Sensors and Actuators B 223 (2016) 359–364

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Electrochemiluminescence detection of cancer cell based on an electrode surface-related "one-pot" cascade DNA auto-machine

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ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form
21 September 2015
Accepted 22 September 2015
Available online 25 September 2015

Keywords:
Electrochemiluminescence
Hybridization chain reaction
Cancer cell

ABSTRACT

An ingenious strategy of an electrode surface-related "one-pot" cascade DNA auto-machine cooperated with electrochemiluminescence (ECL) is proposed for the detection of cancer cell in this study. The tedious operation of separation procedure is circumvented by the step of hybridization chain reaction (HCR) attached on the electrode surface, offering the advantages of operation-simple and time-saving. Notably, as compared to traditional fluorescent signal of DNA machine in which high nonspecific background could be induced by excess molecular beacon or fluorescent dye, in the present study nonspecific background can be markedly reduced by introducing directly HCR on the surface of the electrode. Overall, the system exhibits high sensitivity and specificity with the detection limit as low as single cell level (5 Ramos cells).
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1. Introduction

Cancer is the most deadly diseases in the world. Early and accurate diagnosis and therapeutic evaluation of cancer plays an important role in the improvement of the effective and ultimately successful treatment of cancer [1,2]. The cancer cells have attracted much attention due to their malignant proliferation behavior. Besides, the number of cancer cells has been shown to be a significant predictor of progression-free survival and overall survival, and was suggested as an indicator for clinical management such as the determination of success/failure of therapeutic intervention [3,4]. How to detect cancer cells with high sensitivity and specificity is a critical issue for prognostic and therapeutic implications of cancer and a platform capable of distinguishing trace cancer cells is imperative. Accordingly, tremendous efforts have been devoted to the development of approaches that can be used to analysis various cancer cells, such as electrochemical biosensing and imaging, colorimetric assay, fluorescence analysis, microtube device, surface-enhanced Raman scattering assay et al. [1,2,5–13].

Based on the specific affinity, target cancer cells can be specifically recognized and then sorted for further analysis. Nonetheless, modifications on affinity probes (aptamers or antibodies) may lead to significant loss of their affinity and specificity to targets [14]. Furthermore, the complex operations of the immobilized cells in above-mentioned methods limit the application of this technique in the diagnostic. Additionally, the proportion of false positive and false negative results in practical application is higher. Therefore, there is an urgent need to develop a strategy for detecting cancer cells from a heterogeneous mixture of bodily fluids with a higher sensitivity, such that a small number of cancer cells can be detected.

A molecular machine is constructed by molecular components which perform mechanical-like movements in response to specific stimulin. DNA is an excellent raw material for molecular machine due to the unique molecular recognition properties and its particular biophysics properties. A wide variety of DNA molecular machines have been designed and used for biorecognition and biosensing events in proteins analysis, DNA and RNA quantitation, and other small molecules detection [15–19]. In addition, aptamers are nucleic acid-based (DNA or RNA) affinity sequences that provide a number of advantages including specificity, temperature stability and reusability [20,21]. Because the aptamer-ligand recognition event can be easily amplified via nucleic acid sequence-based amplification strategy [22,23], there is no doubt that DNA machine

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http://dx.doi.org/10.1016/j.snb.2015.09.109
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will possess more broad applications with the incorporation of aptamer into the DNA machine.

So far, various designs of DNA machine have been advanced for transducing target-DNA interactions into fluorescence signal [16–19]. Fluorescent possesses adequate transducing elements to generate physically detectable signals from the recognition events. Most detections have been achieved by switching molecular beacon or intercalating fluorescent dye. However, the sensitivity for target detection is mainly limited by nonspecific background, which appears to involve excess molecular beacon or fluorescent dye [24]. Therefore, the development of a feasible detection method with low background, high sensitivity and simplicity has become highly focused which can provide an alternative detection approach to the fluorescence system. Electrochemiluminescence (ECL) is a form of chemiluminescence reaction which is preceded by an electrochemical reaction [25,26]. It has been one of the most attractive analytical tools due to its many advantages (low background optical signal, low cost, wide range of analytes and easing of reaction controlled by applying electrode potential). In addition, ECL intensity can be significantly increased by enhancers, such as H_2O_2 [27], $S_2O_8{}^{2-}$ [28], amine [29], Au nanoparticle [30], graphene oxide [31–33].

Inspired by those observations, we designed a sensitive strategy for ECL detection of cancer cell on the construction of an "one-pot" electrode surface-related cascade DNA auto-machine based on cancer cell aptamer (Fig. 1). Among the DNA machine, hybridization chain reaction (HCR) is artfully attached on the electrode surface with the advantages of separation-free and low background, which is unprecedented to the best of our knowledge. In the present study, Ramos cell was used as a model target cell to demonstrate proof of principle. This system exhibits high sensitivity and specificity with the detection limit as low as 5 Ramos cells.

