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ORIGINAL PAPER

Dual-aptamer based electrochemical sandwich biosensor for MCF-7 human breast cancer cells using silver nanoparticle labels and a poly(glutamic acid)/MWNT nanocomposite

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Abstract

This paper reports on a sensitive and selective method for the detection of *Michigan Cancer Foundation-7 (*MCF-7) human breast cancer cells and MUC1 biomarker by using an aptamer-based sandwich assay. A biocompatible nanocomposite consisting of multiwall carbon nanotubes (MWCNT) and poly(glutamic acid) is placed on a glassy carbon electrode (GCE). The sandwich assay relies on the use of a mucin 1 (MUC1)-binding aptamer that is first immobilized on the surface of modified GCE. Another aptamer (labeled with silver nanoparticles) is applied for secondary recognition of MCF-7 cells in order to increase selectivity and produce an amplified signal. Differential pulse anodic stripping voltammetry was used to follow the electrochemical signal of the AgNPs. Under the optimal condition, the sensor responds to MCF-7 cells in the concentration range from 1.0×10^2 to 1.0×10^7 cells·mL^{-1} with a detection limit of 25 cells. We also demonstrate that the MUC1 tumor marker can be detected by the present biosensor. The assay is highly selective and sensitive, acceptably stable and reproducible. This warrants the applicability of the method to early diagnosis of breast cancer.

Keywords MUC1 aptamer · Michigan Cancer Foundation-7 · Electrochemical aptasensor · Aptamer based sandwich assay · Differential pulse anodic stripping voltammetry · Poly(amino acid) nanocomposite · Carbon nanotube · Aptamer-AgNP bioconjugates

Introduction

Breast cancer is the most common non-skin cancer in woman, accounting for 23% of all causes of cancer in woman [1, 2]. Early diagnosis and timely therapy can be useful for providing proper treatment at the early stage and saving the patients [3, 4]. Despite the diagnostic advancement, highly sensitive and selective detection of cancer cell is essential to disease extent and appropriate treatment plan.

Aptamers have a range of analytical and therapeutic applications [5]. This group of synthetic nucleic acid molecules can be selected from the systematic evolution of ligand by exponential enrichment (SELEX). For cellular study, aptamers have been applied to distinguish the native protein target on the surface of cellule and identify different type of cell lines [6].

In this work, a DNA aptamer targeting mucin 1 (MUC1) was selected that binds with high affinity and selectivity the MUC1 tumor marker with the aim of detecting human breast carcinoma *Michigan Cancer Foundation-7* (MCF-7) cells. The MUC-1 is a cell-surface associated glycoprotein, that is aberrantly expressed on greater than 90% of human breast carcinomas and it has been a target of interest for many years [7]. Considering the key role of tumor cell

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in diagnosis and treatment of cancer, developing high sensitive and selective biosensor is of great importance. Various techniques, including piezoelectric microcantilever sensors (PEMS) [8], surface plasmon resonance [9], electrochemiluminescence [10] and electrochemistry [11–13] have been used for analysis of cancer cells. Among the different sensors have been applied for cancer cell detection, the electrochemical biosensors are preferred due to their higher sensitivity and cost-effectiveness [14–16]. In 2015, Tao et al. fabricated a selective biomembrane mimetic surface (SBMMS) and a graphene oxide/AgNPs nanohybrid for cellular molecule sensing [17]. Determination of adenocarcinoma gastric cancer cell was done using aptamer-Au@Ag nanoparticles by Amouzadeh Tabrizi [18]. Electrochemical detection of colorectal cancer (CT26) cells was performed by an aptasensor based on sandwich architecture [19]. The electrochemical biosensors have potential application for the clinical diagnosis because the electrical signals can be collected quickly and easily in real time [20].

Nanomaterials with unique physical and chemical properties play an important role in improving the sensitivity for cellular detection. Among the known nanomaterial, MWCNT had been paid more attention due to their highly conductive nature, enlarged surface area and unusual biomolecule binding capabilities [21]. Our attention is directed toward the application of a nanocomposite containing PGA and MWCNT to modify the surface of glassy carbon electrode (GCE). Poly(glutamic acid) (PGA) is a natural biopolymer made up of repeated glutamic acid monomers connected by amide linkages between α-amino and γ-carboxyl groups [22]. According to the reported results, the use of carbon nanotube/polymer composite for electrode modification improved the performance compared to individual polymer or carbon nanotubes modified electrodes. Indeed, a carbon nanotube-PGA composite, provides outstanding properties, such as durability and activity with large surface area which can facilitate the electron-transfer reaction.

In this study, we have proposed a dual-aptamer-based sandwich biosensor for electrochemical detection of MCF-7 breast cancer cell and MUC1 tumor marker. In the sandwich bioassay method, cancer cells are sandwiched between capture aptamer and AgNPs-labeled aptamer (detection aptamer) and the electrochemical signal of detection aptamer attached to the biological target is determined. In aptasensor designing, a MWCNT-PGA nanocomposite modified electrode was used as biomolecules immobilization matrix. Using of a nanocomposite containing PGA, providing appropriate carboxylic functional groups and a biocompatible platform for aptamer immobilization. First, MUC1 aptamer (as capture aptamer) is immobilized on the surface of MWCNT-PGA/GCE via covalent bonds between free carboxylic group of polymer and the 5′-NH2-terminal end of synthetic aptamer. When a proper concentration of MCF-7 cell was put on the surface of electrode, the cells is captured through the specific interaction between the aptamer and the target. Then, in order to complete the sandwich architecture, the aptamer conjugated AgNPs (Apt-AgNPs) was added to the bio complex for cell detection (Scheme 1). Differential pulse anodic stripping voltammetry (DP-ASV) of AgNPs was evaluated for quantification of cancer cell in the sample. The accumulation potential of −1.0 V was applied for 120 s and a differential pulse voltammogram was then recorded at a scan rate of 100 $mV{\cdot}s^{-1}$ by sweeping the potential from −0.1 to 0.5 V (vs. Ag/AgCl). In this work we used AgNPs as a signaling probe due to its extraordinary electrochemical behavior and good biocompatibility. We have tried to improve the signal amplification using biomolecules labeled with nanomaterials that effectively increase the biomolecules loading towards a sandwich complex reaction, and result in an improvement of detection sensitivity. It is noticeable that the use of MWCNT-PGA nanocomposite and Apt-AgNPs as a signal tag can improve the sensitivity of the constructed aptasensor for determination of MCF-7 cancer cells and MUC1 biomarker. The applicability of the fabricated aptasensor in cancer screening was evaluated by analyzing the MUC1 serum levels in patient woman who have been subjected to breast cancer.

