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A novel electrochemical biosensor based on polyadenine modified aptamer for label-free and ultrasensitive detection of human breast cancer cells

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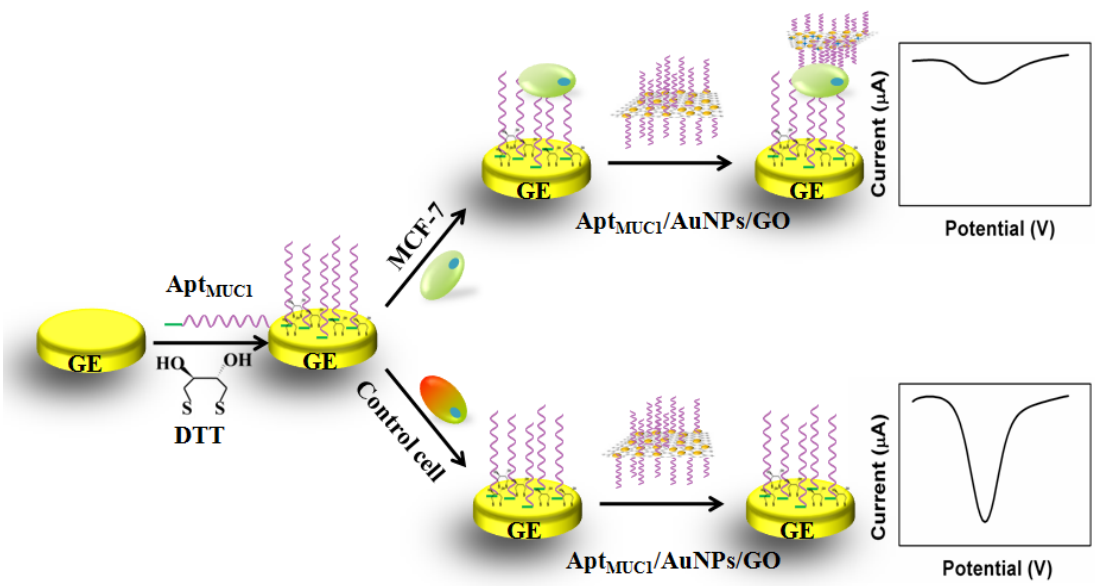
Abstract

Simple, rapid, sensitive, and specific detection of cancer cells plays a pivotal role in the diagnosis and prognosis of cancer. A sandwich electrochemical biosensor was developed based on polyadenine (polydA)-aptamer modified gold electrode (GE) and polydA-aptamer functionalized gold nanoparticles/graphene oxide (AuNPs/GO) hybrid for the label-free and selective detection of breast cancer cells (MCF-7) via a differential pulse voltammetry (DPV) technique. Due to the intrinsic affinity between multiple consecutive adenines of polydA sequences and gold, polydA modified aptamer instead of thiol terminated aptamer was immobilized on the surface of GE and AuNPs/GO. The label-free MCF-7 cells could be recognized by polydA-aptamer and self-assembled onto the surface of GE. The polydA-aptamer functionalized AuNPs/GO hybrid could further bind to MCF-7 cells to form a sandwich sensing

system. Characterization of the surface modified GE was carried out by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using $Fe(CN)_6^{3-/4-}$ as a redox probe. Under the optimized experimental conditions, a detection limit of 8 cells•mL^{-1} (3σ/slope) was obtained for MCF-7 cells by the present electrochemical biosensor, along with a linear range of 10 to 10^5 cells•mL^{-1}. By virtue of excellent sensitivity, specificity and repeatability, the present electrochemical biosensor provides a potential application in point-of-care cancer diagnosis.

Graphical abstract



Keywords: biosensor, polydA, aptamer, MUC1 protein, breast cancer cell.

