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Sensitive detection of human breast cancer cells based on aptamer–cell–aptamer sandwich architecture

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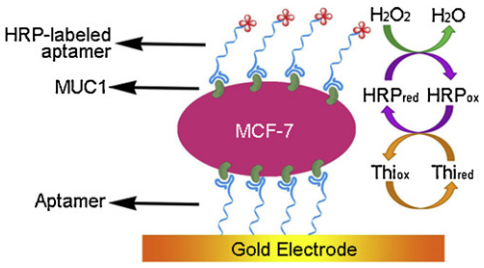
HIGHLIGHTS

- An electrochemical biosensor for the detection of MCF-7 cells was fabricated.
- An aptamer–cell–aptamer sandwich architecture was constructed on an electrode.
- Dual-recognition and enzyme-linked amplification were well integrated.
- Favorable sensitivity and selectivity can be achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

Breast cancer is one of the most critical threats to the health of women, and the development of new methods for early diagnosis is urgently required, so this paper reports a method to detect Michigan cancer foundation-7 (MCF-7) human breast cancer cells with considerable sensitivity and selectivity by using electrochemical technique. In this method, a mucin 1 (MUC1)-binding aptamer is adopted to recognize MCF-7 human breast cancer cells, while enzyme labeling is employed to produce amplified catalytic signals. The molecular recognition and the signal amplification are elaborately integrated by fabricating an aptamer–cell–aptamer sandwich architecture on an electrode surface, thus a biosensor for the detection of MCF-7 is fabricated based on the architecture. The detection range can be from 100 to 1×10^7 cells, and the detection limit can be as low as 100 cells. The method is also cost-effective and conveniently operated, implying potential help for the development of early diagnosis of breast cancer.

1. Introduction

Breast cancer is the most common type of malignant tumor in women (comprising 22.9% of all cancer types) [1]. Although significant progress has been made in both the understanding and treatment of this cancer during the last few decades, it still remains one of the leading causes of death [2]. Early diagnosis and timely treatment have been considered to be the most effective ways to improve survival rate at present [3]. However, despite the diagnostic improvements [4–7], the detection of breast cancer in early stages is still a challenge [8]. A main bottleneck is the lack of effective molecular contrast agents, which can disclose the presence of early-stage malignancies, tracking cell migration, and monitoring surgical and pharmacological response [9].

The discovery of aptamer, especially those selected for binding tumor markers or cancer cells, may provide a potential solution for early diagnosis and therapy for cancers [10,11]. Aptamer-based detection may not only exhibit desirable selectivity, specificity, but

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also show some distinguished advantages, such as ease in molecular design and modification, and the capability of passing through some barriers due to its low molecular weight [12,13]. Therefore, based on the discovery of DNA aptamers targeting mucin 1 (MUC1) [14], which is a glycoprotein expressed on the apical surface of epithelial cells and whose overexpression is often associated with some kinds of cancers [15], we and some others have developed a few methods to detect MUC1-overexpressed breast cancer cells, in which MUC1-binding aptamers and different measuring techniques have been well applied [16–19]. These efforts have provided a novel concept for the early diagnosis of breast cancer. However, it is also noticed that the sensitivity, which is critical for early diagnosis, might still have space for further improvement. For example, although electrochemical technique is well known for its high sensitivity, previous studies could only have obtained a detection range from 10^4 to 10^7 cells [16,17].

Here, we propose a novel electrochemical method for the detection of Michigan cancer foundation-7 (MCF-7) human breast cancer cells, in which dual-recognition and enzyme-linked amplification are well integrated by using an aptamer–cell–aptamer sandwich architecture. Favorable detection selectivity and superior sensitivity can thereby be achieved. The detection range can be as wide as $100 - 1 \times 10^7$ cells, which is the best among the aptamer-based biosensors as we know. Therefore, it may have great potential for the development of early diagnosis of breast cancer.

2. Experimental

2.1. Materials

Thiolated MUC1 DNA aptamer (HPLC purified) were manufactured by Invitrogen Biotechnology Co. Ltd. The sequence was 5′-HS-GCA GTT GAT CCT TTG GAT ACC CTG G-3′. Horse radish peroxidase (HRP, approx. 300 units per mg solid using pyrogallol as substrate), albumin from bovine serum (BSA), 6-mercapto-1-hexanol (MCH), tris(2-carboxyethyl) phosphine (TCEP), N-(ε-maleimidocaproyloxy) sulfosuccinimide (Sulfo-EMCS), and thionine were purchased from Sigma, and used without further purification. Other chemicals were all of analytical grade and used as received. All solutions were prepared with double-distilled water, with a specific resistance of >18 MΩ cm, and stored at 4 °C.

