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A repeatable assembling and disassembling electrochemical aptamer cytosensor for ultrasensitive and highly selective detection of human liver cancer cells

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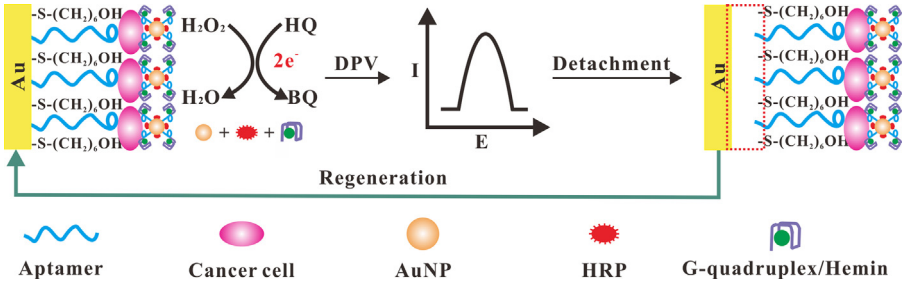
HIGHLIGHTS

- An electrochemical aptasensor was developed for the detection of HepG2 cells.
- Dual recognition and enzymatic signal amplification were well designed.
- The cytosensor performed well in sensitivity and selectivity.
- A repeatable assembling and disassembling cytosensor can be achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a repeatable assembling and disassembling electrochemical aptamer cytosensor was proposed for the sensitive detection of human liver hepatocellular carcinoma cells (HepG2) based on a dual recognition and signal amplification strategy. A high-affinity thiolated TLS11a aptamer, covalently attached to a gold electrode through Au–thiol interactions, was adopted to recognize and capture the target HepG2 cells. Meanwhile, the G-quadruplex/hemin/aptamer and horseradish peroxidase (HRP) modified gold nanoparticles (G-quadruplex/hemin/aptamer–AuNPs–HRP) nanoprobe was designed. It could be used for electrochemical cytosensing with specific recognition and enzymatic signal amplification of HRP and G-quadruplex/hemin HRP-mimicking DNAzyme. With the nanoprobes as recognizing probes, the HepG2 cancer cells were captured to fabricate an aptamer-cell-nanoprobes sandwich-like superstructure on a gold electrode surface. The proposed electrochemical cytosensor delivered a wide detection range from 1×10^2 to 1×10^7 cells mL^{-1} and high sensitivity with a low detection limit of 30 cells mL^{-1}. Furthermore, after the electrochemical detection, the activation potential of −0.9 to −1.7 V was performed to break Au–thiol bond and regenerate a bare gold electrode surface, while maintaining the good characteristic of being used repeatedly. The changes of gold electrode behavior after assembling and desorption processes were investigated by electrochemical impedance spectroscopy and cyclic voltammetry techniques. These results indicate that the cytosensor has great potential in disease diagnostic of cancers and opens new insight into the reusable gold electrode with repeatable assembling and disassembling in the electrochemical sensing.

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1. Introduction

Recent statistical reports show that human cancer is a major public health problem and has become a leading cause of death worldwide. Among them, liver cancer is the sixth most common type of cancer and the second leading cause of cancer death worldwide. The most frequent liver cancer, approximately 75–90% of all liver cancers is hepatocellular carcinoma (HCC), also called malignant hepatoma [1,2]. As is well-known, early detection or diagnosis of cancer greatly increases the chances for effective treatment and improves patient survival rate at present. In particular, the accurate qualitative detection of cancer cells represents a critical step in cancer diagnosis. To achieve the goal of early diagnosis, it is essential to introduce a straightforward strategy for ultrasensitive detection of human liver hepatocellular carcinoma cells (HepG2). In the past two decades, a series of typical approaches, including immunohistochemistry [3], polymerase chain reaction (PCR) [4], flow cytometry [5] and mass spectrometry [6] have been developed for cancer cell detection. However, despite the diagnostic improvements, early stage recognition of liver cancers remains a major challenge. Most of those approaches often require time-consuming and labor-intensive processes, special technical skills and instruments that mean high costs. Therefore, it is necessary to develop simple, rapid, specific and sensitive methods for cancer cell detection.

Recently, there have been some attempts for cancer cell detection using electrochemical detection methods, for example electrochemical impedance spectroscopy (EIS) [7–10], differential pulse voltammetry (DPV) [11–14], cyclic voltammetry (CV) [15], stripping voltammetry (SV) [16,17] and electrochemical luminescence (ECL) [18–19]. Compared with the typical methods, electrochemical methods do offer the advantages of acceptable sensitivity, operational simplicity, rapid response and low cost. Besides that, the detection sensitivity can be improved by using designed electrocatalysts for signal amplification in electrochemical sensing. The widely used method for signal amplification is to load enzymes onto electrodes or nanomaterials through enzymatic electrochemical processes. In comparison with natural enzymes, DNAzymes take the advantages of high chemical stability, cost-effectiveness, easy modification, and simple synthesis. Particularly, G-quadruplex/hemin DNAzymes, a complex between hemin and a single-stranded guanine-rich nucleic acid, has been widely used as horseradish peroxidase (HRP)-mimicking DNAzymes electrocatalysts for electrochemical sensing [20]. Significant advances have been made in several areas related to the design of electrochemical biosensors. However, the sensitivity and selectivity of most electrochemical biosensors is not satisfactory, and these methods are still in the development phase. From this perspective, it remains a challenge to develop electrochemical biosensors with excellent specificity and sensitivity to capture and detect targeted cancer cells [21].