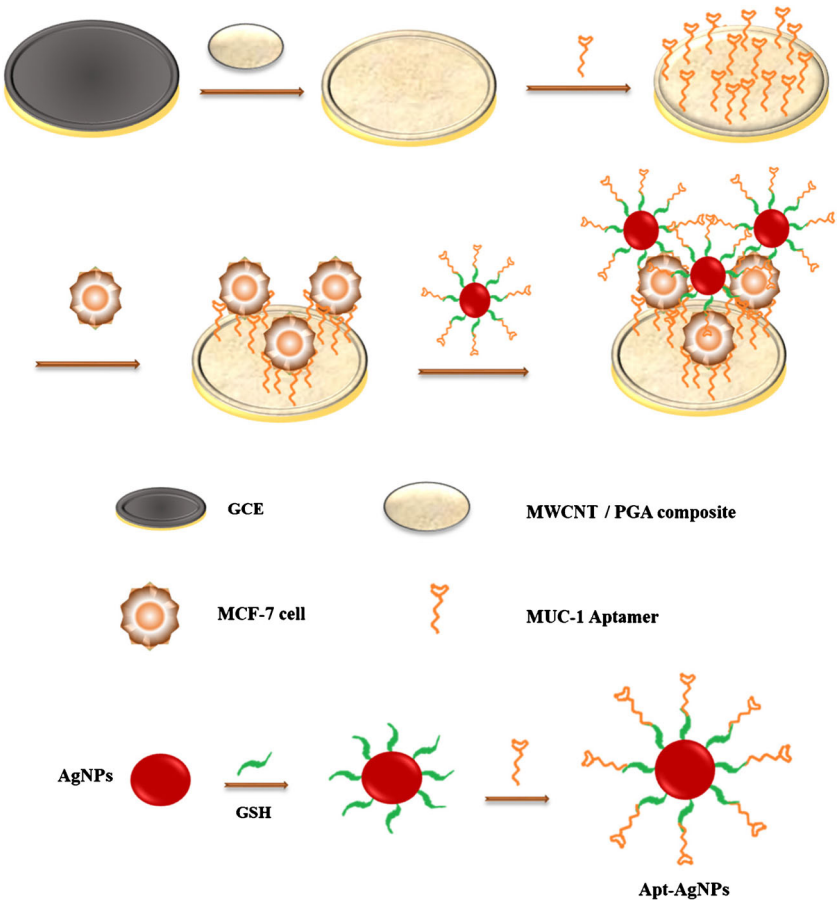
Experimental section

Chemicals and reagents

All the used chemicals had analytical grade and used without purification. MWCNT, sodium dihydrogen phosphate, disodium hydrogen phosphate, poly(glutamic acid), silver nitrate, sodium borohydride, bovine serum albumin (BSA), glutathione (GSH), potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$), potassium hydroxide (KOH), 1-ethyl-3-(3-dimethylaminopropyl) carbo-diimide hydrochloride (EDC), Trypan blue, N-hydroxysuccinimide (NHS), 6-mercapto-1-hexanol (MCH) were purchased from commercial sources Sigma Aldrich (https://www.sigmaaldrich.com/european-export.html). MUC1 protein was provided by Novus Biological (Cambridge, UK, https://www.novusbio.com/). All the buffer and the double distilled water were sterilized using an autoclave.

The human breast cancer cells MCF-7 as target cell and SKBR3, HeGp2, HEK, Hela cells as control cells were received from Iranian Biological Research Centre (Tehran, Iran, http://en.ibrc.ir/). MCF-7 cells were cultured in a 5% CO_2 incubator at 37 °C and supplemented with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, 1% penicillin/streptomycin.

All oligonucleotides used in this study were synthesized and purified by Bioneer Company (Korea) and the sequences



Scheme 1 Schematic representation of the aptamer-base sandwich biosensor for MCF-7 cancer cell detection

of oligonucleotides were as follows: MUC-1 aptamer: 27 mer, 5′-NH2-TGC AGT TGA TCC TTT GGA TAC CCT GGT- 3′ (Mw (8451.8), Tm (64.9 °C)).

Instrumentation

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, Netherlands). The experimental conditions were controlled with Nova 1.7 software. All experiments were carried out with a conventional three-electrode system composed of glassy carbon electrode (GCE) as the working electrode (WE), a platinum counter electrode (CE) and an Ag/AgCl (sat. KCl) reference electrode (RE). The Metrohm model 691 pH/mV meter was applied for pH measurements. The experimental conditions included a frequency between 100 kHz and 0.01 Hz, AC amplitude of 10 mV for EIS, and a scan rate of 100 $mV{\cdot}s^{-1}$ for the cyclic voltammetry. The impedance spectra were shown as Nyquist plots and fitted by a Randles equivalent circuit. The SEM images were recorded using a TESCAN instrument model VEGA3. The MCF-7 cancer cells were observed on a slide under a microscope and counted using a Neubauer chamber or Hemocytometer. Trypan blue has been used as a dye to detect dead cells.

