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Mini review

Electrochemical Biosensors for Detection of Tumor Cells: A Review

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The spontaneous circulation of tumor cells is a sign of "invasive behavior" of some cancer cells. The acquisition of strong invasive tumor cells and their subsequent metastasis lead to the death of 90% of cancer patients. Electrochemical biosensors have been explored to detect tumor cells with high sensitivity. In this review, we summarized the progress in electrochemical biosensors for the detection of tumor cells, including direct detection, sandwich-type detection, magnetoelectric detection.

Keywords: tumor cells; electrochemical biosensors; impedance; magnetoelectric biosensors

1. INTRODUCTION

Cancer is one of the most deadly diseases in the world. The early diagnosis of cancers has aroused widespread concern. Identification of tumor cells in the early state is of importance to the diagnosis and treatment of cancers [1]. For example, circulating tumor cell (CTC) spreading from tumor to blood is related to a metastatic disease. Recently, CTC has been considered as the biomarker for attractive prognosis and diagnosis of cancer patient [2]. However, CTC is rare in whole blood. There are billions of healthy blood cells in a sample that may contain only a small amount of CTC [3]. In recent decades, many novel techniques have been developed to detect cell viability and proliferation, including immunohistochemistry, immunofluorescence, flow cytometry, reverse transcription-polymerase chain reaction (RT-PCR), and immunization-magnetic separation [4]. The electrochemical sensing technique is one of the most sensitive biosensors due to its remarkable advantages. All kinds of biometric elements can be fixed at the electrode for precise interactions between biometric elements and targets. The electrochemical signal is proportional to the target concentration. Electrochemical biosensors can be categorized as voltammetry/amperometry, potentiometry, impedimetry and conductometry, which are dependent upon the parameters [5-7]. In

this review, we reviewed several aspects of electrochemical methods for the determination of tumor cells, classified as direct detection, sandwich-type detection, and magnetoelectric detection.

2. DIRECT DETECTION

Cells captured by sensor electrodes can prevent the electron transfer of electroactive substances in solution. Herein, the methods of direct detection by limiting the electron transfer are classified according to the types of receptors modified on electrode surface, such as antibody, aptamer, folic acid, lectin and so on.

2.1 Antibody as the receptor

In cell identification, especially in tumor cells, a large number of membrane proteins are emphasized. They include epidermal growth factor receptor (EGFR) of lung cancer, MUC1 for breast cancer, glypican-3 (GPC3) for liver cancer and so on [8-10]. The anti-EpCAM-labeled magnetic nanospheres have been used for the capture of rare tumor cells in whole blood.[3] Arya et al. reported the direct detection of MCF-7 cells by CV technique using anti-EpCAM/LC-SPDP/Au electrodes [11]. The method showed a linear range of $1 \times 10^5 \sim 1 \times 10^8$ cells/mL with a detection limit of 1×10^5 cells mL^{-1}. Based on the special recognition of anti-EpCAM to ovarian cancer cells (SKOV3), Venkatanarayanan et al. has reported the impedance detection of SKOV3 using platinum microelectrodes modified with anti-EpCAM (Figure 1) [12]. The detection limit of 4 cells was achieved and the linear dynamic range is 4 ~ 650 cells/mL, which is approximately equivalent to fractional coverages from 0.1% to 29%. Nwankire and co-workers, for the first time, reported the label-free detection of $SKOV_3$ in whole blood. The method based on the eLoaD platform is highly sensitive with fully integrated liquid handling [13]. It can detect five different samples simultaneously in the linear range of 1.6×10^4 and 2.67×10^6 cells/mL. By the specific interaction between cell surface melanocortin 1 receptor (MC1R) antigen and anti-MC1R antibody (MC1R-Ab), Seenivasan et al. reported an electrochemical immunosensor for the detection of melanoma cells by using melanoma-specific MC1R as the target marker [14]. MC1R-Abs were immobilized on screen-printed electrodes (SPEs) modified with amino-functionalized silica nanoparticles (n-SiNPs)-polypyrrole (PPy) nanocomposite thin film. Damiati et al. reported the detection of human hepatocarcinoma cell (HepG2) by using anti-CD133 antibody/rSbpA/ZZ lattice/Au electrodes to recognize and capture the highly expressed tumor marker [15]. Zhang et al. reported the direct detection of A549 cells using 3D carbon nanospheres and gold nanoparticles (AuNPs)-modified electrode [16]. The method used for early diagnosis of lung cancers exhibits a linear range 4.2 to 4.2×10^6 cells/mL with a detection limit of 14 cells/mL.

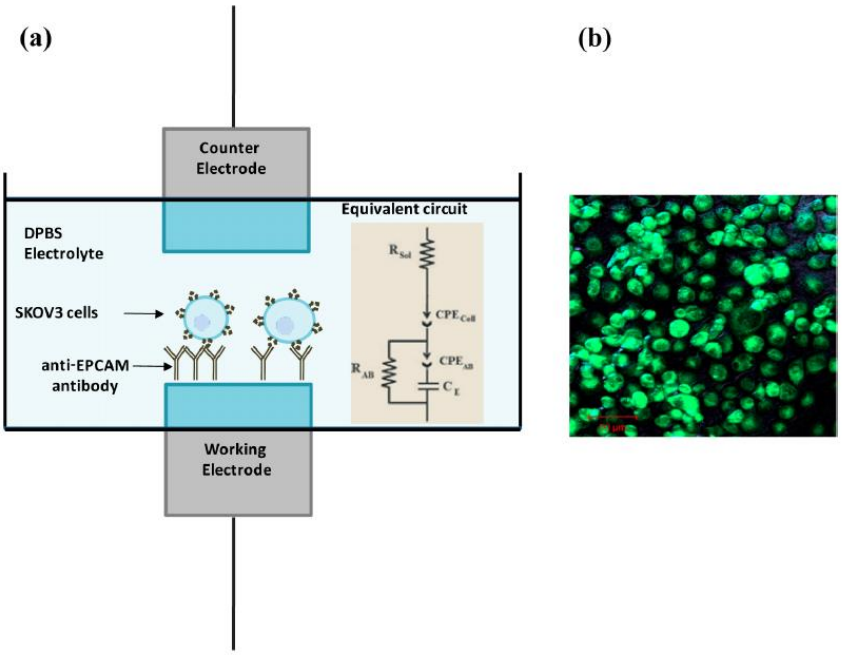

Figure 1. Schematic of (a) the electrochemical cell used for the impedance measurements with the equivalent circuit model on the right, and (b) confocal fluorescence image of the captured SKOV3 cells on the platinum electrode. Reprinted with permission from reference [12]. Copyright 2013 American Chemical Society.