1. Introduction

In 2008, it was reported that more than 12 million people suffered from cancer and approximate 7.6 million deaths were caused by cancer [1]. Among these deaths, breast cancer took up the second major cause for women, and most cancer deaths resulted from neoplasm metastasis [2,3]. Since early diagnosis and treatment are vital in the improvement of cancer survival rate [4], the precise detection of cancer cells is highly desired for reducing mortality from cancer [5,6]. In this context, an increasing number of biosensors have been developed for detection of cancer cells, including colorimetric [7,8], electrochemical [9,10] and fluorescent biosensors [11,12]. Among these biosensors, electrochemical biosensors show a promising approach in biochemical analysis due to their excellent advantages of ultrasensitivity, simple preparation, low cost and low background [13-16]. So far, electrochemical biosensors have made great development in detection method, electrode material and capture. Li *et al.* reported an anodic stripping voltammetry method for the detection of breast cancer cell number by immobilizing cancer cells onto gold electrode (GE) with hydrosulfuryl functional aptamer [17]. Tiwari *et al.* reported a sandwich architecture for the capture of target cancer cells by immobilizing an amino-labeled aptamer onto the carboxylic acid modified GE via coupling chemistry [18].

Recently, aptamer-based electrochemical biosensors have been paid much attention due to their excellent properties [19,20]. Aptamer is a single strand nucleic acid synthesized using SELEX (systematic evolution of ligands by exponential enrichment) [21]. In comparison to antibody, aptamer has a few unique advantages

such as easy synthesis, good stability and high selectivity [22,23], which is widely applied in the cellular study and bioassay [24,25]. For the achievement of bioconjugation with the electrode, aptamer usually has to be modified with functional groups, e.g., amino and hydrosulfuryl [26,27]. However, it is a complicated and high-cost process for the modification of aptamer with functional groups. In addition, the functional group may affect the affinity of aptamer to target analyte. It was reported that polyadenine (polydA) sequences containing multiple consecutive adenines could be immobilized on gold surface by virtue of a high affinity, even comparable to the Au−S chemical interaction [28]. Therefore, analogous to the role of the hydrosulfuryl group, polydA was employed to immobilize aptamer onto the surface of GE for the development of an electrochemical biosensor.

Graphene oxide (GO) has been applied to construct various biosensors due to its unique electronic, optical, mechanical and catalytic properties [29-32]. Dong *et al.* reported a novel sandwich system for detection of thrombin by the amplification of aptamer functionalized gold nanoparticles/reduced graphene oxide hybrid [33]. Considering the complexity and high cost for modification of hydrosulfuryl, we developed a simple, ultrasensitive, high selective and low cost electrochemical biosensor based on the strongly binding affinity between polyadenine (polydA) aptamer and GE, and the outstanding performance of graphene oxide (GO). The mucin 1 protein (MUC1) was selected as a biomarker of breast cancer cell [34]. The MUC1 aptamer (Apt_{MUC1}) was modified with polydA and afterwards immobilized on the surface of GE. The Apt_{MUC1} functionalized gold nanoparticles/GO (AuNPs/GO)

hybrid could further bind to breast cancer cell to form a sandwich sensing system, which was beneficial to significantly enhance the signal response. It was noteworthy that the Apt_{MUC1} used in our strategy saved the modification with any functional groups (e.g. –SH, -NH_2). The present electrochemical biosensor exhibited an excellent analytical performance for the detection of MCF-7.

2. Experimental

2.1. Chemicals and materials

Ultrapage purified aptamer for MUC1 (5'-GCAGT TGATC CTTTG GATAC CCTGG $TTTTTA_{10}$-3') was synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). DL-Dithiothreitol (DTT) was purchased from Sigma aldrich. Oligonucleotides were stored at -20°C, and were heated to 95°C for 5 min and gradually cooled to room temperature before use. Cary 60 UV/Vis Spectrophotometer (Agilent, USA) was used to quantify the oligonucleotides at 260 nm. The other chemicals were purchased from Aladdin reagent (Shanghai, China) and used as received without further purification. Milli-Q ultrapure water was used throughout (specific resistance > 18 MΩ).

2.2. Preparation of Apt_{MUC1}/AuNPs/GO

GO was prepared according to a modified Hummer's method [35]. 10 mL of 0.25 mg•mL^{-1} GO and 40 mg of polyvinyl pyrrolidone (PVP) were mixed by magnetic stirring. After washed with ultrapure water and ethanol for three times, separately, a mixture of PVP/GO was dispersed in 2.5 mL of ultrapure water. Then, 0.1 mL of 10 wt % PDDA, 8.4 mL of 0.625 M KCl and 2.1 mL of PVP/GO were mixed under

sonication for 90 min to obtain PDDA/GO. AuNPs were prepared by citrate reduction. 100 mL of 1 mM $HAuCl_4$ aqueous solution was stirred in a round-bottom flask and heated to boil. Then, 10 mL of 40 mM citrate was added to form AuNPs. AuNPs/GO hybrid was prepared by mixing 1 mL of PDDA/GO and 10 mL of AuNPs under stirring for 24 h. The obtained product was washed for 3 times and dispersed in 2 mL of ultrapure water.