2.2. Instrumentation

Double distilled water was purified with a Direct-8 Millipore purification system. The state of cells was observed under an inverted microscope (DMIRB, Leica). The cell density was determined by an automated cell counter (TC10, Bio-RAD) and further confirmed with hemocytometer counting. An Amicon filtration device 30,000 cut-off from Millipore was used to separate out impurities with small molecular weight by centrifuge filtration during the synthesis of HRP-labeled MUC1 aptamer. Electrochemical measurements were performed on a model 660C Electrochemical Analyzer (CHI Instruments).

2.3. Cell culture and preparation

MCF-7 human breast cancer cells and SP2/0 mouse myeloma cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and incubated at 37 °C in a humidified incubator (5% CO_2–95% air). Those cells at the end of the log phase were selected for the following experiments. Cells were detached with 0.02% EDTA and concentrated by centrifugation at $1000 \times g$ for 5 min. The obtained cells were dispersed in sterile pH 7.4 PBS and then concentrated by centrifugation again. Finally, the sediment was re-suspended to PBS containing 0.5% Tween-20 and 1% BSA.

2.4. Bioconjugation of horse radish peroxidase with MUC1 aptamer

The conjugation of HRP with thiolated MUC1 aptamer was achieved by using a cross-linker sulfo-EMCS [20]. Firstly, 2 μM HRP was incubated with 10 μM Sulfo-EMCS in a final volume of 0.5 mL for 1 h at room temperature to form HRP-Sulfo-EMCS. Redundant Sulfo-EMCS was removed by centrifuge filtration. The HRP-Sulfo-EMCS was dissolved to a concentration of 2 μM in PBS (0.1 M, pH 7.4). Then, 10 μM MUC1 aptamer, which was protected by TCEP (1 mM) to prevent the formation of disulfide groups, was allowed to react with the HRP-Sulfo-EMCS for 2 h at room temperature. Excess free aptamer and TCEP were removed from the solution also by centrifuge filtration. The produced HRP-labeled MUC1 aptamer was finally dissolved to a concentration of 2 μM in PBS (0.1 M, pH 7.4).

2.5. Preparation of MUC1 aptamer functionalized gold electrode

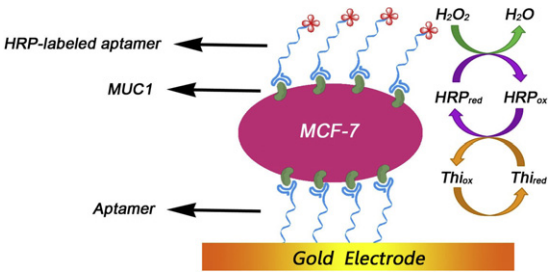
A substrate gold electrode (2 mm in diameter) was firstly polished on microcloth (Buehler) with gamma micropolish deagglomerated alumina suspension (particle size of ~0.05 μm), and the residual alumina powder was removed by sonicating in both ethanol and water for 5 min, respectively. Then, the electrode was electrochemically cleaned in 1 M H_2SO_4 to remove any remaining impurities. After being rinsed with water and dried with nitrogen, the electrode which was ready for chemical modification was incubated with thiolated MUC1 aptamer (1 μM in a buffer solution containing 10 mM Tris–HCl, 1 mM EDTA, 0.1 M NaCl, and 1 mM TCEP) for 16 h, followed by a treatment of an aqueous solution of 1 mM MCH for 2 h. After being thoroughly rinsed with pure water and dried with nitrogen, the MUC1 aptamer functionalized electrode was prepared and could be ready for use.

2.6. Assembly of aptamer–cell–aptamer sandwich

The cells dispersed in RPMI cell media buffer were firstly centrifuged at 920 rpm for 5 min, and washed by a phosphate buffered solution (PBS, 0.01 M, pH 7.4) for twice. Then, they were incubated in a PBS containing 0.5% Tween-20 and 1% BSA for 30 min. After that, the cells were separated from the PBS by centrifugation and resuspended into a sterile PBS containing 1 mM Ca^{2+} and 1 mM Mg^{2+}. The cell suspension was then incubated with the above prepared MUC1 aptamer functionalized electrode at room temperature for 2 h to allow the capture and immobilization of the cells onto the electrode surface. After being rinsed with 0.01 M pH 7.4 PBS containing 0.5% Tween-20 to remove the nonspecific adsorbed cells, the electrode was incubated with the HRP-labeled MUC1 aptamer (20 μl, 2 μM) for 1 h to allow the formation of an aptamer–cell–aptamer sandwich.