2.2 Aptamer as the receptor

Aptamers are nucleic acids or peptides with high affinity for specific targets. DNA or RNA aptamers usually exhibit specific structures in view of their inherent tendency to form base pairs within complementary nucleotides. They can be folded into different secondary structures, such as stem, ring, horn, false knot, G-quadruple and kissing hairpin [17]. Aptamers are one of the most exciting recognition elements for building biosensing devices because of their characteristics of easy synthesis, high sensitivity and specificity in diagnosis, and high stability in various environments. Electrochemical aptasensor is a type of biosensor using an aptamer-immobilized electrode for selective capture of target analyte. In order to detect CTCs accurately and quickly, kinds of aptasensors have been widely developed. Min et al. reported the simulataneous detection of PSMA (+) and PSMA (-) prostate tumor cells with dual RNA and peptide aptamer probes [18]. Based on the recognition between TLS11a aptamer and HepG2 cells, Sun et al. developed a competitive electrochemical biosensor for the capture, detection and release of HepG2 tumor cells. The detection limit was 5 cells/mL and the detection range was 10 ~ 10^6 cells/mL [19]. According to the type of proteins over-expressed on cell surface, there are several reports for MCF-7 detection by electrochemical methods. Due to the special recognition of MUC1 aptamers for MCF-7 cells, In 2010, Li et al. proposed an electrochemical biosensor for the detection of breast CTCs by determining two tumor biomarkers (human mucin-1 and carcinoembryonic antigen) overexpressed on cell surface [20]. Recently, Liu et al. reported an antifouling interface based on the covalent adsorption of a branched zwitterionic peptide on electrodeposited polyaniline film (Figure 2A) [21]. The antifouling performance of the

branched peptide is better than that of PEG and linear peptide. Such an interface is very effective in decreasing the non-specific adsorption of proteins and cells. MUC1-positive MCF-7 breast tumor cells in human serums have been detected with MUC1 aptamer as the bioreceptor. The linear range is from 50 to 10^6 cells/mL, and the detection limit is as low as 20 cells/mL.

Wang et al. suggested the direct detection of CTCs by plasmon-enhanced electrochemistry (DPEE) (Figure 2B) [22]. The aptamer probes were immobilized on the electrode surface of plasmonic gold nanostars (AuNSs). The results showed that CCRF-CEM cell at the concentration of 5 cells/mL can be readily measured. Qu et al. designed an electrochemical biosensor by simultaneously conjugating two different anti-MEAR cell aptamers, TLS1c and TLS11a, to the surface of a GCE via ssDNA and dsDNA, respectively (Figure 2C) [23]. The biosensor made the recognition of sensor surface to tumor cells more effective. As a result, a single MEAR cell in 10^9 whole blood cells has been readily measured. In constrast to the single-aptamer modification method, the work presented a well-designed and ultra-sensitive identification platform. It brings encouraging possibility for further clinical application.

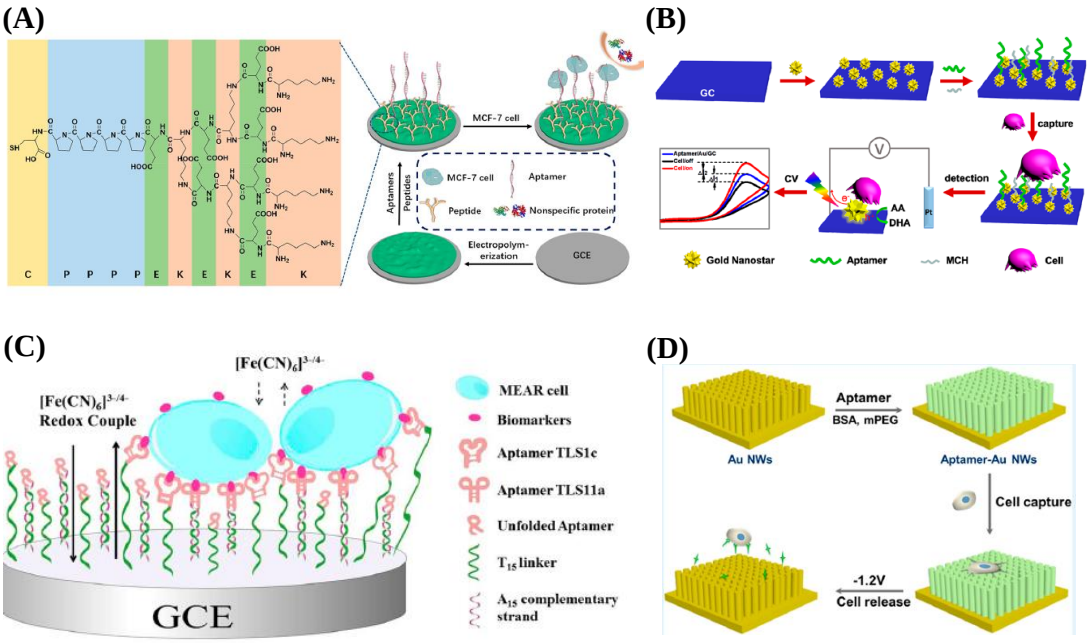

Figure 2. (A) Schematic illustration of the preparation of a PANI-supported branched peptide-based amperometric cell sensor. Reprinted with permission from reference [21]. Copyright 2019 American Chemical Society. (B) Schematic illustration of the strategy for ultrasensitive and label-free detection of CTCs by the DPEE mechanism. Reprinted with permission from reference [22]. Copyright 2019 American Chemical Society. (C) Scheme showing the designed ss-TLS1c/ds-TLS11a dual-modified electrode for specific and sensitive detection of MEAR tumor cells. Reprinted with permission from reference [23]. Copyright 2014 American Chemical Society. (D) Representative process of capturing and releasing CCRF-CEM on aptamer-sgc8c modified AuNWs. Reprinted with permission from reference [24]. Copyright 2017 American Chemical Society.

Furthermore, a few of novel nanostrutures and nanocomposites as the electrode modifiers have been explored to develop aptasensors for the detection of tumor cells, such as metal−organic

frameworks (MOFs), covalent organic, TiO_2 nanotube/reduced graphene oxide and so on [25-29]. For instance, He et al. proposed a bifunctional biosensor by bimetallic NiCo Prussian blue analogue (NiCoPBA) nanocubes for the determination of CEA and H460 tumor cells with a detection limit of 47 cells/mL [29]. Zhou et al. reported a highly sensitive HER2 aptamer-based assay by using MnFePBA@AuNPs as the sensitive scaffold materials.[28] Zhai et al. have demonstrated that the captured CTCs from human leukemic lymphoblasts (CCRF-CEM) by aptamer-modified gold nanowire array (AuNW) can be released through an electrochemical desorption process (Figure 2D) [24]. This work provides valuable information for CTCs isolation and diagnosis as well as therapy of cancers.