Choice of materials

MWCNT and graphene have been used extensively as electron transfer facilitators in fabrication of breast cancer biosensors [23]. We used a nanocomposite made of MWCNT and PGA for modifying the surface of glassy carbon electrode (GCE) to benefit from the properties of both nanomaterials and natural polymer materials. PGA is a good candidate for biosensing assay because of having numerous free protonated carboxyl groups (pKa = 4.45) on the side chain that can link to biomolecules through electrostatic and covalent bonds [24]. It is highly useful due to the abundant presence of carboxylic groups needed for grafting aptamers to the electrode surface. We used a nanocomposite made of MWCNT and PGA for modifying the surface of glassy carbon electrode (GCE) to benefit from the properties of both nanomaterials and natural polymer materials. The probe sequence was immobilized onto MWCNT-PGA/GCE surface by covalently attachment between free carboxylic group of polymeric layer onto the electrode surface and the amino-terminal end of the aptamer. Indeed, the presence of MWCNT- PGA composite, on the surface of GCE provides outstanding properties, such as durability and activity with a large specific surface area. AgNPs as a signaling probe was preferred other nanoparticles in the

present work due to its admirable electrochemical behavior. This nanoparticle exhibits lower oxidation potential and facile electron transfer, so it can produce a defined and resolved voltammetric signal in electrochemical-stripping detection. Silver metal can be oxidized readily and there is no need to use poisonous oxidants. Besides that, the oxidation peak corresponding to the silver has more intensity than the AuNPs, so a signal improvement can be achieved by using AgNPs [25]. Therefore, compared with Au NP, Ag NP can be directly detected using a sensitive electrochemical-stripping detection due to their lower oxidation potential and facile stripping conditions. Glutathione has been used as a linker to prepare the Apt-AgNPs bioconjugate. Amino acids are the ideal agents for the functionalization of nanoparticles; because different functional groups are presented in their structure. Glutathione is a tripeptide (−Glu-Cys-Gly) that contains a thiol group which can be easily adsorbed onto the surface of AgNPs. Meanwhile, there are also many other functional groups such as $–NH_2$ and –COOH which have strong affinity to biomolecules coupling.

Procedure

Preparation of the MWCNT–poly(glutamic acid) dispersion

Preparation of MWCNT/polymer nanocomposites was performed using solution mixing which is the most common method for CNT/polymer nanocomposites fabrication because it is amenable to small sample sizes [26]. In this method, a dispersion of MWCNT in a suitable solvent and polymers are mixed in solution. Due to the difficult dispersion of MWCNT in a solvent by simple stirring, the necessary weight fractions of MWNTs (1.0 mg) were dispersed in 1.0 mL of distilled water by ultrasonic processor at room temperature for 1 h. Then PGA (0.20 mg) was added to this solution and the mixture was stirred for 1 h followed by sonication again for 30 min. finally, a nanocomposite consisting of MWCNT in a polymer matrix was applied for electrode modification process.

Preparation of the modified glassy carbon electrode (MWCNT-PGA/GCE)

Pretreatment of a GCE is the first step in fabrication processes of the aptasensor. The surface of GCE was polished by 0.05 μmol·L^{-1} Al_2O_3 and sonicated in anhydrous alcohol and water solutions for 5 min until a mirror-like surface was obtained. The polished electrode was modified with MWCNT-PGA/GCE nanocomposite. For this purpose, 20 μL of MWCNT-PGA suspension was dropped on the surface of GCE and exposed to air for solvent evaporation.

Bioconjugation of AgNPs with MUC1 aptamer

In order to supply the Apt-AgNPs bioconjugate, glutathione has been used as a linker. At first the synthesis of glutathione functionalized silver nanoparticles (GSH-AgNPs) was carried out based on the method described in the literature [27]. A 250 μL of the GSH-Ag NP solution was added to the 50 μL of MUC1 aptamer solution (0.1 μmol·L^{-1}) in the presence of EDC and NHS (1.0 mmol·L^{-1}). The resulting solution was kept for 2 h under moderately shaking in the dark for the assembling of AgNPs to amine group of MUC1 aptamer segment. The reaction is based on the interaction between terminal amine group of aptamer and the free carboxylic group of glutathione. To remove the non-conjugated nanoparticles, the Apt-AgNPs were centrifuged (5000 rpm for 20 min) and subsequently washed with phosphate buffer (pH 7.4). The precipitation was suspended into 50 μL of phosphate buffer (pH 7.4) and stored at 4 °C. The synthesized Apt-AgNPs is ready for modification of electrode.

Test protocol

Immobilization of MUC1 aptamer on the surface of the modified GCE

The terminal carboxylic acid groups of MWCNT-PGA modified GCE was activated by immersion in an aqueous solution of NHS/EDC (1.0 mmol·L^{-1}) for 1 h at room temperature. The electrode was then washed quickly with dual distillated water and 10 μL of an aptamer solution (5 μmol·L^{-1}) was dropped on the surface of the electrode for 16 h in a chamber saturated with water vapor. There was an important point before the last step in order to obtain the operative conformation of MUC1 aptamer for binding to the target of MCF-7 cells. The MUC1 aptamer had to be prepared as follows: denaturation of anti-TET aptamer at 90 °C for 15 min, cooling at 4 °C for 10 min and placing it at 25 °C for 15 min. The probe sequence immobilization onto the surface of MWCNT-PGA/GCE is based on covalently attachment of free carboxylic group of polymer (-COOH) and the 5′-NH2-terminal end of synthetic aptamer. Each modification step was followed by CV and EIS measurements. After being thoroughly rinsed with distillated water, the MUC1 aptamer functionalized electrode can be ready for use.

The formation of aptamer–cell–aptamer sandwich architecture

After washing with water, 20 μL of phosphate buffer containing MCF-7 breast cancer cell with specific concentration was dropped on the surface of MUC1 aptamer functionalized electrode. It was incubated at 37 °C for 60 min to allow the

immobilization of the target cells onto the surface of electrode. After capturing the cells onto the electrode surface, the electrode was immersed in a phosphate buffer containing 0.5% Tween-20 and 1% BSA at the room temperature for 30 min to remove the nonspecific adsorbed cells on the surface of electrode. In the last step, the electrode was incubated with Apt-AgNPs bioconjugates for 40 min which leads to the formation of an aptamer–cell–aptamer architecture. After being rinsed with the phosphate buffer, the prepared aptasensor was used for electrochemical measurements.