Apt_{MUC1}/AuNPs/GO hybrid was prepared by mixing 0.1 mL of 100 μM Apt_{MUC1} and 0.9 mL of AuNPs/GO in phosphate buffered saline (PBS) buffer containing 0.3 M NaCl. After continuous shaking for 24 h, the obtain product was washed with PBS buffer for 3 times. Then, 50 μL of 0.1 mM bull serum albumin (BSA) was added to the above mixture to block the remaining bonding sites on the surface of AuNPs/GO. The prepared Apt_{MUC1}/AuNPs/GO hybrid was dispersed in 1 mL of PBS buffer for the ensuing experiment.

2.3. Surface modification of electrode

GE with a diameter of 2 mm was initially polished with 0.3 and 0.05 μm alumina slurry until a mirror plane was formed. The electrode was afterwards sonicated in the ethanol and ultrapure water for 1 min, separately, to remove the excess Al_2O_3 powder. After that, GE was electrochemically cleaned in 0.1 M H_2SO_4 solution with successive cyclic voltammetry (CV) scans from 0 V to +1.5 V until reproducible voltammograms were observed. Finally, GE was washed with ultrapure water and dried with nitrogen gas.

1 μM Apt_{MUC1} and 200 μM DTT were dissolved in PBS buffer. 10 μL of the

above mixture solution was taken out and dropped onto the surface of GE within 16 h at 4°C. After surface modification, the electrode was rinsed thoroughly with PBS buffer to remove the excess aptamers and DTT, and dried with nitrogen gas. Thus, a sensing interface for the electrochemical detection of MCF-7 was successfully prepared.

2.4. Electrochemical detection

The prepared GE was immersed in various concentrations of the MCF-7 solution and incubated for 20 min at 37°C. Then, GE was rinsed with ultrapure water to remove the nonspecific binding cells. Subsequently, the GE bonding MCF-7 cells was immersed in an Apt_{MUC1} functionalized AuNPs/GO solution. GE was used as the working electrode.

The measurements of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on an electrochemical workstation (CH Instruments 660D, Shanghai Chenhua Equipment, China) equipped with three-electrode system including a sandwich GE working electrode, a platinum wire counter electrode and a silver/silver chloride reference electrode. The characterization of electrochemical impedance spectroscopy (EIS) was performed on an electrochemical workstation (Autolab, Metrohm). All the measurements were conducted in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. CV was carried out within a potential range from −0.3 V to +0.6 V at a scan rate of 0.1 V/s. DPV was carried out within a potential range from −0.1 to +0.6 V at a scan rate of 4 mV/s and a modulation amplitude of 50 mV. Electrochemical impedance spectra for GE were

carried out in a frequency range of 1 MHz to 0.1 Hz at AC amplitude of 5 mV.

2.5. Cell culture

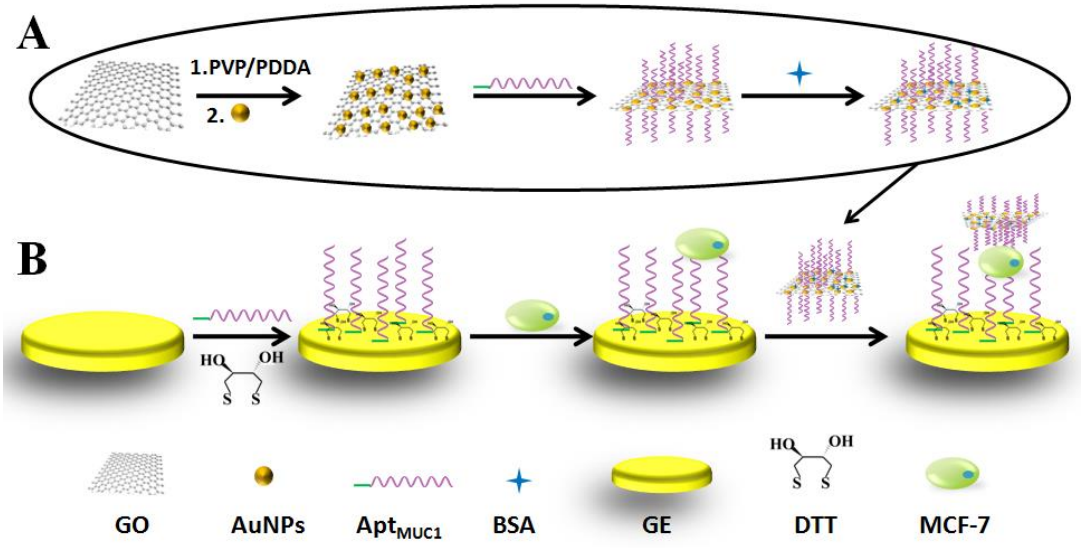
MCF-7 human breast cancer cells were cultured in RoswellPark Memorial Institute (RPMI 1640) medium supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The cell density was determined using a Petroff Hausser cell counter (USA).