2.3 Folic acid as the receptor

Folate plays an important role in protein synthesis, cell division and growth. There are three kinds of folate binding proteins: high affinity folate binding proteins, membrane related binding proteins and cytoplasmic binding proteins [30-33]. Zheng et al. found that CNTs@PDA-FA keep the characteristics of CNTs and show a strong binding with the folate receptor overexpressed on tumor cells [4]. Then, they designed a new nanoprobe for label-free and sensitive detection of HeLa and HL-60 cells with electrochemical impedance technique. This is the first work in which polydopamine-coated carbon nanotubes was used for electrochemical detection of tumor cells. The BSA-stabilized Au and Ag nano-clusters have been widely explored for the applications of fluorescent imaging and cancer therapy. Based on this fact, Hu et al. synthesized FA/Ag@BSA for the detection of KB cells with a detection limit of 20 cells/mL [34]. Folic acid has also been covalently modified onto SWNTs surface to specifically recognize tumor cells by the high affinity of folic acid to its receptor on cellular surface [35]. A label-free cytosensor was proposed with surface-confined ferrocene as the signal indicator to determine HeLa cells without external chemicals effect. The method shows a linear range of $10 \sim 10^6$ cells/mL. The detection limit reached to 10 cells/mL even in the presence of numerous non-cancerous cells. In addition, Du et al. proposed an efficient impedance cytosensor with FA-functionalized zirconium MOFs (UiO-66) [36]. The method exhibited a linear range of $10^2 \sim 10^6$ cells/mL. The detection limit was calculated to be as low as 90 cells/mL. Hu et al.synthesized Ag@BSA composite microspheres as a novel electrochemical biosensing interface for the sensitive detection of KB cells [34]. The Ag@BSA composite microspheres were immobilized on gold electrode surface via Au-S bonds. CV and EIS results indicated that the biosensor can detect KB cells in the range of 60 to 1.2×10^8 cells/mL with a detection limit of 20 cells/mL.

2.4 Lectin as the receptor

Lectin is a kind of glycoprotein or sugar-binding protein from various plants, invertebrates and higher animals. Lectin has been used as the receptor of tumor cell based on its interaction with glycoprotein on cell surface. Zhang et al. reported the electrochemical detection of cancer-associated glycosylation using lectin-Au-Th [37]. The lectin-based biosensors have different detection limits for various cells according to mannose and sialic acid expressed on normal and tumor cells from human lung, liver, and prostate. Cao et al. developed a microfluidic platform by combining the techniques of

EIS and optical microscopy on an ITO electrode array (Figure 3) [38]. This work provided an optical and electronic sensor paltform to evaluate the carbohydrate expression on K562 cells. In the microfluidic channel, four ITO electrodes were modified with three lectin molecules and one passivation agent. In the whole experiment, the sample consumption of each sensing interface is only 5 × 10^3 cells. The proposed method facilitated multiple unmarked and noninvasive analysis on a single microfluidic chip. The method can be further used to evaluate the changes of glycan expression in living cells under the action of drugs. This is of great importance to study the rare cell sample by the electrochemical and optical signals simultaneously. Moreover, Zanghelini et al. have proposed a first tridimensional biosensor platform as the electrochemical point-of-care device by a distinction between highly invasive (T47D) and less invasive (MCF7) tumor cell lines captured by vegetal lectins [39].

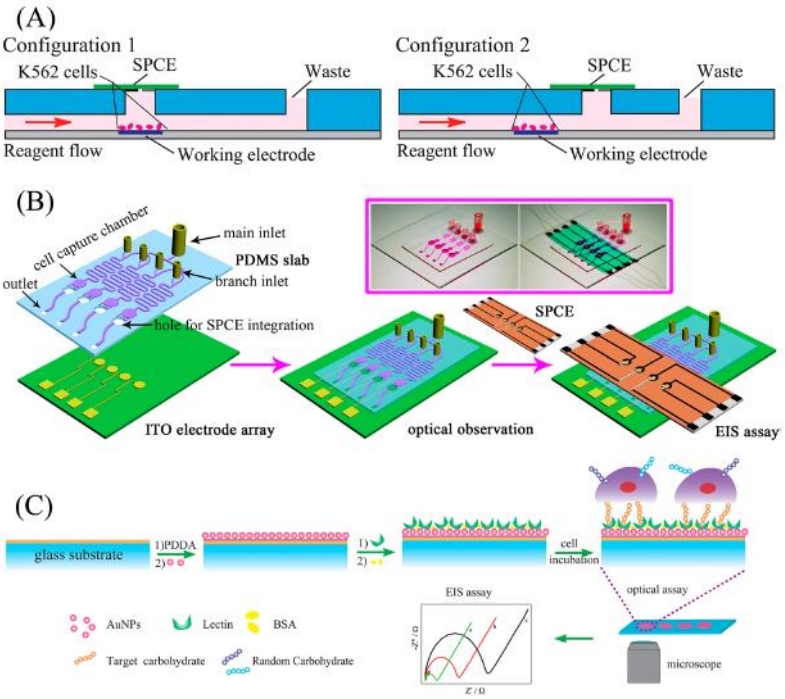

Figure 3. (A) Side view of one channel in the two microfluidic configurations. (B) The construction process of the device for optical−electrochemical monitoring according to configuration 2. (C) Schematic representation of lectin-based array for cell surface glycan evaluation. Reprinted with permission from reference [38]. Copyright 2012 American Chemical Society.

Concanavalin A (ConA) is one of the carbohydrate binding proteins extracted from concanavalin. ConA has high specificity in binding to carbohydrate such as sugar, glycoprotein and glycolipid. Because tumor cells related to glycoprotein and glycolipid glycosylation play an important role in the formation of most tumor cells, high specific affinity of ConA to glycosylation has been used as the receptor to monitor glycoprotein expression in tumor cells. For example, Chowdhury et al. developed an unmarked electrochemical biosensor for rapid detection of tumor cells with ConA-GQD@Fe_3O_4 as the electrode material.[40] The detection limits for HeLa and MCF-7 cells were 246 and 367 cells/mL, respectively, with a linear range of 5 × 10^2 ~ 1×10^5 cells/mL.

2.5 Other receptors

Besides the aforcementioned bioreceptors for cell capture, tetrathiafulvalene derivative, peptide nanoparticles, mannosyl, boronic acid, poly-L–lysine and clay-protein can also be suggested as cell receptors [26, 41-48]. For example, vicinal-dithiol-containing protein (VDP) is over-expressed in tumor cell and is potential biomarker for aggressive tumor; the synthesized 2-p-aminophenyl-1, 3, 2-dithiarsenolane (VTA2) was proved to be a highly selective ligand for VDP. In 2015, Xu et al. reported a sensitive cytosensor with VTA2-conjugated MCNTs (VTA2@MWCNT) array for selective detection of VDP-overexpressed HL-60 cells [47]. Zhang et al. developed a label-free electrochemical sensor for CD44 by ligand-protein interaction (Figure 4) [48]. Carbon nanotube composites were assembled on the electrode surface to improve the conductivity. Hyaluronic acid (HA) was coupled to the surface of CNTs by electrostatic interaction with PDDA. Therefore, they directly detected $CD44^+$ cells by electrochemistry with a detection limit of 5.94pg/ml. This method does not need any labeling for signal amplification.