Direct electrochemistry of AgNPs aggregations

The prepared electrode was firstly immersed in HNO_3 solution (0.5 mol·L^{-1}) for about 10 min to fully dissolve AgNPs loaded on the surface of electrode. Then, the phosphate buffer was added to the resulting solution for electrochemical analysis using a three-electrode system and anodic stripping voltammetry. After an acid-dissolution of AgNPs, differential pulse anodic stripping voltammetry was performed for quantification of released Ag^+ from the complex. Anodic stripping voltammetry is a voltammetry method for quantitative determination of trace amount of ionic species. The analyte of interest is accumulated on the working electrode during a deposition step for a known period of time and stripped from the surface into the solution by applying an oxidizing potential. Constant potential of −1.0 V was applied to the working electrode for 120 s and potential was then swept from −0.1 to 0.5 V (vs. Ag/AgCl) with modulation amplitude of 25 mV. The electrochemical signal of Ag oxidation, was proportional to the concentration of Ag ions in the solution and further corresponded to the amount of the concentration of captured MCF-7 cells. The control experiments were performed in a similar manner but without adding the MCF-7 cells. For the detection of MUC1 marker, the aptasensor was prepared in a similar way as mentioned above except MCF-7 was replaced by MUC1 protein.

Results and discussion

Electrochemical characterization

CV and EIS are two effective techniques have been adopted to get information on the changes of interfacial properties in the aptasensor assembling processes. Figure 1 displays the CV and EIS responses of the stepwise modification process on the GCE (a), MWCNT-PGA/GCE (b), MUC1 aptamer/MWCNT-PGA/GCE (c), MCF-7/MUC1 aptamer/MWCNT-PGA/GCE (d) and Apt-AgNPs/MCF-7/MUC1 aptamer/MWCNT-PGA/GCE (e) in a solution containing electrochemical probe of $K_4Fe(CN)_6$ (0.5 mmol·L^{-1}) and $K_3Fe(CN)_6$ (0.5 mmol·L^{-1}).

Electrochemical characterization of the aptamer-cell-aptamer sandwich

Cyclic voltammetry characterization study the formation of the cancer aptasensor on the surface of electrode was confirmed by the commonly used CV technique (Fig. 1a). Figure 1a shows that the peak current (Ip) of MWCNT-PGA/GCE (curve b) is higher than that observed for the bare GCE (curve a). Considerable increase in the peak current intensity can be related to the large surface area and high electron conductivity of nanocomposite. The presence of free carboxylic groups on the polymeric layer provides a suitable platform for aptamer stabilization. By immobilization of the MUC1 aptamer on the surface of modified electrode (Fig. 1a, curve c), the peak current is decreased significantly. The $[Fe(CN)_6]^{3-/4-}$ ions cannot reach to the electrode surface due to the electrostatic exclusion interaction between the probe and MUC1 aptamer. Based on the recorded data (Fig. 1a, curve d), further decrease in the peak current is observed after incubation of immobilized aptamer with target cells for 60 min. The results confirm the successfully capturing of MCF-7 breast cancer cells and the formation of aptamer–cell–aptamer sandwich architecture.

EIS characterization

EIS signals were applied to evaluate the electron transfer properties of the modified GC electrodes (Fig. 1b). The Nyquist diagram obtained for bare GCE in 0.5 mmol·L^{-1} $K_3Fe(CN)_6$ shows a small electron transfer resistance (Fig. 1b, curve a). By modification of the GCE with MWCNT-PGA composite, the conductivity and electron transfer process are improved and the R_{ct} value is decreased. After the aptamer immobilization, by the formation of aptamer monolayer on the surface of modified electrode (Fig. 1b, curve b), the semicircle diameter in the impedance spectrum is increase which can be related to the electrostatic repulsion between the aptamer and electrochemical probe. Subsequently, due to the nonconductive properties of biomacromolecule, further increase of the charge transfer resistance can be observed when the MCF-7 breast cancer cell was incubated with MUC1 aptamer (Fig. 1b, curve c). The last step involves the recognition of the reporter Apt-AgNPs bioconjugates by MCF-7 cells (Fig. 1b, curve d) which leads to a decrease of the impedance value interestingly. This is due to high electron transfer efficiency of the synthesized Apt-AgNPs bioconjugates, although the aptamer layer acts as an insulating barrier which impairs the interfacial charge transfer process. The results indicate that the formation of aptamer-cell-aptamer structure was performed successfully.

