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An aptamer-based quartz crystal microbalance biosensor for sensitive and selective detection of leukemia cells using silver-enhanced gold nanoparticle label

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ABSTRACT

An aptamer-based quartz crystal microbalance (QCM) biosensor was developed for the selective and sensitive detection of leukemia cells. In this strategy, aminophenylboronic acid-modified gold nanoparticles (APBA-AuNPs) which could bind to cell membrane were used for the labeling of cells followed by silver enhancement, through which significant signal amplification was achieved. Both the QCM and fluorescence microscopy results manifested the selectivity of the sensor designed. A good linear relationship between the frequency response and cell concentration over the range of 2×10^3–1×10^5 cells/mL was obtained, with a detection limit of 1160 cells/mL. This approach provides a simple, rapid, and economical method for leukemia cell analysis which might have great potential for further use.

1. Introduction

Leukemia, a cancer of the blood or bone marrow, is still one of the most common and aggressive cancers [1]. For the purpose of efficient cure of this disease, sensitive and accurate diagnosis is essential and important. Methods currently used for the detection of leukemia cells include flow cytometry [2], polymerase chain reaction [3], and fluorescence measurement [4]. Nevertheless, many of these methods have associated disadvantages; for example, they are time-consuming, costly and require sophisticated instrumentations. Hence, there is still a need to develop simple and economical methods for selective recognition and sensitive detection of leukemia cells.

Aptamers, obtained by an in vitro process named SELEX (systematic evolution of ligands by exponential enrichment) [5,6], are a kind of artificial single-stranded oligonucleotide strands with special three-dimensional structures. They can selectively recognize and bind to a wide range of targets, such as drugs [7], proteins [8] and even whole cells [9]. In comparison with traditional antibodies, aptamers exhibit several advantages including easy and economical synthesis, ease of purification to a high degree, good stability, and lack of immunogenicity [10]. These unique properties make them useful in the fabrication of various biosensors for leukemia cell detection [11,12]. However, in many of these methods, the aptamers were further conjugated with other materials, such as nanoparticles, which might to some extent decrease the performance of the aptamers. What is more, few efforts have been made in the development of an aptamer-based quartz crystal microbalance (QCM) biosensor for leukemia cell detection. Therefore, the construction of a QCM biosensor using the unmodified aptamer for cancer cell detection is still encouraging.

In recent years, due to the unique optical, chemical and biological attributes, gold nanoparticles (AuNPs) are finding more and more applications in chemical and biological fields, such as metal ions and DNA hybridization detection [13–16], drug delivery [17], cancer diagnostics and therapy [18,19]. Besides, the advantages of AuNPs including facile synthesis, ease of functionalization and good biocompatibility make them suitable to serve as binding agents towards specific targets such as whole cells through appropriate modification. In this article, *p*-aminophenylboronic acid conjugated gold nanoparticles (APBA-AuNPs) were synthesized and used for capturing leukemia cells and then as substrates for silver enhancement, which is an appealing way for signal amplification, owning to a variety of advantages such as simplicity, low cost and high sensitivity. According to a previous report [20], AuNPs could serve as nucleation sites and automatically catalyze the chemical reduction of silver ion into silver metal in the

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presence of a reducing agent such as hydroquinone (HQ). The QCM technique was applied for detection of the deposited silver metal since it was a device sensitive to the mass change on the electrode surface. It is also worth mentioning that the synthesized particles were very stable when stored at 4 °C.

QCM, a well-known nanogram mass sensing device, has been widely used in biological research fields due to its satisfactory performance, e.g., high sensitivity, rapid and facile operation. Many reports on the design of label-free signal transduction platform for cell-based biosensing have been presented. It has been applied to study the attachment, adhesion and spreading of cells on different substrates [21–23] and response of cells to exogenous stimulations [24–26]. The advantages of QCM in these studies are that it can be used as a continuous monitoring device and can detect cumulative effects in a non-invasive way with high sensitivity. However, there are few reports [27] on the analysis of leukemia cells using QCM.

Herein, an aptamer-based QCM biosensor was fabricated for selective and sensitive determination of acute leukemia cells based on silver-enhanced AuNP label. In this strategy, target leukemia cells were selectively captured by the aptamer immobilized on the QCM sensor surface, then APBA-AuNPs were used to label the cells followed by silver enhancement, and the resonant frequency change of the QCM caused by the deposition of silver metal was monitored in real-time.