3. Results and discussion

3.1. Principle of a sandwich electrochemical biosensor for MCF-7 detection

In a traditional method, aptamer has to be modified with functionalized groups before use [26,27]. For instance, a strong Au-S binding was widely utilized to self-assemble thiolated aptamer onto the surface of GE and functionalize AuNPs. In view of the high cost of aptamer modification, we developed a new strategy for evading the modification at aptamer terminal to construct a sandwich electrochemical biosensor. The principle of a sandwich electrochemical biosensor for MCF-7 detection is depicted in Scheme 1. Since polydA could be adsorbed on the surface of GE by virtue of a high affinity, polydA was first bound at Apt_{MUC1} terminal and followed by being immobilized onto the surface of GE [28]. This strategy saved the cost for aptamer functionalization, and reduced the potential influence of affinity between aptamer and target analyte. In order to eliminate superfluous signal produced by nonspecific adsorption, DTT was used as masking agent to cover the remaining bonding sites on the surface of GE [36]. Subsequently, Apt_{MUC1} functionalized AuNPs/GO hybrid was bound to GE binding MCF-7 cells to form a sandwich sensing

system. Since negative Apt_{MUC1} on the surface of AuNPs/GO hybrid repelled the redox couple, the sandwich sensing system possessed a high sensitivity. In the absence of MCF-7 cells, the redox couple could pass through the modification layer to generate an electrochemical signal. In the presence of MCF-7 cells, MCF-7 cells could be captured based on a specific interaction between Apt_{MUC1} and MUC1 on the surface of MCF-7 cells. The binding between GE bound MCF-7 cells and Apt_{MUC1} functionalized AuNPs/GO hybrid would bring a dramatic steric hindrance on the redox couple transfer through GE, leading to an enhanced signal change. Therefore, MCF-7 cells could be detected sensitively and specifically by the present sandwich sensing system.



Scheme 1. Schematic illustration of a sandwich electrochemical biosensor for MCF-7 detection.

3.2. Morphology of AuNPs/GO hybrid

As shown in Scheme 1A, AuNPs/GO hybrid was prepared by self-assembly of

AuNPs and GO. TEM image in Fig. 1A indicates that the prepared AuNPs had a larger dispersibility with an average diameter of 13 nm. As shown in Fig. 1B/C, the as-prepared GO with a smooth surface would become rough after loading AuNPs. Furthermore, few free AuNPs could be observed outside of GO, indicating AuNPs were successfully adsorbed on the surface of GO. Subsequently, Apt_{MUC1} was functionalized on the surface of AuNPs/GO hybrid by the interaction between polydA and Au.

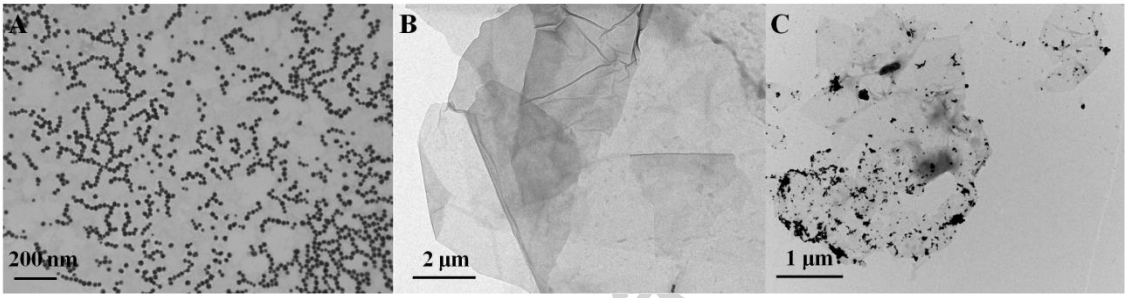


Fig. 1. TEM images of the as-prepared AuNPs (A), GO (B), and AuNPs/GO (C).

