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Application of Au cage/Ru(bpy)$_3^{2+}$ nanostructures for the electrochemiluminescence detection of K562 cancer cells based on aptamer

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ABSTRACT

In this work, an electrochemiluminescence (ECL) cytosensor for ultrasensitive and selective cytosensing of K562 cancer cells was developed. Pt nanoparticles (PtNPs) dotted carbon nanotubes (CNTs) was immobilized on the working electrode which can not only improve the electronic transmission rate but also increase the surface area. And aptamers modified electrode was employed for specific and efficient cancer cell capture. A new class of nanoprobes were also prepared by integrating the functions of specific recognition of concanavalin A (Con A) and signal amplification of Au cage/Ru(bpy)$_3^{2+}$ nanostructures. With a sandwich-type cytosensor format, the amount of Au cage/Ru(bpy)$_3^{2+}$-labeled Con A increased with the increment of K562 cancer cells in the samples, resulting in the increase of ECL signals. The as-proposed cytosensor exhibited excellent analytical performance toward the cytosensing of K562 cells in a wide detection linear range from 500 to 5.0×10^6 cells mL^{-1} with a detection limit of 500 cells mL^{-1}. Moreover, the proposed method showed good precision, acceptable stability and reproducibility.

1. Introduction

Human chronic myelogenous leukemia (CML) is a malignancy of pluripotent hematopoietic cells. It is caused by the dysregulated activity of the tyrosine kinase, which was encoded by the chimeric bcr/abl oncogene. K562, as one of the most aggressive human CML cell lines, was therefore chosen for detection [1,2]. It has been reported that early and accurate cancer diagnosis could provide an easier and more effective way to monitor progression of the disease and ultimately successful treatment of cancer [3,4]. Recently, great efforts have been directed to develop novel immunoassays combined with fluorescence [5], electrochemical impedance spectroscopy [6], mass spectrometry [7], and electrochemical [8] methods to detect the cancer cells. However, there are many limitations of their practical usages, including poor sensitivity or selectivity. Compared with the traditional methods, electrochemiluminescence (ECL), a chemiluminescence reaction initiated and controlled by the application of an electrochemical potential, is a promising technique in biochemical analysis due to its distinct advantages of simplicity, rapidity, sensitivity, controllability and low background [9,10]. In spite of above advantages in this field, it is still a challenge to explore new protocols and strategies for the further improvement of the sensitivity.

Carbon nanomaterials, such as carbon nanotubes, carbon nanodots and carbon nanofibers, have been widely used in both analytical and industrial electrochemistry due to its chemical inertness, low residual current, excellent conductivity, wide potential window, and electrocatalytic activity to a variety of redox reactions [11]. Recently, carbon nanotube (CNT) composites or multilayers have attracted much attention in biosensor due to the high surface area-to-weight ratio, and fast electron-transfer capabilities [12,13]. Moreover, the combination of two (or more) types of materials, such as CNTs and gold nanoparticles might add new functionalities or extend the practical applications of these nano-objects. On the other hand, noble metal nanostructures, such as gold, platinum or palladium nanoparticles (AuNPs, PtNPs and PdNPs), are of general interest mainly due to their remarkable high catalytic activity and their special electronic and optical properties. During these years, PtNPs have stimulated extensive research in electrochemical applications, attributing to their high electrocatalytic efficiency and selectivity [14]. In this work, a facile ultrasound method has been introduced to fabricate the nanohybrids of PtNPs dotted CNTs (Pt-CNTs) as cell sensing platform. And then, aptamer was applied to capture cancer cells. Aptamer, a kind of synthetic oligonucleotides

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discriminated by in vitro screening and systematic evolution of exponential enrichment technology (SELEX), which have better specificity due to their ability to fold into distinct secondary and tertiary structure [15,16]. Aptamers also have many other advantages over antibodies, such as low molecular weight, easy but reproducible production and low cost [17,18]. In view of above advantages, aptamers were modified on the electrode for cancer cell recognition and capture.

There is still a need for new methods to improve the sensitivity of ECL cytosensor. Hollow micro-/nanostructures have attracted great attention over the past few decades due to their unique properties, such as designable morphology, void space, low density, low coefficients of thermal expansion and refractive indexes, which are attributed to the special construction and composition [19]. These attractive characteristics give them potential for applications in photonic crystals, catalysis, drug delivery and biomedical therapy, sensing and rechargeable batteries. Further, the tiny component units, like nanoparticles, nanocubes and nanosheets, which assemble the porous walls of hollow structures, endow them with distinguished performance resulting in widespread potential applications [20]. Many efforts have been devoted to the exploration of the synthesis of an increasingly broad array of hollow structures using a diverse range of methods, including using hard templates [21], sacrificial templates [22] and soft template approaches [23]. Cu_2O polyhedron crystals are ideal sacrificial templates due to their rich variety of shapes, easily "prepared/removed" features and their reductive activity [24]. Herein, Au cages with hollow structures using Cu_2O cubes as sacrificial templates have been prepared, which was employed to load $Ru(bpy)_3^{2+}$ and promote the ECL sensitivity.

