of electrochemical methods. The detection limit of this biosensor was found 2.92 pM in a reaction volume of 100 μL. Fayazfar et al. developed a DNA impedance biosensor for tumor protein 53 (TP53) gene mutation detection, which is an important marker in cancer diagnosis. Fabrication process allows the AuNPs growth on MWCNTs and captured the complementary DNA sequence over probe DNA with an excellent response following a linear range of 1.0×10^{-15} - 1.0×10^{-7} M and a detection limit of 1.0×10^{-17} M (Fayazfar et al., 2014). The outcome of different reported biosensors and their analytical per-formance are summarized in Table 1.

4. Conclusion and future outlook

To ultimately improve the prognosis and treatment strategies of cancers, there is a high demand for efficient biosensors for rapid analysis of cellular alterations to detect related biomarkers. This review discusses different types of recently reported electrochemical biosensors and their working principles with designs to detect cancer biomarkers. However, new approaches such as nanofabrication and clinical applications are needed for the development of lab-on-chips and next-generation novel biosensors at low cost. Interestingly, electrochemical biosensors are the mostly reported sensing method for the detection of cancer biomarkers. Nanostructured materials and nanocomposites are playing an essential role in the fabrication and designing of various electrochemical bio-sensors and are expected to be used more in future research. Further studies are required in the fabrication of electrochemical transducers to enhance the stability and reproducibility of the sensing device. Commercialization of biomarker biosensors is still in their infancy because of their challenges in miniaturizing of the device and the inte-gration of microfluidics technologies toward the development of the clinical rationale.

Declaration of competing interest

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

Acknowledgment

No financial support was received from any source.

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