3.3. Electrochemical characterization of the sensing interface

Cyclic voltammetry and alternating-current impedance were utilized for characterizing the surface modification of GE with Apt_{MUC1}/DTT, MCF-7 and Apt_{MUC1}/AuNPs/GO. $[Fe(CN)_6]^{3-/4-}$ was used as an efficient indicator for electrode surface characteristics [37]. As shown in Fig. 2A, a pair of symmetrical redox peaks was observed using unmodified GE (black curve). When GE was modified with Apt_{MUC1} and DTT, the peak-to-peak potential difference increased obviously and the peak current decreased in the meantime, indicating that the electron transfer was restrained. Upon the addition of MCF-7 cells, larger potential difference and smaller

peak current were observed, indicating the successful immobilization of breast cancer cells on GE. After the conjugation of Apt_{MUC1}/AuNPs/GO and GE binding MCF-7 cells, Apt_{MUC1} on the surface of AuNPs/GO repelled the redox couple. The variations of potential difference and peak current showed a reasonable trend in line with the estimate.

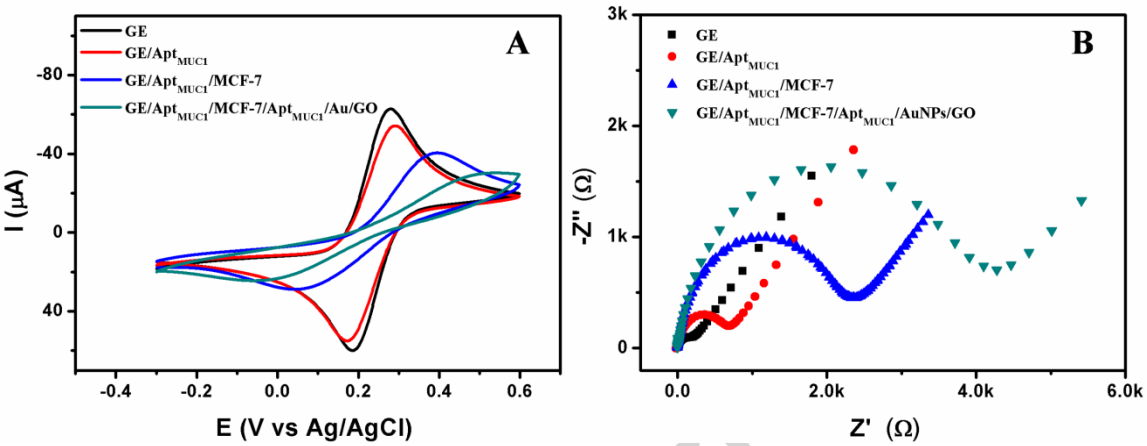


Fig. 2. (A) Characterization of GE after each step of surface modification by cyclic voltammetry measurements. (B) Characterization of GE after each step of surface modification by alternating-current impedance measurements. The concentration of MCF-7 was 10^4 cells•mL^{-1}.

EIS was also used to characterize the process of surface modification with the aid of the redox couple $[Fe(CN)_6]^{3-/4-}$. As shown in Fig 2B, a minor increase of impedance could be observed after the immobilization of Apt_{MUC1} onto GE, due to a higher resistance of electron transfer (red curve). While MCF-7 cells were immobilized onto Apt_{MUC1} modified GE, a further barrier for the electrochemical process was produced, resulting in a significant increase of the electron-transfer resistance (black curve). After the formation of sandwich architecture, a further

increase in electron-transfer resistance was observed. According to the data of CV and EIS, Apt_{MUC1} was confirmed to be modified on the surface of GE through a high affinity between polydA and Au. Meanwhile, Apt_{MUC1} would not be separated from the surface of GE in the presence of MCF-7 cells.