2.7. Electrochemical measurements

A three-electrode system was used for the measurements, in which the sandwich architecture modified gold electrode was adopted as the working electrode. A saturated calomel reference electrode (SCE) and a platinum electrode served as the reference and counter electrode, respectively. The parameters for electrochemical measurements have been described in the captions of figures without repetitious description here.



Scheme 1. Schematic illustration of the aptamer sandwich-based and enzyme-linked electrochemical detection of MCF-7 human breast cancer cells.

3. Results and discussion

As is shown in Scheme 1, only in the presence of the target cells, the sandwich architecture can be formed on the electrode surface, and the electrochemical response catalyzed by HRP, which is labeled on the MUC1 aptamer, may thereby be obtained with the help of an electron mediator thionine. Because the aptamer recognizes the target cells twice, the specificity is better than a single recognition. Furthermore, benefited from the electrochemical catalysis of HRP, enzyme-linked signal amplification is also achieved. So, the sensitivity of this method will be highly improved compared with previous reports.

3.1. Characterization of the aptamer–cell–aptamer sandwich

In order to confirm the formation of the sandwich architecture on the working electrode surface, the commonly used electrochemical technique, electrochemical impedance spectroscopy (EIS, Nyquist plots) has been adopted to characterize the sandwich architecture. As is shown in Fig. 1, the bare electrode exhibits only a tiny semicircle domain (dots a, charge-transfer resistance (Rct) is calculated to be 72 Ω), which implies fast electron transfer process. After the monolayer of aptamer is formed on the electrode surface, due to both the steric and electrostatic exclusion between the aptamer and the electrochemical probe $[Fe(CN)_6]^{4-/3-}$, an increase of the electron transfer resistance can be observed (dots b, Rct is 1.0 kΩ). Moreover, further increase of the resistance can be observed when target cells and HRP-labeled aptamer are introduced in sequence (Rct is 5.6 kΩ and 7.7 kΩ, respectively), indicating the capture of target cells and the formation of aptamer–cell–aptamer sandwich. It is also noticed that the degree of the increase (b–c (4.6 kΩ) > c–d (2.1 kΩ) > a–b (0.9 kΩ)) coincides with the size of the substances in different layers (cells > HRP-labeled aptamer > aptamer), which verify the composition of the sandwich.

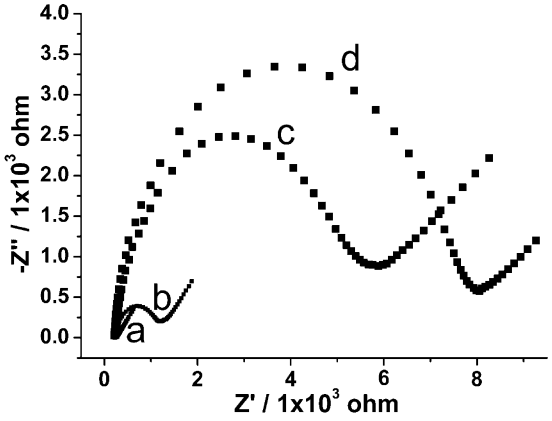


Fig. 1. Electrochemical impedance spectra (Z_{im} vs. Z_{re}, Nyquist plots) of (a) bare electrode, (b) aptamer monolayer modified electrode, (c) aptamer–cell bilayer modified electrode, and (d) aptamer–cell–HRP-labeled aptamer sandwich modified electrode. Test solution: 10 mM PBS, pH 7.4, containing 0.1 M NaCl, and 5 mM $[Fe(CN)_6]^{4-/3-}$ probe. The bias potential was 0.224 V vs. SCE. The frequency was from 100 kHz to 1 Hz and the amplitude was 5.0 mV.

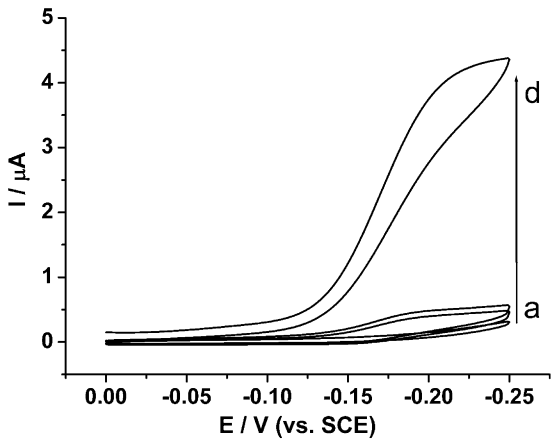


Fig. 2. Cyclic voltammograms obtained at the aptamer–cell–HRP-labeled aptamer sandwich modified electrode (a) in the absence of both thionine and H_2O_2, (b) in the presence of thionine. Curves (c) and (d) show the cases in the presence of both thionine and H_2O_2, while an aptamer–cell bilayer modified electrode is employed for (c). Test solution: 10 mM PBS containing 20 μM thionine and 6 mM H_2O_2. Scan rate was 5 mV s^{-1}.

