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Label-free and competitive aptamer cytosensor based on layer-by-layer assembly of DNA-platinum nanoparticles for ultrasensitive determination of tumor cells

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ABSTRACT

A label-free and competitive electrochemical cytosensor based on layer-by-layer (LBL) assembly of DNA-platinum nanoparticles (DNA-PtNPs) for signal amplification was developed for the tumor cell determination. In this study, three different sizes of PtNPs were synthesized and successfully characterized. The PtNPs with the highest electrocatalytic activity were selected as nanocarriers. The thiolated TLS11a aptamer, with high affinity to human liver hepatocellular carcinoma (HepG2) cells, was covalently attached to the gold nanoparticles (AuNPs) deposited on indium tin oxide (ITO) glass. Meanwhile, nanoprobes were fabricated through ferrocene-labeled complementary DNA (cDNA-Fc) immobilized on the surfaces of the PtNPs. LBL technology could provide abundant signal tags and efficient signal amplifiers for electrochemical cytosensing. When the target cells competed with cDNA to bind with aptamer, double-stranded DNA was denatured and PtNPs-DNA bioconjugates were released from the ITO electrode, resulting in decreased current response. Thus the current change was related linearly to the logarithm of cell concentration from 50 to 1×10^6 cells mL^{-1} with a low detection limit of 15 cells mL^{-1}, acceptable stability and reproducibility. Furthermore, the enzyme-free cytosensor could be regenerated through an electrochemical reductive desorption technique. The cytosensor possesses potential applications in early cancer diagnosis and treatment.

1. Introduction

Cancer has the second highest death rate in worldwide diseases, and attracts more concerns [1,2]. It is acknowledged that early diagnosis and detection of tumor can provide an effective means of monitoring the disease progress, increasing greatly the opportunities for clinical treatment and improving significantly the survival rate of patients. Thus, accurate and early detection of cancer is of utmost importance in cancer diagnosis and therapy. Over the past few decades, various techniques have been applied in cancer cell research, such as immunohistochemistry [3], fluorescence measurement [4], polymerase chain reaction (PCR) [5], and flow cytometry [6]. Nevertheless, most of the techniques are laborious, poorly selective, and time-consuming or involve high-cost instrumentation. Since the density of cancer cells in the peripheral blood is relatively low, early stage determination still remains a major challenge that it is urgently desired to develop a fast, cost effective, specific and ultrasensitive method of monitoring cancer.

So far, electrochemical detection methods have gained much attention in cancer cell detection owing to their preponderant features [7–10]. Compared with other detection methods, electrochemical biosensors provide an attractive solution due to the advantages of rapid response, operational simplicity, acceptable sensitivity, and cost effective. In spite of above advantages in cancer diagnosis, the challenge for cytosensors is still to develop new protocols for the improvement of selectivity, sensitivity, and reliability [11]. Further, these electrochemical cytosensors are basically based on a traditional sandwich structure. Herein, a competitive strategy was introduced into our cytosensing platform. The competition assay is simple, fast, highly sensitive and specific, and reliable, reducing the problem of false-positive results [12–14]. Incorporation of the competition assay into electrochemical cytosensing is rarely reported at present. As a promising tool, it could open new insight into cancer detection.

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Aptamers (Apt) play essential roles in the competition assay development. They are the artificial single-stranded nucleic acid molecules, which have the capability to specifically recognize and bind to various targets, including small molecules, proteins and cells [15,16]. They have shown low immunogenicity, good stability, and ease of chemical modification compared to antibodies. In electrochemical sensing, based on these salient properties, aptamers have been widely used as promising molecule probes for the detection of cancer cells [17–19]. Many electrochemical aptasensors have emerged in the past years, and they are still in constant development and improvement. They integrate the advantages of electrochemical sensors and the high selectivity to target cells of aptamer. For instance, Feng's group developed a label-free electrochemical impedance spectroscopy ultrasensitive cytosensor for CTCs combined with specific recognition of EpCAM aptamer [20].

In addition, the use of specifically designed signal-amplifying electrocatalysts can improve the detection sensitivity of electrochemical biosensors. Loading enzymes onto electrodes or nanomaterials is a common strategy for signal amplification based on enzymatic eletrocatalysis [21–23]. Nevertheless, enzymes are relatively costly, unstable and susceptible to slight changes in the environment, leading to inactivation [24]. Therefore, nonenzymatic electrocatalysts with robust and high electrocatalytic activities are highly desirable [25–27]. Especially, noble-metal nanoparticles have exhibited outstanding catalytic efficiencies with great potential in the application of electrochemical biosensors [28,29]. Up to now, Pt nanoparticles are still an ideal catalyst on account of good electrical conductivity, superior electrocatalytic activity, high specific surface area, and satisfactory biocompatibility [30]. Generally, the electrocatalytic performances depend intimately on the particle size and dispersity. To further enhance signal amplification efficiency of electrocatalysts, there is a need for available methods to immobilize catalysts and signal tags. Compared with other signal amplification strategies including hybridization chain reaction (HCR) [31], rolling circle amplification (RCA) [32], and strand displacement amplification (SDA) [33], the layer-by-layer (LBL) assembly technique may provide abundant signal amplifiers and signal tags to improve the detection sensitivity, which has aroused more interests [34,35]. The development of microelectrode arrays has generated a dramatic increase in electrochemistry research programs [36–38]. There are numerous advantages to microelectrode array-based detection systems, such as reducing sample and reagent consumption, increasing sensitivity, integrating multiple processes, parallel analysis and high throughput.