3.4. Optimization of experimental variables

The incubation time of MCF-7 cells was a key factor for both capturing breast cancer cells and improving the performances of biosensor. The effect of the incubation time was investigated within 0-30 min in the presence of MCF-7 cells with a certain concentration. As shown in Fig. S1, the electrochemical signal was gradually enhanced with increasing incubation time of MCF-7 cells from 0 min to 20 min, and then leveled off within 20-30 min, indicating a thorough capture of MCF-7 cells on the surface of GE. Therefore, 20 min of incubation time was selected for capturing MCF-7 cells on the surface of GE. In order to amplify the signal response, the effect of the binding time of Apt_{MUC1} functionalized AuNPs/GO hybrid was also investigated by monitoring the signal response of DPV. In Fig. S2, peak current was gradually enhanced with increasing the binding time up to 40 min, and then leveled off within 40-50 min. Therefore, 40 min was used as an optimal binding time of Apt_{MUC1} functionalized AuNPs/GO hybrid.

3.5. Quantitative detection of MCF-7 cells

The sensitivity of the present sandwich sensing system as an important feature for biosensors was investigated by using DPV. In this case, DPV as an extremely sensitive electrochemical detection method for trace analysis was carried out by

monitoring the current change induced by binding MCF-7 cells and Apt_{MUC1}/AuNPs/GO to Apt_{MUC1} modified GE. The redox couple $[Fe(CN)_6]^{3-/4-}$ was utilized as an indicator for the electrochemical measurement. Moreover, the process of DPV measurement was so rapid that MCF-7 cells would hardly be seriously injured, which was conducive to maintain the activity of MCF-7 cells. To estimate the sensitivity and linear range of the electrochemical biosensor for the detection of MCF-7 cells, various concentrations of MCF-7 cells (0, 10, 10^2, 10^3, 10^4, 10^5 cells•mL^{-1}) were examined. Fig. 3 illustrates that the responses of DPV curves increased apparently with the increase of MCF-7 cell concentration from 0 cells•mL^{-1} to 10^5 cells•mL^{-1}.

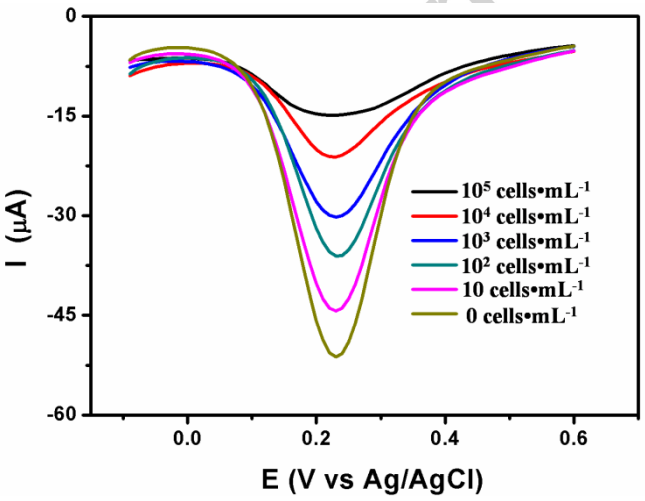


Fig. 3. Responses of DPV curves to the different concentrations of MCF-7 cells ranging from 0 to 10^5 cells•mL^{-1} by the present sandwich sensing system.

In order to quantify the ratio of peak current change, the ratio $(I_{MCF\text{-}7}\text{-}I_{blank})/(I_{10^5MCF\text{-}7}\text{-}I_{blank})$ was utilized to elucidate the results (Fig 4A). Where I_{blank} is the peak current of DPV curve in the absence of MCF-7 cells, $I_{10^5MCF\text{-}7}$ is the

maximum peak current after GE is saturated by 10^5 cells•mL^{-1} MCF-7 cells, and I_{MCF-7} is defined as the corresponding peak current exposed to the solution with a certain concentration of MCF-7 cells. As shown in Fig. 4A, the rate of peak current change is in a logarithm relation with the number of MCF-7 cells. Therefore, a calibration curve is obtained between the ratio of peak current change and the logarithm number of target cells ranging from 10 cells•mL^{-1} to 10^5 cells•mL^{-1} (Fig 4B). A detection limit (LOD) as low as 8 cells•mL^{-1} with a wide detection range was obtained based on the calculation of 3σ/slope. The sensitivity of the present sandwich electrochemical biosensor is superior to that of many reported biosensors for MCF-7 detection, as listed in Table 1. The results demonstrate that the proposed biosensor presents a more sensitive sensing platform for the detection of cancer cells.

