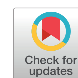
Sensors and Actuators B 270 (2018) 1–8

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Ultrasensitive and versatile homogeneous electrochemical cytosensing platform based on target-induced displacement reaction for "signal-on" bioassay



Chengcheng Gu[a,1], Panpan Gai[a,1], Xiaojuan Liu[a], Jing Liu[b], Feng Li[a,*]

[a] College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, PR China
[b] Central Laboratory of Qingdao Agricultural University, Qingdao 266109, PR China

ARTICLE INFO

Article history:
Received 3 November 2017
Received in revised form 4 April 2018
Accepted 28 April 2018
Available online 4 May 2018

Keywords:
Homogeneous electrochemical cytosensing platform
Target-induced displacement reaction
Signal-on assay
Tumor cell detection

ABSTRACT

We proposed, for the first time, a ultrasensitive amplification-free, label-free, and enzyme-free homogeneous electrochemical cytosensing platform based on the target-induced displacement reaction for tumor cell "signal-on" assay. To realize this proposal, the electroactive probe, $[Fe(CN)_6]^{3-}$, was entrapped in the pores of positively charged MSN (PMSN), and then the negatively charged aptamer of the tumor cell was adsorbed on the surface of PMSN as a biogate. Once the tumor cells were recognized and captured by the aptamer, the biogate could be efficiently opened and the entrapped $[Fe(CN)_6]^{3-}$ released, which can be ascribed to the decreased adhesion between the aptamer-cell complex and PMSN. Consequently, the electrochemical signal dramatically increased. Overall, the "signal-on" strategy was conveniently realized. Notably, the detection limit for tumor cells assay (13 cells/mL) is comparable to those of the reported methods Furthermore, based on the similar proposal, this biosensor also can be extended for the ultrasensitive detection of microRNA (38 aM, S/N = 3) and Hg^{2+} (0.47 pM, S/N = 3). Therefore, this biosensing platform has the great potential to be a powerful tool in the applications of disease diagnostics and environmental monitoring.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Cancer has become one major cause of mortality for human beings, and every year, more than eight million people died of cancer [1]. Therefore, it is imperative to develop reliable methodology for early diagnosis of cancer. To date, many methods, including real-time-polymerase chain reaction (RT-PCR)-based DNA tests [2,3], protein microarray [4], serum proteomic patterns [5–7], computer tomography [8], have been applied in cancer diagnosis. However, these methods suffer from such drawbacks as complex sample treatment and/or expensive instrumentation. Recently, electrochemical cytosensors have drawn great attention because of their noticeable merits of high sensitivity, rapid response, simple instrumentation and low cost [9–15]. Unfortunately, the relatively complicated immobilizing and washing procedures lead to long assay time, poor reproducibility or even low electrochemical signal, which restrict their further development and wider applications. By comparison, the homogeneous and immobilization-free electrochemical biosensors avoid the aforementioned drawbacks, and have the noticeable advantages of operational simplicity and improved target recognition efficiency [16]. Moreover, they have been widely applied in the detection of various targets, such as metal ions, ATP, DNA methyltransferase activity, alkaline phosphatase activity, cancer biomarkers, microRNAs and telomerase activity [17–27], exhibiting the merits of rapidness, high sensitivity and good selectivity. However, there also existed some shortcomings, such as sophisticated amplification procedures [28], relatively high cost of the labeling of the oligonucleotides or the strict reaction conditions of the tool enzymes [22]. Therefore, to circumvent the aforementioned issues and achieve breakthroughs in practical application, it is urgently needed to develop a homogeneous electrochemical cytosensing platform for accurate and sensitive bioassay of tumor cells.

As we know, mesoporous silica nanoparticles (MSN) can effectively achieve the loading of substrates due to the porous structure and the capped and gated MSN have attracted substantial research interest in the controlled release systems [29–32]. Among them, DNA could not only specifically recognize the targets, but also capsulate the guest molecules as an ingenious bio-gate [29,32]. Hence,

* Corresponding author.
E-mail address: lifeng@qust.edu.cn (F. Li).
[1] C. C. Gu & P. P. Gai contributed equally to this work.

https://doi.org/10.1016/j.snb.2018.04.167
0925-4005/© 2018 Elsevier B.V. All rights reserved.

homogeneous electrochemical "signal-on" assays could be easily performed, if the electroactive probes could be sealed in the pores of MSN by DNA-biogates, and then released when the target was present.

Herein, we proposed, for the first time, a new homogeneous electrochemical cytosensing platform based on the target-induced displacement reaction for "signal-on" assay of the tumor cells, which was an amplification-free, label-free, and enzyme-free strategy. In details, as shown in Scheme 1A, the electroactive probe $[Fe(CN)_6]^{3-}$ was entrapped in the pores of positively charged MSN (PMSN), and the negatively charged aptamer of the tumor cell was adsorbed on the surface of PMSN as a bio-gate through the electrostatic interaction. As a result, the bioconjugate Apt-capped PMSN formed and played the key role in the as-proposed strategy. Taking the breast cancer cells (SK-BR-3) as a model target, initially, in the absence of the target, $[Fe(CN)_6]^{3-}$ was encapsulated in the pores of PMSN, and almost no $[Fe(CN)_6]^{3-}$ was present in the solution. Thus, only a small electrochemical signal was observed. Once the cells were recognized and captured by the aptamer (Scheme 1B), the bio-gate aptamer could be opened efficiently due to the decreased adhesion between the Apt-cell complex and the bioconjugate, which would then trigger the controlled release of the entrapped $[Fe(CN)_6]^{3-}$. Subsequently, the electrochemical signal significantly increased, thus realizing the "signal-on" bioassay. Furthermore, to verify the versatility of the as-proposed strategy, we employed different ssDNA caps to realize the ultrasensitive determination of other targets, such as microRNA and heavy metals ions. This effective biosensing strategy possesses the unique advantages of high sensitivity, simplicity, low cost, rapidness and high accuracy, thus, having great potential to be used as a versatile tool for bioassay and playing an important role in the early diseases diagnosis and environmental monitoring.