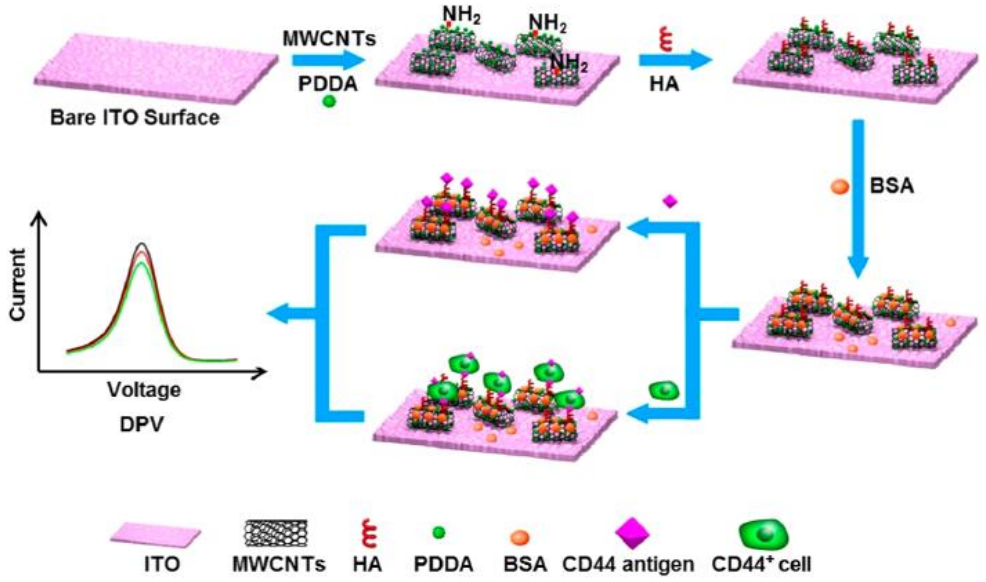

Figure 4. Fabrication process and sensing mechanism. Reprinted with permission from reference [48]. Copyright 2019 American Chemical Society.

3. SANDWICH-TYPE DETECTION

Sandwich-type structure is an important method to amplify the signal of electrochemical biosensors. The signal labels usually include enzymes, nanocatalysts, electroactive materials, quantum dots and so on [49-52]. The progress in these methods have been summarized according to the difference of signal labels as follows.

3.1 Enzymatic amplification

Enzyme-based sandwich-type electrochemical biosensors have attracted intensive attention

because of its highly catalytic amplification property. The commonly used natural enzyme labels for electrochemical biosensors including horseradish peroxidase (HRP), glucose oxidase (GOx) and alkaline phosphatase (ALP) [53, 54]. There are also several reports about the detection of tumor cells by sandwich-type electrochemical biosensors [53, 55-58]. For example, Zheng et al. fabricated the HRP-TRAIL-Fe_3O_4@Au hybrid nanoprobe by the co-immobilization of HRP and TRAIL on the surface of Fe_3O_4@Au nanocomposite by layer-by-layer (LBL) assembly (Figure 5) [58]. Then, the nanoprobe was used to develop a novel electrochemical sensing platform. Selective detection of different types of leukemia cells and quantitative analysis of DR4/DR5 expressed on the cell surface were carried out.

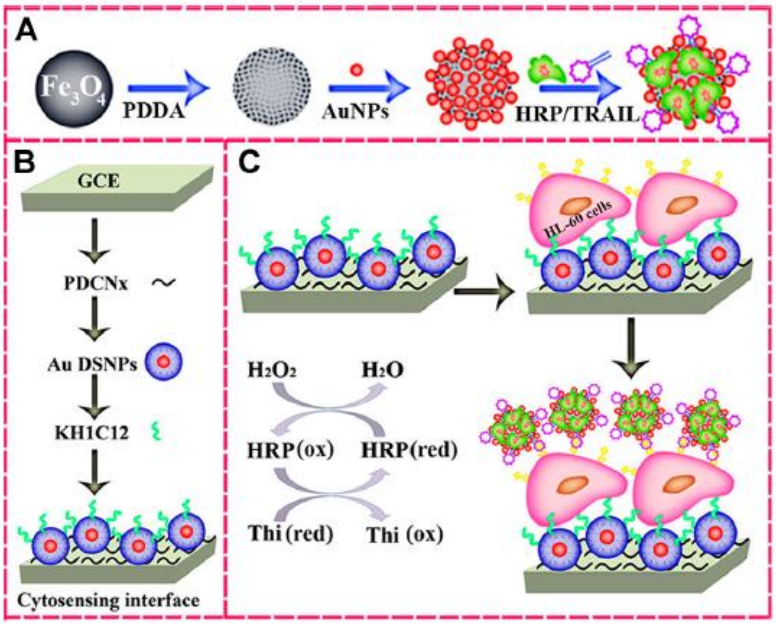

Figure 5. Schemes of (A) fabrication of HRP-TRAIL-Fe_3O_4 @Au hybrid nanoprobe, (B) assembly of the electrode interface, and (C) sandwich-like nanoarchitectured electrode geometry for the cytosens-ing of HL-60 cells. Reprinted with permission from reference [58]. Copyright 2013 American Chemical Society.

With HRP-aptamer-AuNP as the nanoprobe, Chen et al. reported the signal-amplified detection of cell surface N-glycan expression using Con A as the receptor (Figure 6) [55]. The detection limit was found to be 10 cells/mL for CCRF-CEM cells. Sheng et al. have demonstrated an ultrasensitive electrochemical cytosensor based on signal amplification of rolling circle and HPR [56]. The method was successfully applied for the electrochemical sensing of MCF-7 cells ranging from 20 to 5×10^6 cells/mL^{-1} with a detection limit of 12 cells/mL^{-1}. Amouzadeh Tabrizi et al. reported an amperometric cytosensor for the detection of AGS tumor cells [59]. The aptasensor exhibited a good response with a linear range of 10 to 5×10^5 cells/mL and a detection limit of 6 cells/mL. Chen et al. developed a platform by immobilizing a three-dimensional DNA nanostructure on the gold electrode [57]. The DNA nanostructured aptasensor was used for sensitive detection of HepG2 by multibranched hybridization chain reaction (HCR) amplification strategy. The detection limit of the aptasensor is 5

cells/mL with a broad detection range from 10^2 to 10^7 cells/mL.

Chen et al. By immobilizing DNA tetrahedron with three-dimensional DNA nanostructure on gold electrode, a well-designed platform was established to capture HepG2 cells more concretely and effectively [57]. The aptasensor with this DNA nanostructure was applied to the sensitive electrochemical detection of human hepatocarcinoma cells (HepG2) based on the multi branch HCR amplification technique. The detection limit of the established cell sensor is 5 cells / ml, and the detection range is from 102 to 107 cells / ml.