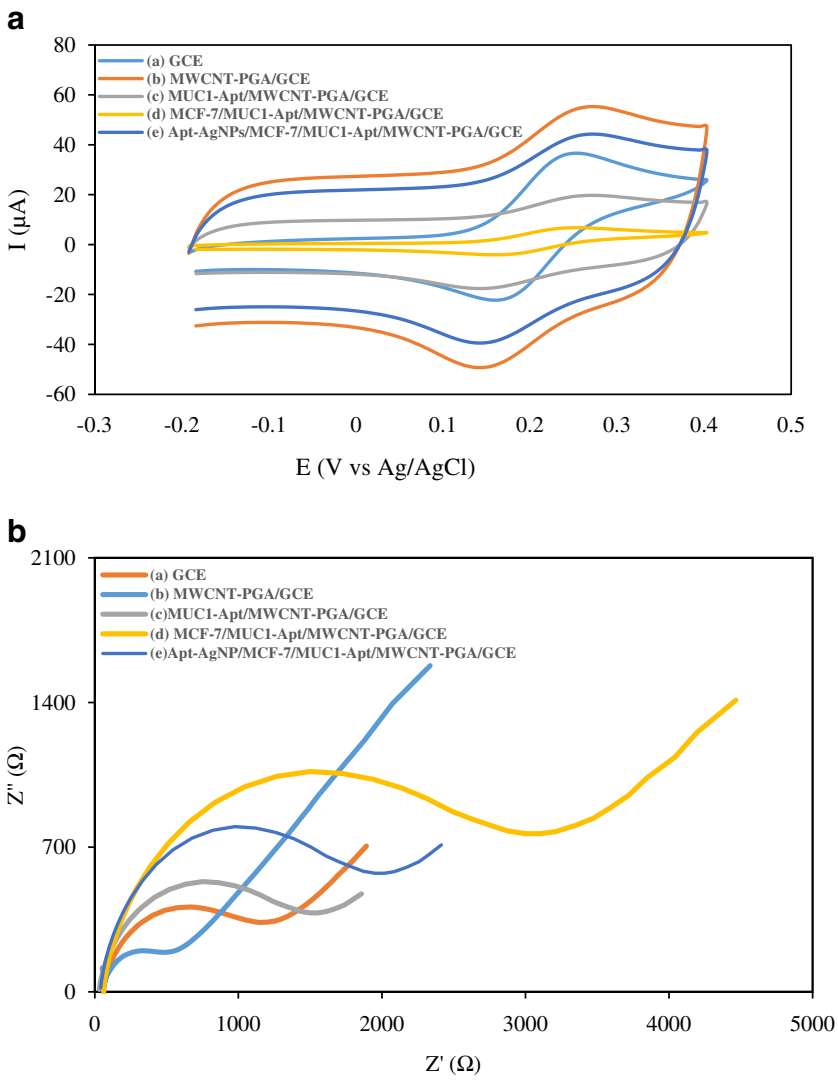


Fig. 1 Cyclic voltammograms (**a**) and Nyquist diagrams (**b**) of the modification steps of Apt-AgNPs/MCF-7/MUC1-Apt/MWCNT-PGA/GC electrode in 0.5 mmol·L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol·L^{-1} KCl: (a) bare GCE, (b) MWCNT-PGA/GCE, (c) MUC1-Apt/MWCNT-PGA/GCE (d) MCF-7/MUC1-Apt/MWCNT-PGA/GCE (e) Apt-AgNPs/MCF-7/MUC1-Apt/MWCNT-PGA/GCE. Inset (b): The Randles equivalent circuit applied to model impedance data in the presence of redox couples

The results obtained by EIS were in good agreement with the CV experiments.

Scanning Electron Microscope (SEM) analysis of MWCNT-PGA/GCE

The surface morphologies of modified bioelectrodes were investigated by SEM technique. According to Fig. 2, the unmodified GCE surface of is completely smooth which can create a suitable platform for stabilization of polymeric nanocomposite on it. After covering the electrode surfaces by MWCNT-PGA nanocomposite film, the SEM image indicates a porous network structure. MWCNT show a high degree of dispersion and remain successfully embedded all over the polymeric matrix. A high interface area and highly porous structure of the polymeric nanocomposite provides more active sites for satisfactory immobilization of aptamer molecules through the covalent interaction onto surface of the prepared nanocomposites.

Optimization of experimental variables

In a sandwich-type aptasensor, several experimental conditions can influence the sensor performance which should be optimized (Fig. S1). The following parameters were optimized: (a) concentration of MUC1 aptamer (b) incubation time for capturing MCF-7 and (c) incubation time for recognizing bioconjugates. Respective data and Figures are given in the Electronic Supporting Material. The following experimental conditions were found to give best results: (a) concentration of aptamer: 5.0 μmol·L^{-1} (b) Optimal incubation time for capturing MCF-7: 60 min (c) Optimal incubation time for recognizing bioconjugates: 40 min.

Analytical performance of the assay

Under optimum conditions, the performance of the sandwich type aptasensor for determination of MCF-7 breast cancer cell was investigated using DPV analysis (Fig. 3). The electrochemical measurements were performed after the Apt-

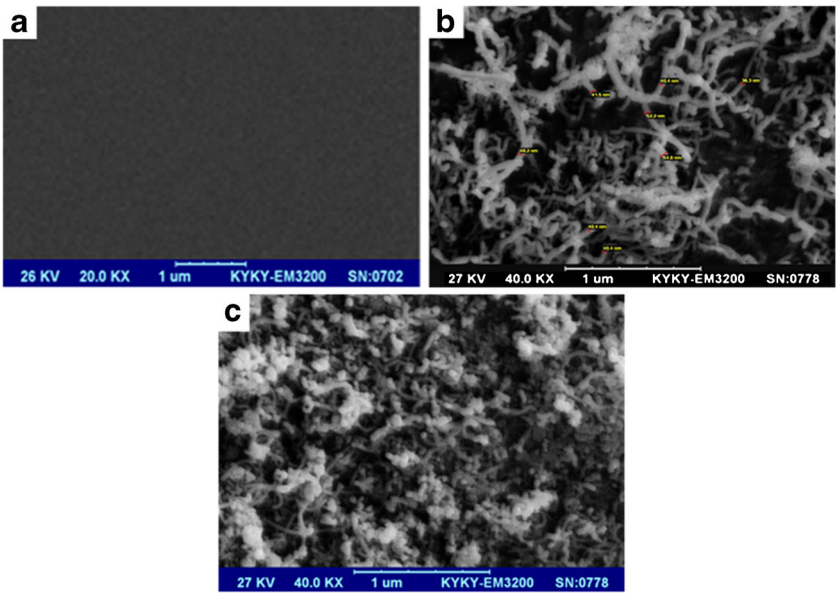


Fig. 2 SEM images of GCE (**a**) MWCNT/GCE (**b**) MWCNT-PGA/GCE (**c**), respectively

AuNPs/MCF-7/MUC1 aptamer/PGA-MWCNT/GCE electrode was incubated with different concentration of cancer cells for 60 min. The DP-ASV signals related to the Apt-AgNPs complexes were recorded and applied for evaluation of biosensor sensitivity. It can be seen that the peak currents of the biosensor increase by increasing the concentration of MCF-7 cells. Due to Fig. 3a, the fabricated biosensor shows a good linear response versus the logarithm of concentration of MCF-7 cell ranging from 1.0×10^2 to 1.0×10^7 to cells·mL^{-1}. The linear regression equation was calculated as y = 14.943× - 11.946 with a correlation coefficient of 0.9949 and detection limit of 25 cells·mL^{-1} (Fig. 3b). Table 1 shows the analytical characteristics of the fabricated aptasensor in comparison to other similar investigations for cellular analysis. The results show that the detection limit in this study is better than other reported works. It is believed that the introduction of Apt@AgNPs improves the sensitivity of the aptasensor for breast cancer cell detection.