3.2. Obtaining of the enzyme-linked catalytic signals

We have then studied the catalysis of HRP in the sandwich to obtain the catalytic signals for the detection of cells. As is shown in Fig. 2, no catalytic wave can be observed in a scan range from 0 V to −0.25 V in the absence of the catalytic substrates (curve a). Upon addition of both hydrogen peroxide (H_2O_2) and thionine to the test solution, an apparent reduction current with the shape of a catalytic wave can be observed (curve d), suggesting the occurrence of the HRP-catalyzed and thionine-mediated electrochemical reduction of H_2O_2.

As controls, in the case that only thionine is present (curve b), or if HRP-labeled aptamer is not introduced (curve c), only a tiny reduction peak current can be observed, owing to the direct electrochemical reduction of thionine (oxidation state). The net currents at −0.25 V (background current subtracted) are ~17 and ~26 times smaller than that both the enzyme and the two substrates have been introduced in the system. These results have also confirmed that HRP labeling has not only benefited the obtaining of the measured signals, but also provided the opportunities for the sensitive detection MCF-7 cells.

Next, we have studied the influence of the quiet time (time between the addition of the substrates and the starting of the electrochemical measurements) on the electrochemical response. As is shown in Fig. 3A, the HRP-linked sandwich presents a maximum current response if the electrochemical measurements are launched immediately on addition of the substrates. With the extension of the quiet time, it can be observed that the current decreases accordingly. Amperometric *i*–*t* curve shows the tendency of the current changes more intuitively (Fig. 3B), which also confirms the observation from cyclic voltammogram. Since a high current response is beneficial for the detection sensitivity, electrochemical measurements are performed immediately after the addition of the substrates in the following experiments.

3.3. Detection of the target MCF-7 cells

By making use of the catalytic signals, we have then introduced different numbers of MCF-7 cells in the sandwich, and studied the

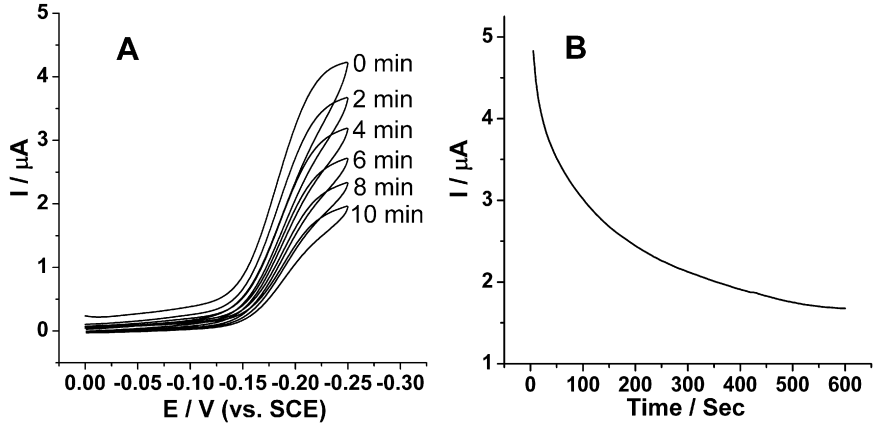


Fig. 3. (A) Cyclic voltammograms and (B) Amperometric *i*–*t* curve obtained at the aptamer–cell–HRP-labeled aptamer sandwich modified electrode with different quiet times. Others same as in Fig. 2(d).