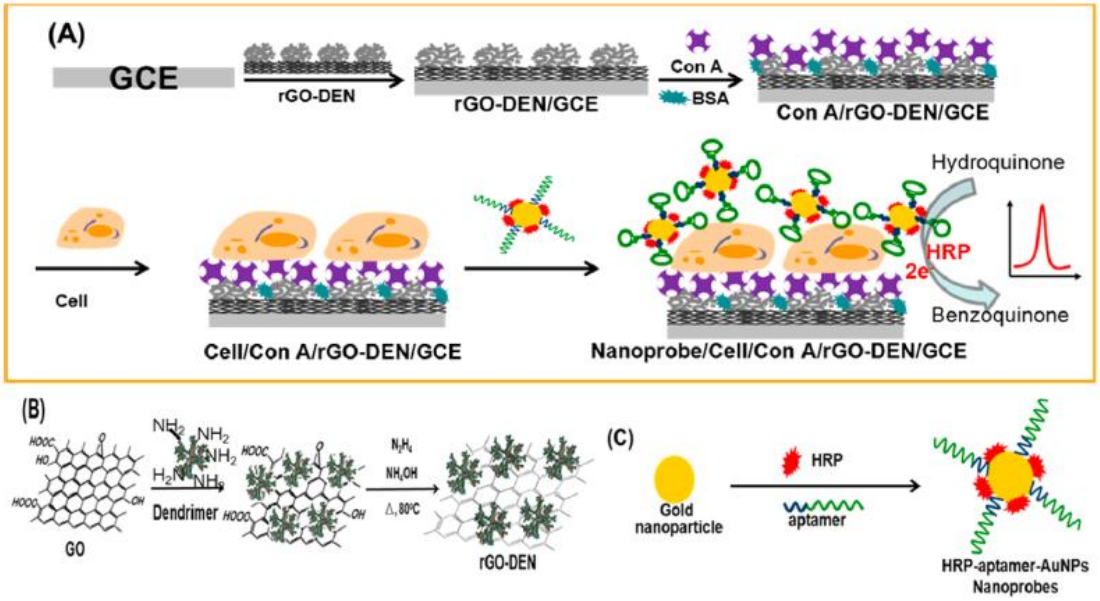

Figure 6. Schematic illustration of the electrochemical aptamer biosensor for dynamic evaluation of cell surface nglycan expression based on multivalent recognition and dual signal amplification. Reprinted with permission from reference [55]. Copyright 2014 American Chemical Society.

3.2 Nanocatalysts

Nanocatalysts with the unique properties of nanomaterials and catalytic function have the characteristics of high catalytic efficiency, stability, economy and large-scale preparation. They have been widely used in medicine, chemical industry, food, agriculture and environment. A few of nanocatalysts have been developed recently for the detection of tumor cells. For example, Zheng et al. discovered a new function of Fe_3O_4 NPs as efficient electrocatalysts for the reduction of small dye molecules (Figure 7) [60]. They suggested that the Fe_3O_4@nanocage core-satellite nanohybrids exhibited more robust electrocatalytic activities than the enzymatic peroxidase/H_2O_2 system. The calibration curves for two types of cells displayed a linear relationship between 50 and 1×10^7 cells/mL with a correlation coefficient of 0.998. The detection limits for MCF-7 and T47D cells were determined to be 34 and 42 cells/mL, respectively. Tang et al. reported a novel ultrasensitive immunsensing protocol for the detection of CTCs by using Pt@Ag nanoflowers (Pt@AgNFs) and AuNPs/acetylene black (AuNPs/AB) nanomaterial [61]. A linear relationship in the range from 20 to 1

× 10^6 cells/mL was obtained and the detection limit is as low as 3 cells/mL with acceptable stability and reproducibility. Moreover, polyhedral-AuPd nanoparticles, Cu_2O@PtPd nanocomposite and trimetallic Au@PtPd nanoparticles have also been used as the signal labels for tumor cell detection with satisfactory results [60, 62-64]. Sun et al. developed a sandwich-like electrochemical aptasensor for detection of HepG2 with hybrid nanoelectrocatalyst/enzyme for signal amplification [65, 66]. In their study, the thiolated TLS11a aptamer was used as the selective bio-recognition element. The electrochemical cytosensor achieved a wide linear range from 100 to 10^7 cells/mL with a detection limit of 15 cells/mL.

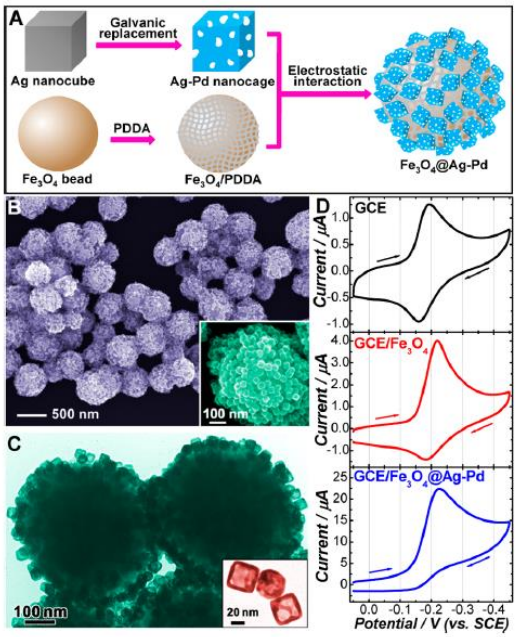

Figure 7. Schematic illustration of the fabrication of Fe_3O_4@Ag−Pd hybrid NPs [60]. Copyright 2014 American Chemical Society.

3.3 Electroactive materials

Nanomaterials have been proved to be promising materials for improving the sensitivity of biosensors. With the rapid development of nanotechnology, various nanomaterials have been applied for designing of signal-amplified electrochemical biosensors. Graphene quantum dots (GQDs) and AuNPs have been used to effectively deliver optical and electrochemical signals for biosensing. Amouzadeh Tabrizi et al. proposed an ultrasensitive sandwich-type immunosensor using rGO-TPA/$FeHCF_{nano}$/Anti-HCT* as the signal tag for the detect of SKBR-3 breast tumor cell in the concentration range of 500 ~ 30,000 cells/mL with a detection limit of 21 cells/mL [67]. Zhang et al. reported a novel lectin-based biosensor for electrochemical assay of cancer-associated glycosylation (Figure 8) [37]. It is based on the different expression of mannose and sialic acid on normal and tumor cells derived from human lung, liver, and prostate. In a sandwich format, high sensitivity and selectivity were achieved by the lectin-Au-Th bioconjugates featuring lectin and thionine (Th) labels linked to AuNPs for signal amplification. The proposed strategy confirmed that mannose was highly

expressed in both normal and tumor cells, while sialic acid was more abundant in tumor cells in contrast to normal cells.