The detection limit of the cell biosensor was compared to other reported biosensor for MCF-7 detection, as indicated in Table 1. The fabricated biosensor shows lower detection limit for detection of MCF-7 cancer cell compared to the previously reported works [28–33].

Selectivity, reproducibility and stability of the sandwich-type biosensor

To investigate the interference effects on the binding specificity of the biosensor to MCF-7 cancer cell, control experiments using SKBR3, HeGp2, HEK and Hela cells was performed. As illustrated in Fig. 4, the control cells cannot be captured by MUC1 aptamer after incubation in the sensing system, leading to no obvious current change. However, a significant current change was obtained for MCF-7cells. The present electrochemical aptasensor exhibits a high selectivity for the detection of MCF-7 cells.

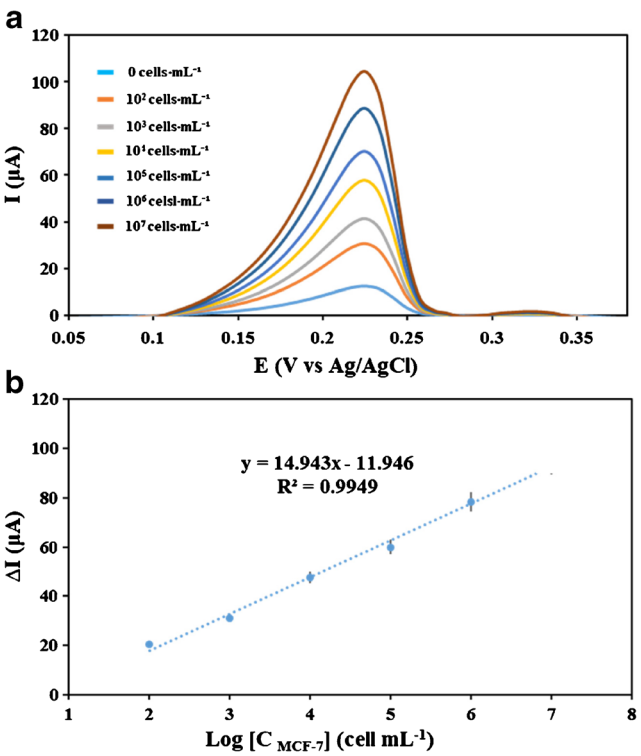


Fig. 3 **a** Differential pulse anodic stripping voltammograms of the sandwich-type biosensor at the optimum condition for different concentrations of MCF-7 cancer cell ($n = 3$), potential sweep from −0.1 to 0.5 V (vs. Ag/AgCl) with potential pulse amplitude of 25 mV, scan rate 100 mV·s^{-1}, accumulation potential of −1.0 V, and accumulation time of 120 s (**b**) The corresponding calibration plot of DP-ASV response versus MCF-7 cancer cell concentration

Table 1 Comparison of the dynamic range and detection limit of several biosensors for determination of MCF-7 breast cancer cell

Method	Signal ammplification	Liner range/ cells·mL^{-1}	Limit of detection/ cells·mL^{-1}	References
Electrochemisrty	Quantum dots	1.0×10^2–1.0×10^6	1.0×10^2	[28]
Electrochemiluminescence	Rul-aptamer	1.0×10^2–5.0×10^7	38	[29]
Electrochemisrty	HRP labeled aptamer	1.0×10^2–1.0×10^7	1.0×10^2	[30]
Electrochemisrty	CdS NPs	1.0×10^4–1.0×10^7	3.3×10^2	[31]
Surface plasmon resonance	NiO NPs	5.0×10^2–4.0×10^3	136	[32]
Electrochemistry	AgNCs	5.0×10^1–5.0×10^3	50	[33]
Electrochemistry	AgNPs labeled aptamer	1.0×10^2–1.0×10^7	25	This work

The long-time stability of the fabricated aptamer biosensor was evaluated by studying its DPV responses during a 28-day period (Fig. S2a). When the biosensor was stored in a 0.1 mol·L^{-1} of phosphate buffer (pH 7.4) at 4 °C for 14-days, no significant change was observed in the current signal and it retained more than 92% of its initial response. The current response was gradually decreased after 21 and 28. This proved that the fabricated biosensor has an acceptable stability for about half month. The reproducibility of the biosensor has been studied at MCF-7 cancer cell concentration of 10^4 cells·mL^{-1}. Five parallel prepared electrodes were used for target (10^4 cells·mL^{-1}) detection in the same day. The relative standard deviation (RSD) for single-electrode repeatability and electrode-to electrode reproducibility were calculated as 2.4% and 5.8% ($n = 5$) respectively. As shown in Fig. S2b, the similar electrochemical responses of the electrodes imply the suitable reproducibility of the cell biosensor.

Detection of MCF-7 cancer cells in human serum

In order to evaluate the potential application of the MCF-7 cell biosensor in complex real sample, three different concentration of MCF-7 cells were spiked in the human serum which was diluted with phosphate buffer and the measurements were performed. As shown in the Table 2, the recovery values indicate the good applicability of the method for cancer cell analysis. Also, a comparative study was performed for a buffer and the human serum solution containing the same MCF-7 cell concentrations. According to Fig. 5, a behavior similar to that found for a cell-containing buffer was observed. So, due to these results, the constructed biosensor can be applied for MCF-7 breast cancer cell detection in a complicated sample matrix.