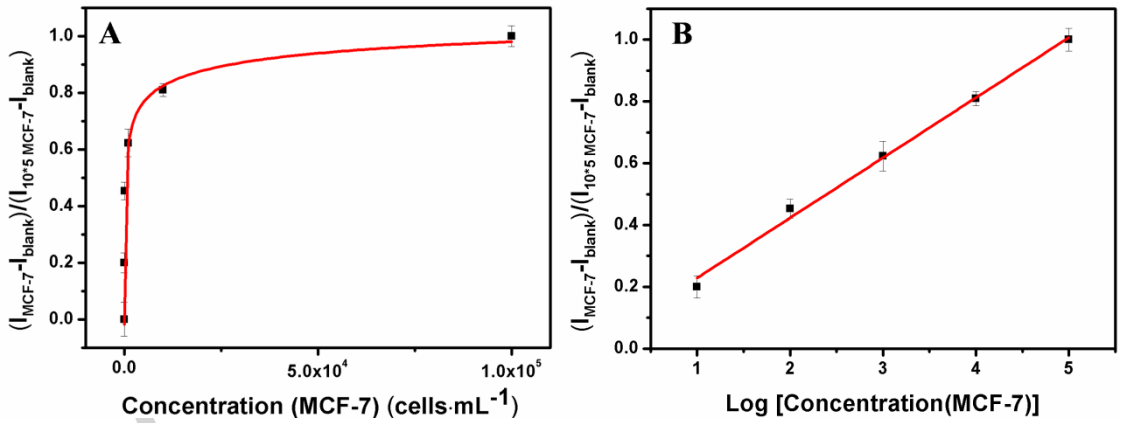


Fig. 4. (A) Nonlinear fitting curve of peak current change ratio versus MCF-7 cell concentration with error bars. (B) Calibration curve of peak current change ratio versus logarithm of MCF-7 cell concentration with error bars.

Table 1. Comparison of various MCF-7 sensors.

Analytical method	Detection limit	Ref.
Colorimetric detection	125 cells•mL^{-1}	[38]
Fluorescence detection	10 cells•mL^{-1}	[39]
Electrochemical detection	32 cells•mL^{-1}	[40]
Electrochemiluminescent detection	230 cells•mL^{-1}	[41]
Our approach	8 cells•mL^{-1}	

3.6. Selectivity of the electrochemical biosensor towards MCF-7 cells

It is significantly important to distinguish target cancer cells from normal cells and other cancer cells for cancer diagnosis and therapy [42]. In order to evaluate the selectivity of the electrochemical biosensor, the measurement was performed by using 10^5 cells•mL^{-1} HL 7702, HEK 293, HeGp2 and 10^2 cells•mL^{-1} Hela, MCF-7, respectively. As illustrated in Fig. 5, HepG2, HEK 293, HL 7702 and Hela cells show a little current change after incubated in the sensing system, but a well-defined current change was obtained for MCF-7 cells. The different current changes depend on the cell type and the selectivity of aptamer. The MUC1 protein was limited expressed on the membrane of HEK 293, HL 7702 and HeGp2 cells. The control cells could not be captured by Apt_{MUC1} due to the lack of a high specific affinity, leading to no obvious current change. Compared with signal response of MCF-7 cells, little changes were observed in the case of 10^2 cells•mL^{-1} HeLa. The higher concentration of Hela cells can lead to some signal response, which resulted from the MUC1 protein over

expressed on the Hela cells [43,44]. However, Hela cells could not affect the selectivity of the electrochemical biosensor for the determination of MCF-7 cells at the same concentration and could be solved by multiplex biomarker detection. This result demonstrates that the present electrochemical biosensor exhibits a high selectivity for the detection of MCF-7 cells and has great potential for practical application.