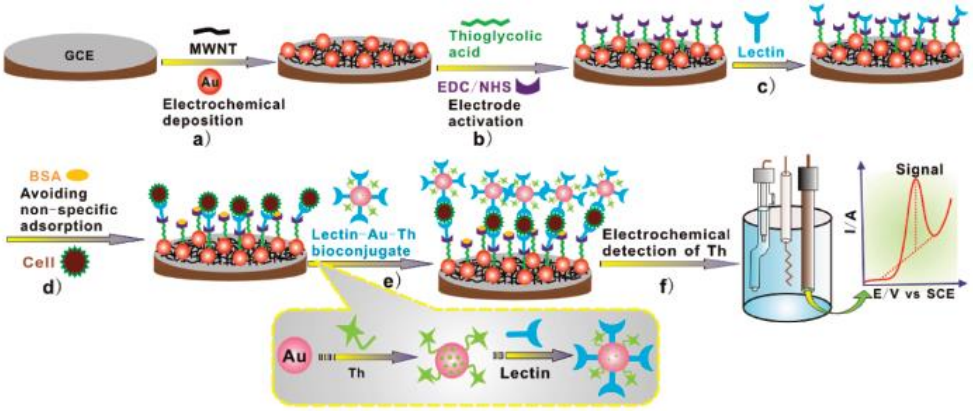

Figure 8. Schematic illustration of the lectin-based biosensor for electrochemical analysis of glycan expression on living cells. Reprinted with permission from reference [37]. Copyright 2010 American Chemical Society.

Silver nanomaterials could be directly electrochemically oxidized with a well-defined stripping voltammetric peak [68-70]. Some silver-based nanostrucutres have been employed as the reporters for tumor cell detection [61, 71-73]. For example, Zhang et al. synthesized p-sulfonatocalix[4]-arene-modified silver nanoparticles (pSC4-AgNPs) and explored as an universal nanoprobe for electrochemical cell analysis (Figure 9A) [71]. pSC4 can recognize and bind to various amino acid residues on the membrane protein, and AgNPs can give a sensitive electrochemical signal. Therefore, a variety of cells can be measured by the probes.

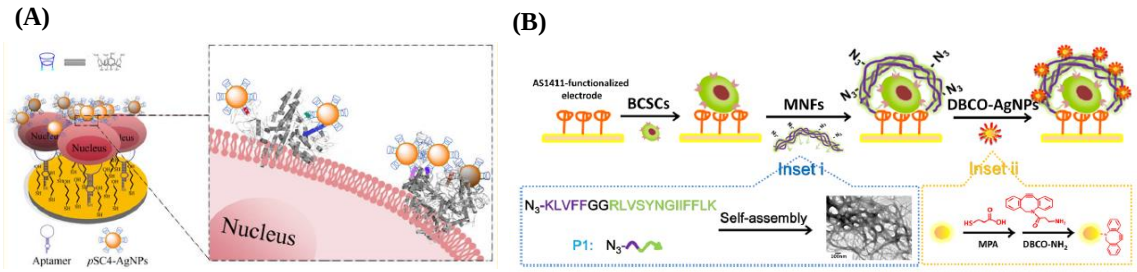

Figure 9. (A) Schematic illustration for mechanism of cell electrochemical detection by using pSC 4 −AgNPs as a universal and sensitive electrochemical probe. Reprinted with permission from reference [71]. Copyright 2019 American Chemical Society. (B) Schematic illustration of the multifunctional nanofiber-assisted electrochemical identification of BCSCs. Reprinted with permission from reference [61]. Copyright 2019 American Chemical Society.

The current values increased linearly with increased HepG2 amounts from 5 to 2.5 × 10^5 cells/mL. The detection limit of 5 cells/mL is lower than previously reported values. Tang et al. proposed an electrochemical method to identify stem-like cells in breast tumor by multifunctional nanofibers (MNFs) (Figure 9B) [61]. The MNFs synthesized through facile self-assembly of peptide

probes performed three functions: specifically targeting surface biomarker, recruiting AgNPs, and providing large amounts of reaction sites. By measuring the electrochemical signal from MNF-recruited AgNPs, the method was used to detect target cells as low as 6 cells/mL within a linear range from 10 to 5×10^5 cells/mL.

Additionally, Jiang et al. developed a label-free and competitive electrochemical biosensor by assembly of DNA-Pt nanoparticle (DNA-Pt NP) for amplified detection of tumor cells [74]. The concentration range varied from 50 to 1×10^6 cells/mL with a detection limit of 15 cells/mL. The thiolated TLS11a aptamer with high affinity to HepG2 cells was covalently attached to the AuNPs deposited on indium tin oxide (ITO) glass. Guo et al. proposed an electrochemical immunoassay for MCF-7 cells based on the signal enhancement of silver nanoclusters (Ag NCs) [75]. MCF-7 tumor cells have been determined with high sensitivity (50 cells/mL).

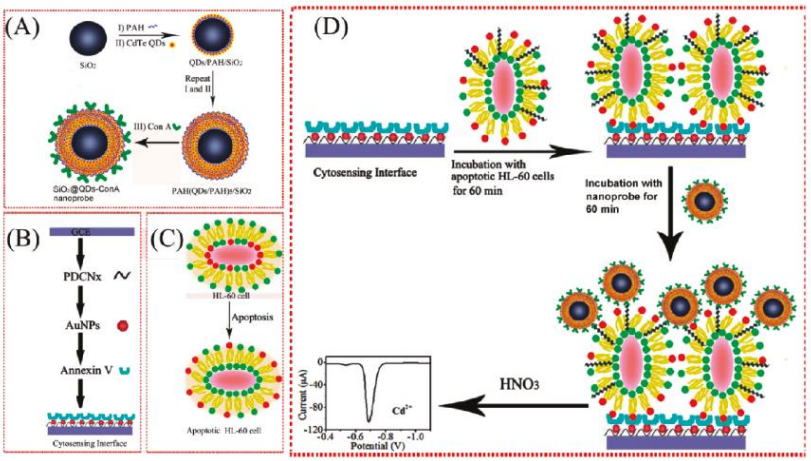

Figure 10. Scheme of the preparationof SiO_2 @QDs-ConA nanoprobe via layer-by-layer (LBL) assembly [76]. Copyright 2011 American Chemical Society.