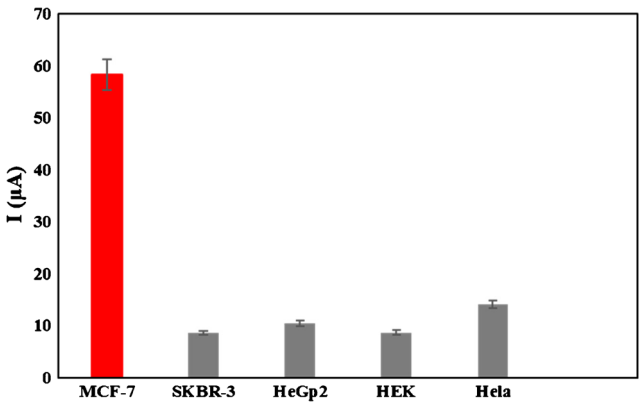


Fig. 4 Selectivity testing of the sandwich-type cell biosensor for MCF-7 cancer cells: SKBR3, HeGp2, HEK and Hela cells were used as control cells. The cell concentrations were 1.0×10^4 cells·mL^{-1}

Detection of MUC1 in human serum

The fabricated aptasensor can also be applied for the detection of MUC1 protein in serum samples. This is due to the fact that the detection of cancer cells by the MUC1 aptamer was carried out through the MUC1 protein presented on the cell surface [34]. The electrochemical aptasensor for detection of MUC1 was prepared completely similar to the MCF-7 cancer cell aptasensor. The MCF-7 cells were replaced by MUC1 and the specific interaction between aptamer and MUC1 ensured capture of MUC1 on the surface of electrode. Fig. S3 shows the current response of the biosensor after incubating with different MUC1 concentration. The peak current was increased proportionally to concentration and a linear response was obtained in the range of 5–200 ng·mL^{-1}, with detection limit of 3 ng·mL^{-1}. In order to demonstrate the utility of this biosensor in clinical assay, a series of serum samples from women patients who have been subjected to breast cancer were analyzed by biosensor and ELIZA method. According

Table 2 The recovery percent values for cancer cell analysis (n = 3)

Sample solution	Added (cells·mL^{-1})	Found (cells·mL^{-1})	Recovery (%)
1	1.0×10^2	$0.98(\pm0.04) \times 10^2$	98.0
2	1.0×10^3	$1.02(\pm0.02) \times 10^3$	102.8
3	1.0×10^5	$1.01(\pm0.03) \times 10^5$	101.0

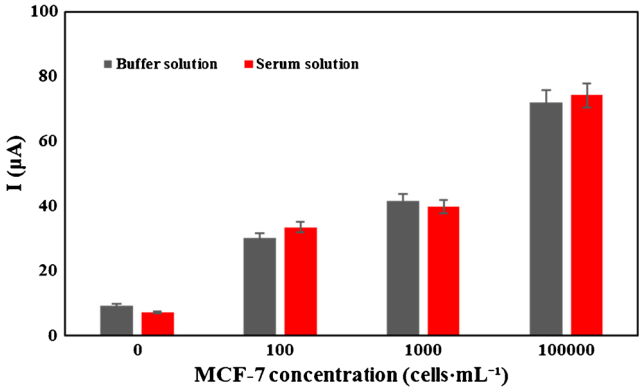


Fig. 5 Comparison of peak current of differential pulse voltammogram for buffer solution and diluted human serum samples containing the same concentration of MCF-7 cancer cell (0, 10^2, 10^3, 10^5 cells·mL^{-1}). Experimental conditions are the same as Fig. 3a

to the results in table. S1, the fabricated biosensor, no significant differences were observed between the values obtained by electrochemical and ELIZA method. The aptasensor bioassay demonstrated a promising potential application to screen clinical samples.

Concluding remarks

In the present work an ultrasensitive sandwich-type biosensor has successfully designed to detect of MCF-7 human breast cancer cell and MUC1 biomarker. A MUC1-binding aptamer is immobilized on the modified GCE surface captures the target and the other aptamer labeled with silver nanoparticles provides a well-defined electrochemical characteristic. The employment of PGA-MWNNT nanocomposite as an electrode modifier increases the loading amount of aptamers on the electrode surface and can preserve their bioactivity. The aptasensor has good selectivity to MCF-7 cancer cell and MUC1 protein. The fabricated sandwich-type aptasensor is successfully used for determination of cancer cells in human serum sample. The electrochemical biosensor is a cost saving method and offers robust results compared with classical analytical techniques such as Enzyme-Linked Immunosorbent Assay (ELISA). However, the main limitations of the method are being time-consuming and having rather complex procedure. Based on the results, we anticipate that the method can be expanded for the detection and quantification of other cancer cells with the aid of related aptamers and provides a promising tool in biomedical and clinical application.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun M (2009) Cancer statistics, 2009. CA Cancer J Clin 59:225–249
2. Berois N, Varangot M, Aizen B, Estrugo R, Zarantonelli L, FernaÂdez P, Krygier G, Simonet F, Barrios E, Muse ÂI (2000) Molecular detection of cancer cells in bone marrow and peripheral blood of patients with operable breast cancer. Comparison of CK19, MUC1 and CEA using RT-PCR. Eur J Cancer 36:717–723
3. Lacroix M (2006) Significance, detection and markers of disseminated breast cancer cells. Endocr Relat Cancer 13:1033–1067
4. Benvidi A, Firouzabadi AD, Tezerjani MD, Moshtaghiun S, Mazloum-Ardakani M, Ansarin A (2015) A highly sensitive and selective electrochemical DNA biosensor to diagnose breast cancer. Electroanal Chem 750:57–64
5. Hamula C, Zhang H, Li F, Wang Z, Le C, Li X (2011) Selection and analytical applications of aptamers binding microbial pathogens. Trends Anal Chem 30:1587–1597
6. Ding DF, Ge Y, Zhang SS (2010) Electrochemical and electrochemiluminescence determination of cancer cells based on aptamers and magnetic beads. Chemistry 16:10707–10714
7. Pieve CD, Perkins AC, Missailidis S (2009) Anti-MUC1 aptamers: radiolabelling with ^{99m}Tc and biodistribution in MCF-7 tumour-bearing mice. Nucl Med Biol 36:703–710
8. Etayash H, Jiang K, Azmi S, Thundat T, Kaur K (2015) Real-time detection of breast cancer cells using peptide-functionalized microcantilever arrays. Sci Rep 5:13967
9. Liu R, Wang Q, Li Q, Yang X, Wang K, Nie W (2016) Surface plasmon resonance biosensor for sensitive detection of microRNA and cancer cell using multiple signal amplification strategy. Biosens Bioelectron 87:433–438
10. Wang YZ, Hao N, Feng QM, Shi HW, Xu JJ, Chen HY (2016) A ratiometric electrochemiluminescence detection for cancer cells using g-C3N4 nanosheets and ag– PAMAM–luminol nanocomposites. Biosens Bioelectron 77:76–82
11. Hao L, Yue H, Yue Y, Weiwei L, Yongmei Y, Genxi L (2015) Peptide-based method for detection of metastatic transformation in primary tumors of breast Cancer. Anal Chem 87:9251–9256
12. Xie H, Li H, Huang Y, Wang X, Yin Y, Li G (2014) Combining peptide and DNA for protein assay: CRIP1 detection for breast Cancer staging. ACS Appl Mater Interfaces 6:459–463
13. Amouzadeh Tabrizi M, Shamsipur M, Saber R, Sarkar S, Zolfaghari N (2017) An ultrasensitive sandwich-type electrochemical immunosensor for the determination of SKBR-3 breast cancer cell using rGO-TPA/FeHCFnano labeled anti-HCT as a signal tag. Sensors Actuators B Chem 243:823–830
14. Chang K, Pi Y, Lu W, Wang F, Pan F, Li F, Jia S, Shi J, Deng S, Chen M (2014) Label-free and high-sensitive detection of human breast cancer cells by aptamer-based leaky surface acoustic wave biosensor array. Biosens Bioelectron 60:318–324
15. Kashefi-Kheyrabadi L, Mehrgardi MA, Wiechec E, Turner APF, Tiwari A (2014) Ultrasensitive detection of human liver hepatocellular carcinoma cells using a label-free aptasensor. Anal Chem 86: 4956–4960
16. Wang K, Fan D, Liu Y, Wang E (2015) Highly sensitive and specific colorimetric detection of cancer cells via dual-aptamer target binding strategy. Biosens Bioelectron 73:1–6
17. Tao G, Fengzhen L, Dawei Y, Yue Y, Zhaoxia W, Genxi L (2015) Assembly of selective biomimetic surface on an electrode surface: a design of nano−bio interface for biosensing. Anal Chem 87:5683–5689
18. Amouzadeh Tabrizi M, Shamsipur M, Saber R, Sarkar S, Sherkatkhameneh N (2017) Flow injection amperometric sandwich-type electrochemical aptasensor for the determination