2. Experimental

2.1. Materials and apparatus

Aptamer sgc8c, a specific recognition probe for CCRF-CEM cells selected by the cell-SELEX method [28], was used in our work for CCRF-CEM cell detection: sgc8c, 5′-AAA AAA AAA AAT CTA ACT GCT GCG CCG CCG GGA AAA TAC TGT ACG GTT AGA-3′ (Sangon Inc., Shanghai, China). A random nucleotide sequence 5′-TTA GCC ATG CAC CGT GAC ACT CCT GTC AGC ATT CAG AAC C-3′ was used as control. Both sequences were terminated with the 5′-thiol modifier C6 and purified with high-pressure liquid chromatography (HPLC). $HAuCl_4 \cdot 3H_2O$ was purchased from Sigma-Aldrich (St. Louis, USA). *p*-Aminophenylboronic acid ($C_6H_8BNO_2 \cdot H_2O$, APBA) was purchased from Frontier Scientific Services, Inc. (Logan, Utah, USA). 1-Ethyl-3-(3′-(dimethylamino) propyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from GL Biochem Ltd. (Shanghai, China). APBA-AuNPs were prepared according to our previous report [29]. Ultrapure water (18.2 MΩ cm) from a Millipore Milli-Q system was used throughout.

Chemicals for cell culture were obtained from Gibco (Grand Island, NY, USA). Phosphate-buffered saline (PBS) solution consisting of 136.7 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, 9.7 mmol L^{-1} Na_2HPO_4 and 1.5 mmol L^{-1} KH_2PO_4 was used.

The piezoelectric quartz crystals (AT-cut, 9 MHz, 12.5 mm diameter) with gold electrodes (6.0 mm diameter) on both sides were used. The resonant frequency and resonant resistance of a quartz crystal were measured simultaneously by using a Maxtek Research Quartz Crystal Microbalance (Inficon).

2.2. Cell culture

CCRF-CEM cells (T-cell, human acute lymphoblastic leukemia) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), and Romas cells (B-cell, human Burkitt's lymphoma) were purchased from the Cell Bank of Nanjing KeyGen Biotech. Co. Ltd., China. Both types of cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (Gibco) and 100 U mL^{-1} penicillin-streptomycin at 37 °C in water-saturated atmosphere containing 5% CO_2. At the logarithmic phase, the cells were collected by centrifugation at 1000 rpm for 5 min, then washed with PBS, and re-dispersed in sterile PBS for further use. The density of cells was determined using a TC10 automated cell counter (BIO RAD). The fluorescence microscopy images of cells were taken by a Leica DMI4000B microscope (Germany).

2.3. QCM measurements

As reported previously [30], the piezoelectric quartz crystal was sandwiched between two glass tubes with only one side of the crystal wafer exposed to the liquid. The reaction chamber above the crystal wafer was held with a chlorinated polyethylene centrifugal tube (12 mm inner diameter, 1.0 cm length). Similar to previous reports [31,32], before use the gold surface of QCM was pretreated with piranha solution, a 7:3 mixture of concentrated H_2SO_4 and 30% H_2O_2 (caution: such a solution should be handled with extreme care), followed by rinsing thoroughly with ethanol and ultrapure water in sequence and finally drying under a nitrogen flow.

The cleaned QCM Au electrode was exposed to 0.2 mM thiol-terminated aptamer solution for 1 h to obtain the aptamer/Au electrode, followed by rinsing with water to remove the unbound or weakly adsorbed aptamers. Then the electrode was immersed into 1.0 mM 6-mercapto-1-hexanol (MCH) solution for about 30 min to block the uncovered gold surface and make aptamers upright on the electrode. The modified electrode was thoroughly rinsed with ultrapure water. Then 470 μL PBS was added onto the QCM Au electrode, followed by adding 30 μL PBS containing different numbers of cells when the resonant frequency (*f*) became stable. Thirty minutes later, the electrode was washed with PBS and then 100 μL APBA-AuNPs was added into the chamber. After incubation for 30 min, the electrode was rinsed with PBS and ultrapure water, respectively. Freshly prepared silver enhancement solution containing silver nitrate (1.7%) and hydroquinone (3.2%) was added into the chamber and the resonant frequency change was monitored. As a control experiment, the cells were directly treated with silver enhancement solution without treatment of APBA-AuNPs. After each measurement, the liquid was let out, and then the quartz crystal gold electrode was washed with H_2SO_4/H_2O_2 (v/v, 7:3) and water successively for reuse. All the QCM experiments were performed at 25 °C.

2.4. Fluorescence microscopy observation

The cells captured on the sensor surface were also characterized by fluorescence microscopy. After incubation with cells for 30 min, the modified electrode was washed twice with PBS, treated with 0.01% acridine orange (AO) dissolved in PBS for 3 min. Then the cells were washed with PBS, and the fluorescence microscopy images of cells were taken under blue light irradiation with a Leica DMI 4000B microscope.

3. Results and discussion

3.1. Characterization of the APBA-AuNPs

UV–vis absorbance spectroscopy, transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FITR) were used for characterization of the APBA-AuNPs synthesized. The results were shown in the recently published report of our group [29].