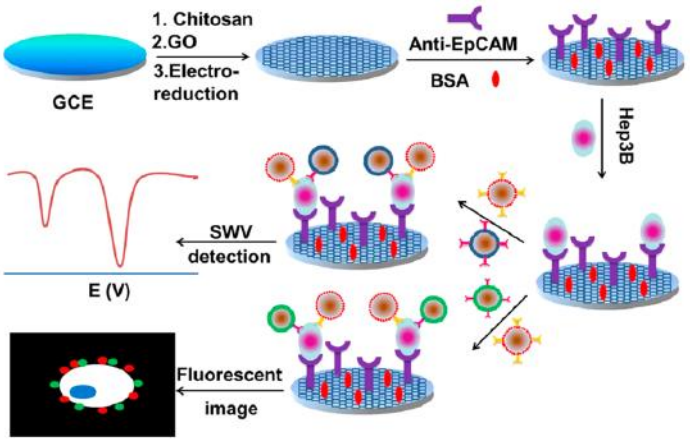

Figure 11. The fluorescent and electrochemical detection procedures of circulating tumor cells. Reprinted with permission from reference [77]. Copyright 2013 American Chemical Society.

Quantum dots (QDs) show a clear stripping voltammetric peak as the metal component. They have also been used as the electroactive reporters for signal-amplified electrochemical analysis of tumor cells [20, 63, 76-80]. Typically, Zhang et al. proposed an ultrasensitive and selective method for the detection of apoptotic cells with lectin-functionalized SiO_2@QDs nanoassemblies as the amplified signal probes (Figure 10) [76]. The nanoprobes combined special carbohydrate recognition and signal amplification of multi-labeled QDs. Based on the specific recognition of annexin V and phosphatidylserine to apoptotic cell membrane, the annexin V/3-D structure interface exhibited predominant ability to capture apoptotic cells.

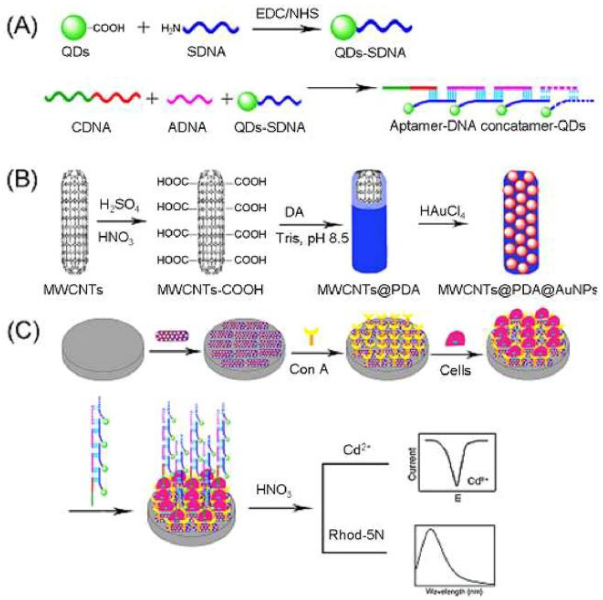

Figure 12. Procedures for the fabrication of aptamer-DNA concatamer-QDs (A), MWCNTs@PDA@AuNPs composites (B), and supersandwich cytosensor (C). Reprinted with permission from reference [80]. Copyright 2013 American Chemical Society.

Wu et al. designed a dual signal amplification immunosensor for highly sensitive and specific determination of a small amount of tumor cells (Figure 11) [77]. In this work, graphene-modified electrode was used to accelerate the electron transfer and two QDs-coated Si nanoparticles were used as the tracking markers. Immunoassay of EpCAM and GPC3 antigens on Hep3B cell line was carried out with anti-EpCAM CdTe- and anti-GPC3 ZnSe-coated Si nanoparticles as the tracers. Liu et al. developed a supersandwich-based signal-amplified method for sensitive detection of tumor cells by aptamer-DNA concatamer-QD probe (Figure 12) [80]. The electrode material of MWCNTs@PDA@AuNPs was fabricated by layer-by-layer assembly of MWCNTs, AuNPs, and polydopamine (PDA). The concatamer-QD probe was designed through DNA hybridization and follow-up covalent coupling. The MWCNTs@PDA@AuNPs composite was applied to amplify the signal and attach Con A for cell recognition. The detection limit was found to be 50 cells/mL. A good linear relationship was achieved with a detection range of 10^2 ~ 10^6 cells/mL.

3.3 Other methods

Recently, DNA hybridization-based techniques such as rolling circle amplification (RCA), DNA nanostrucutres and DNA walkers have been adapted to the detection of tumor cells [19, 81-84]. The electrode can be used for signal output by providing electron transfer region and interface for molecule immobilization and DNA walking. Miao et al. designed a multimodal DNA walker for sensitive determination of CTCs without enrichment step (Figure 13) [85]. The linear detection range is 5 ~ 5000 cells/mL. The detection limit is calculated to be 1 cell/mL, which is superior to most previously reported cytosensors. The group also fabricated a three-dimensional (3D) architecture by combining nitrogen-doped carbon nanotubes, thionine, and AuNPs to evaluate cell surface carbohydrate and glycoprotein [37]. This biosensor showed excellent analytical performance for the detection of HeLa cells ranging from 8×10^2 to 2×10^7 cells/mL with a detection limit of 500 cells/mL. Based on the specific recognition of TLS11a aptamer for HepG2 cells, Sun et al. developed a competitive unmarked electrochemical method for sensitive detection of liver tumor cells by DNA nanotetrahedron (NTH) structure and RCA-directed amplification [83]. The cytosensor was ultrasensitive for HepG2 with a detection limit down to 3 cell/mL.

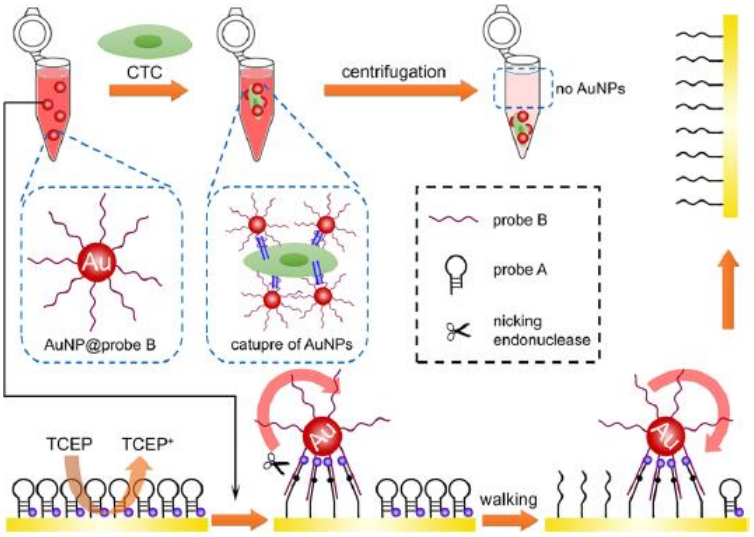

Figure 13. Illustration of the cytosensor based on multipedal DNA walking strategy. Reprinted with permission from reference [85]. Copyright 2019 American Chemical Society.