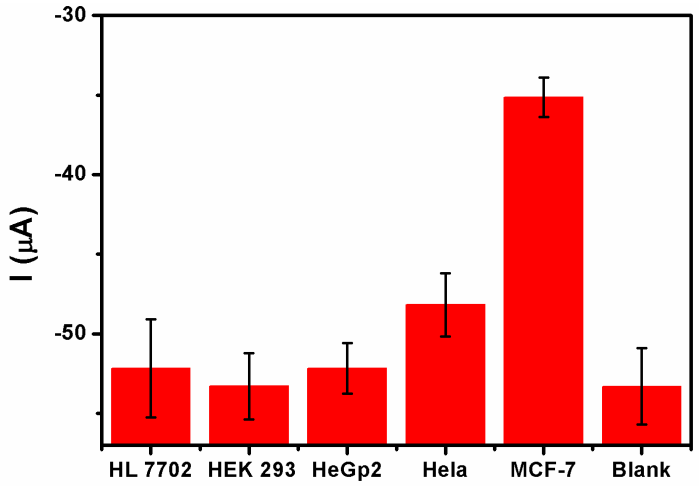


Fig. 5. Selectivity for detection of MCF-7 cells. HL7702 (10^5 cells•mL^{-1}), HEK293 (10^5 cells•mL^{-1}), HeGp2 (10^5 cells•mL^{-1}), Hela (10^2 cells•mL^{-1}), and MCF-7 (10^2 cells•mL^{-1}).

3.7. Detection of MCF-7 cells in human serum

In order to demonstrate the potential application of the electrochemical biosensor for the detection of MCF-7 cells in complicated real sample, MCF-7 cells were spiked in 10% human serum at various concentrations and assayed as outlined above. As shown in Fig. 6, a trend similar to that of the pure buffer solution was observed, confirming that the proposed electrochemical biosensor could be applied for the

detection spiked with different concentrations of MCF-7 cells in a complicated sample matrix. These results confirmed the practical value of the developed biosensor, and the fabricated electrochemical biosensor exhibits great promising in clinical application for the detection of MCF-7 cells.

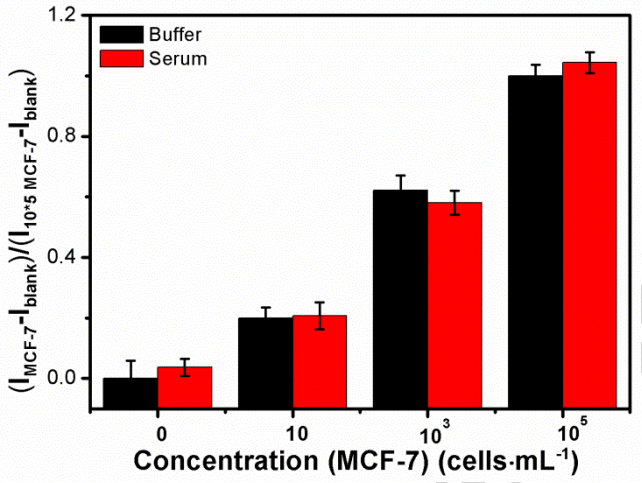


Fig. 6. Results obtained from the buffer solution and diluted human serum samples.

4. Conclusions

In this study, a novel electrochemical biosensor based on polydA modified aptamer was developed for the detection of human breast cancer cells. Since MCF-7 cells over express MUC1 proteins, Apt_{MUC1} modified on GE was used to capture MCF-7 cells by virtue of good specificity, meanwhile Apt_{MUC1} functionalized AuNPs/GO hybrid was used to improve the sensitivity of biosensor. It was demonstrated that the present electrochemical biosensor had a high selectivity in discriminating MCF-7 cells towards normal cells and other cancer cells, and an excellent detection limit of 8 cells•mL^{-1} as well as a wide linear range from 10 to 10^5 cells•mL^{-1}. Compared with traditional methods, the present electrochemical biosensor

based on label-free aptamer could improve the affinity between aptamer and MCF-7 cells and save the cost of aptamer modification. The proposed sensing system provides an ultrasensitive, highly specific, label-free and low cost assay, and has a potential application in point-of-care cancer diagnosis.

Acknowledgements

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Highlights

- A label-free and sandwich electrochemical biosensor was developed for MCF-7 detection.
- Aptamer was anchored on the surface of gold electrode by using polyadenine instead of thiol.
- The electrochemical biosensor offered high sensitivity and selectivity toward MCF-7 breast cancer cells.