of adenocarcinoma gastric cancer cell using aptamer-Au@Ag nanoparticles as labeled aptamer. Electrochim Acta 246:1147–1154
19. Bagheri A, Raoof J, Ojani R, Kavoosian S (2017) Ultrasensitive electrochemical aptasensor based on sandwich architecture for selective label-free detection of colorectal cancer (CT26) cells. Biosens Bioelectron 92:630–637
20. Jing Z, Huinan C, Yingying T, Hong C, Guifang C, Yongmei Y, Genxi L (2018) Research progresses on the functional polypeptides in the detection and imaging of breast cancer. J Mater Chem B 6: 2510–2523
21. Kumar S, Ahlawat W, Kumar R, Dilbaghi N (2015) Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare. Biosens Bioelectron 70:498–503
22. Sung MH, Park C, Kim CJ, Poo H, Soda K, Ashiuchi M (2005) Natural and edible biopolymer poly-gamma- glutamic acid: synthesis, production, and applications. Chem Rec 5:352–366
23. Li Q, Tang D, Tang J, Su B, Huang J, Chen G (2011) Carbon nanotube-based symbiotic coaxial nanocables with nanosilica and nanogold particles as labels for electrochemical immunoassay of carcinoembryonic antigen in biological fluids. Talanta 84:538–546
24. Benvidi A, Yazdanparast S, Rezaeinasab M, DehghanTezerjani M, Abbasi S (2018) Designing and fabrication of a novel sensitive electrochemical aptasensor based on poly(L-glutamic acid)/ MWCNT modified glassy carbon electrode for determination of tetracycline. Electroanal Chem 808:311–320
25. Li H, Sun ZY, Zhong WY, Hao N, Xu DK, Chen HY (2010) Ultrasensitive electrochemical detection for DNA arrays based on silver nanoparticle aggregates. Anal Chem 82:5477
26. Moniruzzaman M, Winey KI (2006) Polymer nanocomposites containing carbon nanotubes. Macromolecules 39:5194–5205
27. Haibing L, Zhimin C, Cuiping H (2009) Glutathione-stabilized silver nanoparticles as colorimetric sensor for Ni^{2+} ion. Sensors Actuators B Chem 143:87–92
28. Li J, Xu M, Huang H, Zhou J, Abdel-Halimb E, Zhang JR, Zhu JJ (2011) Aptamer-quantum dots conjugates-based ultrasensitive competitive electrochemical cytosensor for the detection of tumor cell. Talanta 85:2113–2120
29. Yan M, Sun G, Liu F, Lu J, Yu J, Song X (2013) An aptasensor for sensitive detection of human breast cancer cells by using porous GO/Au composites and porous PtFe alloy as effective sensing platform and signal amplification labels. Anal Chim Acta 798:33–39
30. Zhu X, Yang J, Liu M, Wu Y, Shen Z, Li G (2013) Sensitive detection of human breast cancer cells based on aptamer-cell-aptamer sandwich architecture. Anal Chim Acta 764:59–63
31. Li T, Fan Q, Liu T, Zhu X, Zhao J, Li G (2010) Detection of breast cancer cells specially and accurately by an electrochemical method. Biosens Bioelectron 25:2686–2689
32. Jia Sh LP, Koh K, Chen H (2016) A cytosensor based on NiO nanoparticle-enhanced surface plasmon resonance for detection of the breast cancer cell line MCF-7. Microchim Acta 183:683–688
33. Guo Q, Li X, Shen C, Zhang S, Qi H, Li T (2015) Electrochemical immunoassay for the protein biomarker mucin 1and for MCF-7 cancer cells based on signal enhancement by silver nanoclusters. Microchim Acta 182:1483–1489
34. Hua X, Zhou Z, Yuan L, Liu S (2013) Selective collection and detection of MCF-7 breast cancer cells using aptamer-functionalized magnetic beads and quantum dots based nano-bioprobes. Anal Chim Acta 788:135–140