2. Experimental section

2.1. Materials and reagents

HPLC-purified microRNA and HPLC-purified DNA oligonucleotides were obtained from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Tetraethoxysilane (TEOS), cetyltrimethylammonium bromide (CTAB), and 3-aminopropyltriethoxysilane (APTES), were all purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Mercuric chloride ($HgCl_2$) and tris(hydroxymethyl)aminomethane (Tris) were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Before use, the microRNA and csDNAs were diluted with 100 mM Tris-HCl (pH 7.4) to give the stock solutions. Ultrapure water (resistivity > 18.2 MΩ cm at 25 °C) was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). DEPC-treated ultrapure water was used in all experiments. All reagents were of analytical grade and used without further purification. The sequences of the oligonucleotides are listed in Table S1.

2.2. Apparatus and instrumentation

Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. Dynamic light scattering (DLS) was measured by Malvern Zetasizer Nano ZS90 (Malvern, UK). X-ray photoelectron spectroscopy (XPS) analysis was carried out on a Thermo Fisher X-ray photoelectron spectrometer system. Differential pulse voltammetry (DPV) measurements were performed on a CHI 660E electrochemical workstation (Shanghai CH Instrument Co., China) using a three-electrode system: the Indium Tin Oxides (ITO) as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl as the reference electrode. All experiments were carried out at room temperature (25 ± 1 °C).

2.3. Cell lines and cell culture

SK-BR-3 cells, CCRF-CEM cells and K562 cells were obtained from Nanjing Key Gen Biotech Co., Ltd (Nanjing, China), and cultured in a flask in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) in an incubator (5% CO_2, 37 °C). MCF-7 cells, HL-60 cells, HeLa cells, and 293T cells were obtained from Nanjing Key Gen Biotech Co., Ltd and seeded in DMEM medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), penicillin (100 μg/mL), and streptomycin (100 μg/mL) in 5% CO_2, 37 °C incubator. At the logarithmic growth phase, the cells were collected and separated from the medium by centrifugation at 1000 rpm for 2 min and then suspended in the binding buffer (both 4.5 g L^{-1} glucose and 5 mM $MgCl_2$ dissolved in D-PBS with $CaCl_2$ and $MgCl_2$) to obtain a homogeneous cell suspension.

2.4. Preparation of MSN

Mesoporous silica nanoparticles (MSN) were prepared according to literature with slight modifications [29]. Briefly, 850 μL of NaOH (2.0 M) was added into CTAB solution (0.2 wt%) and stirred for 20 min at 80 °C. And under continuous stirring, 1.25 mL of TEOS was dropped into the above solution and vigorously stirred for 2 h to obtain the white precipitates. Subsequently, the precipitate was collected by centrifugation and washed with ultrapure water and methanol sequentially, and then dried vacuum at 60 °C. Afterwards, the obtained solid product was refluxed for 10 h using a solution composed of hydrochloric acid and methanol to remove the surfactant template. And then the obtained MSN was washed thoroughly with methanol, and dried before using.

2.5. Encapsulation of $[Fe(CN)_6]^{3-}$ into Apt-Capped PMSN

50.0 mg of the as-prepared MSN was dissolved to 1.0 mL of ethanol by sonication and then 1.0 mL of 1.0 M $[Fe(CN)_6]^{3-}$ solution was added into the system with gently shaking at room temperature for 12 h, which resulted in the $[Fe(CN)_6]^{3-}$ into the pores of the MSN *via* diffusion reaction. Subsequently, 0.4 mL of APTES was dropped into the above solution and stirred continuously for 6 h at room temperature. During this process, the amine groups were immobilized on the surface of MSN, thus resulting in the formation of positively charged NH_2-MSN (PMSN). Following that, the precipitate was collected by centrifugation (5000 rpm, 5 min) and washed at least three cycles with ethanol and ultrapure water to remove any un-entrapped $[Fe(CN)_6]^{3-}$, and the precipitate was resuspended into 1.0 mL Tris-HCl buffer (pH 7.4, containing 0.1 M NaCl). 100 μL of aptamer of the tumor cell was incubated with 500 μL of the as-proposed suspension at room temperature for 4 h under gentle stirring. During this process, the aptamer was attached onto the PMSN *via* electrostatic adsorption. And then the mixture was collected by centrifugation (3000 rpm, 2 min) and washed one time to remove redundant aptamer and obtained aptamer-capped PMSN (denoted as Apt-capped PMSN). Finally, the obtained Apt-capped PMSN loaded with $[Fe(CN)_6]^{3-}$ was resuspended into 1.0 mL Tris-HCl buffer (pH 7.4, containing 0.1 M NaCl).

2.6. Electrode pretreatment and homogeneous electrochemical measurement

The indium tin oxide (ITO) electrode was pretreated by being sequentially sonicated in an Alconox solution (8 g of Alconox per

Download English Version:

https://daneshyari.com/en/article/7139013

Download Persian Version:

https://daneshyari.com/article/7139013

Daneshyari.com