Herein, a novel ECL cytosensor is created by dual signal amplification of Pt-CNTs hybrids and Au cage/$Ru(bpy)_3^{2+}$-con A nanocomposites. Pt-CNTs hybrids was employed as platform to realize the capture of the K562 cancer cells. Mannose is the core structure of glycans on membrane glycoproteins which presents in the form of mannose oligosaccharides on the cell surface, and the variation of its expression levels is closely related to such important biological processes as tumor growth and metastasis brain aging, and differentiation [25]. The specific recognition was achieved using lectins, a class of natural nonimmune proteins that can specifically recognize sugar epitopes, as the recognition elements for gaining insight into the biologically relevant surface-accessible glycan motifs by selecting mannose and concanavalin A (a lectin) as an initial proof-of-concept recognition pair [26]. Au cage/$Ru(bpy)_3^{2+}$-con A nanocomposites could be further used for highly sensitive evaluation of carbohydrates through the specific recognition between Con A and mannose. This strategy provides a highly sensitive method for K562 cancer cells detection, and exhibits excellent stability and good reproducibility.

2. Experimental

2.1. Reagents and apparatus

All reagents were of analytical-reagent grade or the highest purity available and directly used for the following experiments without further purification. The aqueous solutions unless indicated were prepared with ultrapure water. Thiolated T2-KK1B10 with high specificity for K562 cancer cells was obtained from Sangon Biotech (Shanghai) Co. Ltd., and the sequence was presented as below: 5′- HS-TTT TTT TTT TAC AGC AGA TCA GTC TAT CTT CTC CTG ATG GGT TCC TAT TTA TAG GTG AAG CTG T-3′ [27]. The random DNA was presented here as below: 5′- NH2- TTT TTT TTT ATC TAA TCT AAC TGA CGC GAA GAG CAT GCA TTC ATC CGA GGC TCA TAC CCT GAC T-3′. 1-ethyl-3-(3dimethylaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were obtained from Alfa Aesar China Ltd. $Ru(bpy)_3^{2+}$-NHS ester were obtained from IGEN (Gaithersburg, MD). Bovine serum albumin (BSA) was obtained from Sigma–Aldrich Chemical Co. (USA). Chloroauric acid ($HAuCl_4{\cdot}3H_2O$), sodium carbonate, Ethylene Glycol (EG), tripropylamine (TPA), $CuSO_4$ and trisodium citrate, K_2CO_3 and Polyvinylpyrrolidone (PVP) were obtained from Shanghai Reagent Company (Shanghai, China). The carbon nanotubes (CNTs) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Phosphate buffered solutions (PBS) (pH 7.4, 10.0 mM) containing NaCl (136.7 mM), KCl (2.7 mM) was used as incubation buffer. The washing buffer was PBS (10.0 mM) containing 0.05% (w/v) Tween-20. PBS (10.0 mM) containing 0.5% (w/v) BSA and 0.5% (w/v) casein was used as blocking solution.

K562 cell line was kindly supplied by the Ministry of Health Key Lab for Otolaryngology, Qilu Hospital of Shandong University, Jinan, China, and maintained in exponential growth in RPMI 1640 medium (GIBCO) containing 10% fetal calf serum (HyClone, Logan, UT), penicillin (100 units mL^{-1}), and streptomycin (100 $\mu g\,mL^{-1}$). The CCRF-CEM (CCL-119, Tcellline, human ALL) cell line was obtained from Sincere Lion Technology (Beijing) Co. Ltd. Cells were cultured at 37 °C in a 5% CO_2/95% air humidified atmosphere. The cells in exponential growth phase were collected and separated from the medium by centrifugation at 1000 rpm for 5 min and then washed thrice with a sterile pH 7.4 PBS. The sediment was resuspended in 10 mM pH 7.4 PBS to obtain a homogeneous cell suspension. Cell number was determined on a Petroff–Hausser cell counter (USA). MCF-7 cell line and SKBR-3 cancer cells were kindly provided from Shandong tumor research institute and maintained in Dulbecco's Modified Eagle Cancer Medium (DMEM) supplemented with 10% fetal calf serum (FBS), 1% penicillin/streptomycin and 1% nonessential amino-acids.

The ECL measurements were carried out on a MPI-E multifunctional electrochemical and chemiluminescence analytical system (Xi'an Remax Analytical Instrument Ltd. Co.) biased at 800 V. Scanning electron microscope (SEM) images were obtained using a QUANTA FEG 250 thermal field emission SEM (FEI Co., USA). All experiments were carried out with a conventional three-electrode system with the modified GCE (3 mm in diameter) as the working electrode (WE), a platinum counter electrode (CE) and an Ag/AgCl (sat. KCl) reference electrode (RE).

2.2. Synthesis of Cu_2O precursor templates

2 mL of mixed solution, composed of 0.74 M sodium citrate and 0.2 M sodium carbonate, was added to 18 mL solution of $CuSO_4$ (0.038 M) dropwise. After that, 3.2 g PVP was added and stirred vigorously until PVP was dissolved completely. Then, the mixed solution in the flask mentioned above was aged in a water bath at 90 °C for 2.5 h. Cu_2O nanoparticles with different morphology could be prepared in the same synthetic system by simply changing the PVP component amount from 2.9 g to 5.2 g. Different amounts of the PVP template could generate various product shapes. The brick-red products were collected and rinsed thoroughly before being dried under vacuum.

2.3. Synthesis of Au cages

The Cu_2O cubic precursors were dispersed in 8 mL of PVP (0.5%) aqueous solution and then 12 mL distilled water was added, followed by adding 2.4 mL of $HAuCl_4$ (10 mM) solution under vigorous stir at room temperature. After 6 h, the black precipitate was collected and dispersed in 40 mL solution containing 0.04% PVP and 1% ammonia for about 12 h. The precipitate was then separated and re-dispersed in 40 mL solution containing 0.04% PVP and 1% ammonia again for another 12 h. At last, the precipitate