In this work, we selected the indium tin oxide (ITO)-based microelectrode array for the operation platform and designed a label-free and enzyme-free electrochemical cytosensor for ultrasensitive detection of cancer cells. The thiolated TLS11a aptamer, which can specifically recognize human liver cancer HepG2 cells [39], was immobilized onto gold nanoparticles (AuNPs) modified ITO glass by gold—thiol bond. Here, it is worthwhile to note that platinum nanoparticles (PtNPs) were employed not only as electrocatalysts to enhance the detection signal, but also as nanocarriers for cDNA-Fc. PtNPs-DNA bioconjugates provided Fc as signal indicator. Particularly, LBL assembly of PtNPs-DNA based on the hybridization of two single-stranded DNAs was employed to achieve signal amplification. Our cytosensing strategy involved competition between the target HepG2 cells and cDNA for binding toTLS11a aptamer, forming an innovative and competitive electrochemical cytosensor for ultrasensitive determination of HepG2 cells. Meanwhile, label-free detection protected the nature property and viability of the cells, which was conducive to do follow-up studies on enriched cancer cells. Moreover, the AuNPs/ITO electrode could be easily regenerated by electrochemical reductive desorption to reutilize the cytosensor.

2. Experiment

2.1. Chemicals and materials

6-Mercapto-1-hexanol (MCH) was purchased from J&K Scientific Ltd. (Guangzhou, China). Chloroplatinic acid hexahydrate ($H_2PtCl_6{\cdot}6H_2O$), chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$), sodium citrate, H_2O_2 (30%, w/w), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), and bovine serum albumin (BSA) were all purchased from Aladin Chemistry Co., Ltd. (Shanghai, China). Sylgard 184 curing agent and Polydimethylsiloxane (PDMS) elastomer were both obtained from Dow Corning (Midland, MI, USA). The DNA oligonucleotides were synthesized and purified from Sangon Biotech. Co., Ltd. (Shanghai, China) and the sequences are listed in Table S1. Phosphate buffer (PB) was prepared with $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$. Phosphate-buffered saline (PBS, pH 7.4, 10 mM) containing 2.7 mM KCl, 137 mM NaCl, 1.4 mM KH_2PO_4, and 8.7 mM Na_2HPO_4 was used as washing solution. PBS buffer (pH 7.0, 100 mM) was chosen as the electrolyte for detection. All chemicals were of analytical grade. Ultrapure water (18.2 MΩ cm, Milli-Q, Millipore) was used during the experiment.

ITO-coated glass (resistance <15 Ω per square and 150 nm thick) was obtained from South China Xiang Science & Technology Co., Ltd. (Shenzhen, China).

2.2. Instrument conditions

The morphologies of samples were investigated by Transmission electron microscopy (TEM, FEI Tecnai G2 Spirit, Netherlands), scanning electron microscopy (SEM, FEI Quanta 400, Netherlands), and scanning probe microscope (SPM, dimension fastscan bio, Bruker). Size Distribution images were obtained using dynamic light scattering (Nano ZS, Malvern). The captured cells were imaged using a fluorescence microscope (Olympus BX53, Japan).

All electrochemical measurements were carried out on a RST5200F electrochemical workstation (Suzhou Risetest Instrument Co., Ltd., Suzhou, China) with a three-electrode system: a platinum wire auxiliary electrode, an Ag/AgCl-saturated KCl reference electrode (Gaoss Union Technology Co., Ltd., Wuhan, China) and a modified or bare ITO working electrode (Φ = 2 mm).

2.3. Synthesis of platinum nanoparticles (PtNPs)

Based on the previously reported methods, PtNPs were prepared with slight modification [40]. Firstly, H_2PtCl_6 solution (1 mL, 1% w/v) was added into 100 mL water. The solution was heated to boiling and sodium citrate solution (3 mL, 1% w/v) was added rapidly. Then, the obtained mixture was kept at a boil with continuous stirring for ca. 30 min. PtNPs with average diameter 5 nm were produced. In the process of the synthesis of larger PtNPs, under vigorous stirring, sodium citrate (0.33 mL, 1% w/v) was added into boiling H_2PtCl_6 solution (100 mL, 0.03% w/v). After 30 min, sodium citrate solution (2.67 mL, 1% w/v) was added into the mixture solution, and then kept boiling for 1 h. PtNPs with average diameter 40 nm were synthesized. To further produce larger PtNPs, 0.5 mL of 1% (w/v) sodium citrate and 3 mL of 1% (w/v) H_2PtCl_6 were used. An average diameter of the resulting PtNPs was 80 nm. Accordingly, three different sizes of PtNPs, which had different catalytic activity, were synthesized and finally stored at 4 °C for subsequent experiments.

2.4. Preparation of PtNPs-DNA1 and PtNPs-DNA2 bioconjugates

First, the prepared 2 mL of PtNPs was mixed with 20 μL of 50 μM cDNA1-Fc which had been pretreated with TCEP (1 mM) for 1 h at room temperature to reduce disulfide bonds, followed by stirring