2. Material and methods

2.1. Chemicals and apparatus

All of oligonucleotides were custom-ordered from SBS Genetech Co. Ltd. (China), and their sequences are listed in Table S1. Nt.Bpu10 I and Klenow fragment exo$^-$ were obtained from Fermentas. Superoxide dismutase, Luminol powder, 3-aminopropyl-triethoxysilane (APS), (3-glycidyloxypropyl)triethoxysilane, thioglycolic acid (TGA), hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich. Luminol stock solution (1.0×10^{-2} M) was prepared by dissolving it in 0.1 M NaOH solution and storing it in dark. Carboxyl group-modified magnetic beads (MBs) were commercially available from BaseLine ChromTech Research Centre (Tianjin, China). Deionized water was used throughout the experiments. All the chemicals employed were of analytical reagent grade. All glassware used in the experiment procedures was cleaned with HNO_3-HCl (3:1, v/v), rinsed thoroughly with deionized water, and dried before use.

The ECL measurements were performed by using a MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) using a three-electrode system. Electrochemical impedance spectroscopy surveys (EIS) were achieved with a CHI 660C electrochemical working station (Shanghai CH Instruments Co., China), using the same three-electrode system as that in the ECL measurements. UV–vis absorption measurement was carried out using a Cary 50 UV-Vis-NIR spectrophotometer (Varian). The spectra of sample were recorded at room temperature. Transmission electron microscopy (TEM) images were taken with a JEOL JSM-6700F instrument (HITACHI).

2.2. Preparation of luminol–capped Au nanoparticles

All kinds of glasswares used in the following procedures were cleaned with HNO_3-HCl (3:1, v/v). Luminol–capped Au nanoparticles (luAu NPs) were prepared by the reduction of $HAuCl_4$ with luminol according to the literature [30]. A 100 mL solution containing 0.01% (w/w) $HAuCl_4$ was heated to boiling point with stirring. 5.0 mL of 0.01 M luminol was rapidly added to the solution. Stop heating until the color changing from yellow to black to purple, indicating the formation of luAu NPs. Subsequently, stirring was continued for 1 h and then stored in the dark at 4 °C for future use.

2.3. Fabrication of luAu-DNA conjugates

LuAu NPs were readily functionalized with thiolated DNA via Au-S chemistry according to the literature [34,35]. Freshly reduced thiolated DNA were used for luAu-H probe (luAu-H1 and luAu-H2) preparation. 100 μL of 1.0×10^{-6} M H1 was added to 3.0 mL of luAu NPs, followed by placing on a shaker overnight at 25 °C. The mixture was adjusted to 0.15 M NaCl, 10 mM phosphate, and 0.01% SDS by simultaneously adding appropriate amounts of 2.0 M NaCl, 0.1 M phosphate buffer (pH 7.4), and 1.0% SDS solution. After a brief shaking, the mixture was allowed to age for 16 h at 25 °C. Finally, luAu-H1 formed. The preparation process of luAu-H2 was the same as luAu-H1, only replacing H1 with H2.

2.4. Cell culture

Ramos cell lines, HeLa cell lines and K562 cell lines were cultured in RPMI 1640 culture medium (10% fetal bovine serum, 2 mM glutamine, 100 IU mL^{-1} penicillin-streptomycin). In all experiments, cells were grown in culture flasks at 37 °C in a humidified atmosphere with 5% CO_2/95% air atmosphere. The cell culture media were supplemented with RPMI 1640 culture medium. Cell suspensions were centrifuged, washed with 4 °C pre-cooled PBS buffer three times and then resuspended in PBS buffer. The cancer cell densities were measured using a hemacytometer.

2.5. Preparation of Au electrode

Au electrode processing is described below briefly. The Au electrode (4 mm in diameter) was first polished with 1.0, 0.3, and 0.05 mm alumina powder sequentially and the residual alumina powder was removed by washing in an ultrasonic bath. The above electrode was cycled between 0 and 1.5 V (versus SCE) in 0.05 M H_2SO_4. The electrode was electrochemically cleaned until a reproducible cyclic voltammogram was obtained, indicating a clean surface of the gold electrode was obtained and then the electrode was rinsed with deionized water. After drying with nitrogen, the electrode was reacted with 1.0×10^{-5} M H0 for 16 h at 37 °C to immobilize H0 onto the electrode surface. The H0-modified electrode was washed with deionized water, and then treated with 1.0×10^{-6} M thioglycolic acid (TGA) at 37 °C for 2 h to obtain well-aligned DNA monolayers on the electrode surface. The above electrode was further rinsed with deionized water.

2.6. Fabrication of MB-DNA duplex conjugates

30 μL of MBs was washed three times with 0.1 M imidazole-HCl buffer (pH 7.4), followed by adding 100 μL of 0.2 M EDC and incubating at 37 °C for 1 h to activate the carboxylate groups on MBs. After washing three times with PBS buffer (pH 7.4), 100 μL of 1.0×10^{-7} M amino-modified aptamer was reacted with activated MBs at 37 °C for 12 h and then the MBs were washed three times with PBS buffer (pH 7.0). The unlinked aptamer was removed by magnetic separation. Subsequently, 100 μL of 1.0×10^{-6} M DNA (2)

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