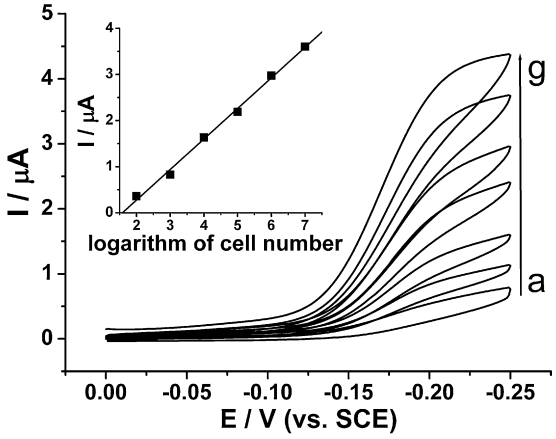


Fig. 4. Cyclic voltammograms obtained at the aptamer–cell–HRP-labeled aptamer sandwich modified electrode fabricated with (a)–(g): 0, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, and 1×10^7 MCF-7 cells. Others same as in Fig. 2(d). The insert shows the relationship between the net peak current (blank response has been subtracted from each data point) and the cell numbers.

electrochemical behaviors to check the possibility to sensitively detect the cells. As is shown in Fig. 4, the catalytic peak currents rise apparently along with the increase of the cell numbers. The results are reasonable and expected. A large number of cells will induce the accumulation of more HRP-labeled MUC1 aptamers onto the electrode, which may greatly accelerate the catalysis and produce more oxidized thionine in a scan cycle. The oxidized thionine subsequently participates in the electrochemical reduction process, making a catalytic current proportional to the amount of the thionine. In a word, the more the target cells are introduced, the more HRP will be recruited, and the higher the catalytic currents will be obtained. The insert of Fig. 4 shows the relationship between the net peak currents (blank response has been subtracted from each data point) at −0.25 V and the cell numbers. It can be observed the currents have a linear relation with the logarithm of cell numbers in a range from 100 cells to 1×10^7 cells, the equation of which is $Y = -1.049 + 0.662X$ ($R = 0.998$). The results show that a favorable detection toward the MCF-7 cells with a large detection range and an excellent detection limit is thereby achieved with the help of the fabrication of HRP-labeled sandwich and the adopting of electrochemical technique.

3.4. Selectivity of the biosensor

Results from the control experiments using SP2/0 cells instead of MCF-7 cells show that the detection also has good selectivity (Fig. 5). Comparing the peak current shown in Figs. 4 and 5, we can know that the net current for 1×10^7 SP2/0 cells is equivalent to that for only 1×10^2 MCF-7 cells. On the other hand, benefited from the dual-recognition of this strategy and the high sensitivity of this method, the specificity of this biosensor can be favorable. The unspecific adsorption of SP2/0 cells and the little expression of

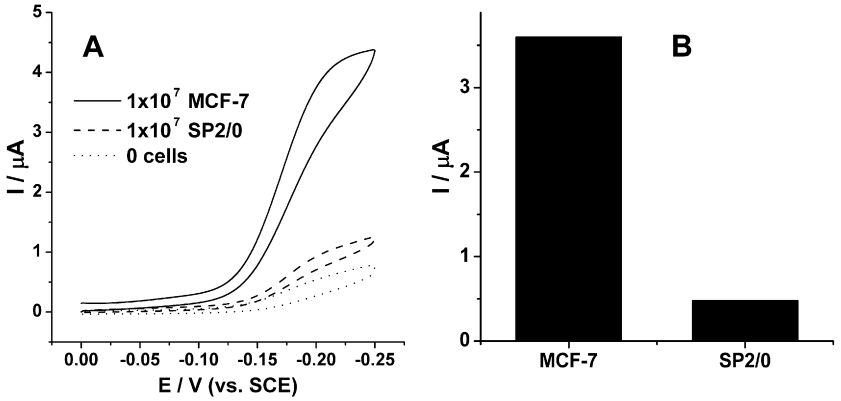


Fig. 5. (A) Cyclic voltammograms obtained at the aptamer–cell–HRP-labeled aptamer sandwich modified electrode fabricated with 0 cells (dot line), 1×10^7 MCF-7 cells (solid line), 1×10^7 SP2/0 cells as control (dash line). Others same as in Fig. 4. (B) Relationship between the net peak currents (current in the case of 0 cells subtracted) and cell types.

MUC1 on the surface of SP2/0 cells may be the cause for the slight catalytic current.

4. Conclusion

In summary, we have fabricated a sandwich architecture and employed electrochemical technique to sensitively detect MCF-7 human breast cancer cells. In the sandwich, the target cells are recognized twice by MUC1 aptamer to provide favorable selectivity. In the meantime, benefited from the catalysis of HRP, enzyme-linked signal amplification can be achieved. So, the detection range of the developed biosensor can be as wide as $100 - 1 \times 10^7$ cells, and the detection limit can be as low as 100 cells, both of which are the best compared with the reported biosensors for the detection of cells. Therefore, it may have great potential for the early diagnosis of breast cancer. Moreover, this proposed method can be also extended for the detection of some other cancer cells by using corresponding aptamers, e.g., Ramos cell-targeted aptamer in the future.

Acknowledgments

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