Aptamers are the artificial single-stranded nucleic acid sequences that fold into secondary and tertiary structures, making them bind to certain targets with extremely high specificity. It is capable of recognizing and specifically binding to a variety of targets ranging from small molecules, proteins to entire cells [22]. Compared to antibodies, aptamers possess low toxicity, are also more thermally stable molecules and usually not detected by the human immune system as foreign agents [23]. Aptamers also have many other advantages, such as low molecular weight, ease of molecular design and modification, easy but reproducible production, and low cost. The discovery of aptamer, especially those selected for binding cancer cells or tumor markers, could provide a potential approach for early diagnosis of cancers [15]. Hence aptamer-based biosensors have recently attracted considerable attention as a promising approach for clinical diagnostics [23,24]. Many different aptameric biosensors based on electrochemistry [24–26] and electrochemiluminescence [18,19,27] and other assays have been developed for cancer cell detection. For example, Peng's group developed an electrochemical aptasensor for the sensitive detection of MEAR cancer cells based on a dual-aptamer recognition strategy. Two types of cell-specific aptamers, TLC1c and TLS11a, offered a unique interface for specifically recognize cancer cells. It is found that such dual-aptamer modified electrodes show greatly improved sensitivity in comparison with those modified with a single type of aptamer. The designed electrochemical cytosensor showed great reliable performance with satisfied sensitivity and specificity and great potential for further CTC-related clinical applications [28]. Therefore, by taking advantages of DNA aptamers targeting TLS11a, which specifically bind to the membrane surface of HepG2 cells [29], an electrochemical aptamer cytosensor (aptasensor) can be designed for sensitive and selective detection of HepG2 cells.

Herein, we proposed a novel electrochemical aptamer cytosensor for highly sensitive and selective detection of HepG2 cells based on a dual recognition and enzymatic signal amplification strategy. With the assistance of TLS11a aptamer and G-quadruplex/hemin/aptamer–AuNPs–HRP nanoprobes, the HepG2 cancer cells were captured to fabricate an aptamer-cell-nanoprobes sandwich-like superstructure on a gold electrode surface. Owing to the designed nanoprobes, both HRP and G-quadruplex/hemin HRP-mimicking DNAzyme could be for the enzyme catalysis and electrochemical signal amplification. The signal of the electrochemical aptasensor can be obtained by measuring the increase in reduction current generated by hydroquinone (HQ) and benzoquinone (BQ), which was generated by HRP and hemin/G-quadruplex DNAzyme in the presence of H_2O_2. As expected, the proposed cytosensor exhibited an excellent sensitivity and selectivity in detection of HepG2 cancer cells. Moreover, after electrochemical detection, an electrochemical reductive desorption protocol was employed to regenerate a bare gold electrode surface, while maintaining the good characteristic for the used repeatedly. Therefore, this cytosensor has potential in early diagnosis of liver cancer and opens new insight into the reused gold electrode function based on electrochemical desorption in the analytical process.

2. Experimental

2.1. Chemicals and materials

Horseradish peroxidase (HRP, 300 units per mg solid), hemin, H_2O_2 (30%, w/w), hydroquinone (HQ), *o*-phenylenediamine (*o*-PD), trisodium citrate, chloroauric acid ($HAuCl_4$) and tris (hydroxymethyl)aminomethane (Tris) were obtained from Aladin Chemistry Co., Ltd. (Shanghai, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and ethylenediaminetetraacetic acid (EDTA) were from Sangon Biotech. Co., Ltd. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) was obtained from J&K Scientific Ltd. (Guangzhou, China). The phosphate buffer saline (PBS, pH 7.4, 10 mM) containing 137 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4 and 1.4 mM KH_2PO_4 was used as a washing solution, and the PBS (pH 7.0, 100 mM) was used as the electrolyte for detection. A solution of hemin (0.1 mg mL^{-1}) was prepared in 20 mM HEPES buffer (pH 7.0, 50 mM KCl, 250 mM NaCl, 1% DMSO). Ultrapure water (18.2 MΩ cm) was made by Millipore Simplicity System (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade and used without further purification.

The thiolated TLS11a DNA aptamer (HPLC purified) were synthesized from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), with the following sequence: 5′-HS-$(CH_2)_6$-ACA GCA TCC CCA TGT GAA CAA TCG CAT TGT GAT