4. MAGNETOELECTRIC DETECTION

When the targets were capured by magnetic nanoparticles (MNPs) or beads (MBs), the minimal matrix interference can be easily obtained by a simple washing step. Thus, magnetic particles have been used to construst electrochemical sensing platforms for cancer diagnosis and analysis [40, 86-90]. Freitas et al. reported the design of electrochemical immunomagnetic bioassays for the detection of the extracellular domain of HER2 (HER2-ECD) in human serum and tumor cells [91].

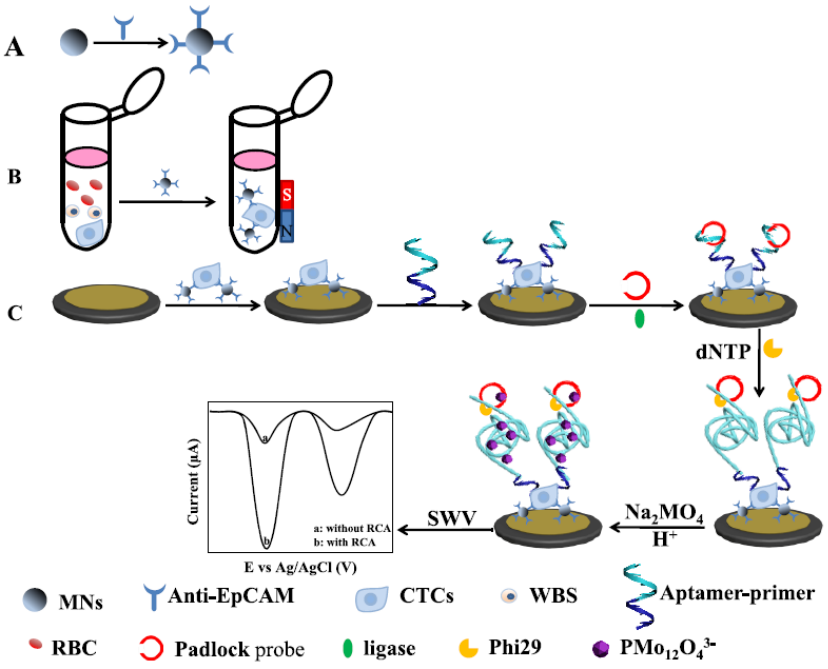

Figure 14. Illustration of CTC Measurement in Whole Blood Based on MN Isolation and RCA Signal Amplification. Reprinted with permission from reference [94]. Copyright 2015 American Chemical Society.

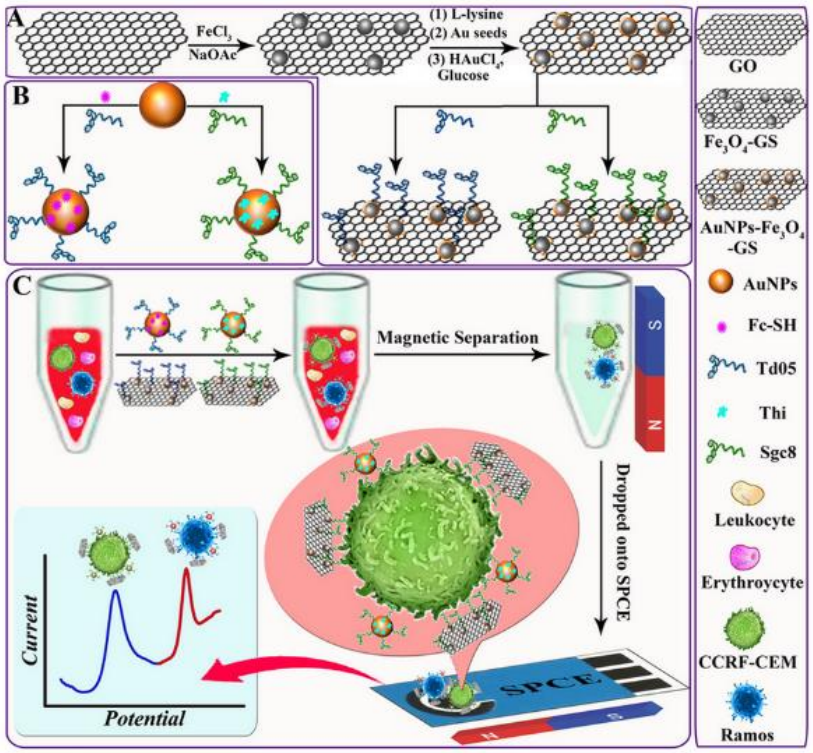

Figure 15. Illustration of the synthesis of (A) the aptamer-functionalized AuNPs-Fe_3O_4-GS capture probes and (B) the aptamer/electroactive species-loaded AuNP amplification signal probes and (C) the capture, isolation, and amplified and multiplexed detection of the target CTCs in whole blood. Reprinted with permission from reference [95]. Copyright 2019 American Chemical Society.

In their studies, carboxylic acid-functionalized magnetic beads (COOH-MBs) were modified with capture probe and alkaline phosphatase (AP)-labeled antibody as the signal reporter. The biosensor was used to detect CTCs in human serum with a detection limit of 3 cells/mL and then used to determine HER^+ breast tumor cell line SK-BR-3. Valverde et al. described an electrochemical immunosensor for the determination of IL-13 receptor Rα2 (IL-13Rα2), an emerging relevant biomarker in metastatic colon cancer [92]. Specific capture antibodies were immobilized onto the COOH-MBs and biotinylated detector antibodies were labeled with Strep-HRP polymer. Safaei et al. reported a simple platform for the isolation and determination of t CTCs by integrating electrochemical ELISA assay witha microfluidic cell capture system [93].

Yang's group reported a magnetic aptasensor for sensitive detection of CTCs (Figure 14) [94]. The method is based on DNA-generated current by rolling circle amplification (RCA) amplification. The commercial EpCAM-modified magnetic nanospheres were applied to capture CTCs. ALP was used as the signal marker to catalyze the generation of large numbers of electroactive p-aminophenol molecules. The low abundance of CTCs in blood limits their detection. Recently, Dou et al. synthesed aptamer/AuNP magnetic graphene nanosheets for capture and seperation of CTCs from human blood (Figure 15) [95]. The target CTCs can be efficiently separated to produce two different voltammetric peaks. The method allowed for the multiplexed detection of Ramos and CCRF-CEM cells with detection limits of 4 and 3 cells/mL, respectively.

5. CONCLUSION

Tumor cells can circulate in body fluids before moving to different parts of the body even in the early stages of cancers,. Therefore, monitoring the cells as a non-invasive cancer diagnosis has important guiding significance for the prognosis and clinical decision-making of local or metastatic tumor patients. However, cancer cells are not easy to be recognized because their number in the blood stream is very small; thus, accurate and extremely effective methods are needed to capture and recognize tumor cells. This work summarized the recent progress in the electrochemical detection of tumor cells, which should be valuable for designing of novel biosensors for clinical diagnosis of cancers.

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