Journal of Pharmaceutical and Biomedical Analysis 137 (2017) 163–169

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Impedimetric nanostructured genosensor for detection of schistosomiasis in cerebrospinal fluid and serum samples

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ARTICLE INFO

Article history:
Received 26 November 2016
Received in revised form 12 January 2017
Accepted 14 January 2017
Available online 20 January 2017

Keywords:
Biosensors
Schistosomiasis
DNA
Cyclic voltammetry
Impedance spectroscopy
Metal nanoparticles

ABSTRACT

Schistosomiasis is a neglected disease closely related to the low levels of social development and a serious public health problem. In this work, we performed an electrochemical detection of *Schistosoma mansoni* DNA with a self-assembled monolayer of mercaptobenzoic acid (MBA) immobilizing nanostructures composed of gold nanoparticles (AuNPs) and magnetite nanoparticles (Fe_3O_4_NPs). Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to monitor the hybridization process. MBA-Fe_3O_4_NPs-AuNPs-DNAprobe system reveals an effective electrochemical response indicating the surface modification. The proposed biosystem was capable to recognize specific nucleotide sequence of *S. mansoni* present in cerebrospinal fluid (CFS) and serum samples at different genome DNA concentrations. The biorecognition resulted in an increase in the electron transfer resistance and a decrease of the current peaks at higher DNA concentrations during electrochemical measurements. The developed platform showed a DNA detection limit of 0.781 and 0.685 pg μL^{-1} for serum and CFS, respectively. Therefore, the obtained biosensor can be considered as a useful tool for specific detection of *S. mansoni* at low concentrations in various biological fluids.

1. Introduction

Schistosomiasis is an important parasitic disease for public health caused by helminth *Schistosoma mansoni* [1], which can be found in many tropical regions of the globe [2]. Some studies have reported six to seven million people affected in Brazil [3,4] with higher prevalence in Northeast states [2] and Southeast states [5,6].

A severe form of *S. mansoni* is the ectopic schistosomiasis that can manifest in different ways, especially involving the nervous system [7]. In these infections, spinal cord is more frequently affected than the brain [8]. The early treatment of the disease is essential for a good prognosis and preventing indiscriminate use of drugs by patients. Therefore, it is necessary the development of affordable diagnostic techniques with high sensibility and specificity [9]. Schistosomiasis diagnosis is based on the analysis of faecal samples and cerebrospinal fluid (CSF) for nervous system manifestation [7,10]. Some diagnostic tests such as ELISA, indirect fluorescence immunoassay, radioimmunoassay are also commonly used [11]. In general, diagnostic tests require expensive equipment and supplies, specialized technicians and are time-consuming [12].

The embolization of eggs during *S. mansoni* infection is the most important mechanism to reach the spinal cord. In addition, it is involved to endemic cases of neuroschistosomiasis in Brazil's Northwest [13]. Diagnostic methods for spinal cord schistosomiasis have low sensitivity and specificity. The definitive diagnosis of schistosomiasis is often a difficult process [14]. Therefore, an effective diagnosis is important, since early treatment can minimize sequelae [15,16].

Polymerase chain reaction (PCR) can be used to diagnose schistosomiasis in several biological samples [17], but its value in the CSF is still unknown. Some authors proposed the use of nested PCR to auxiliary the diagnosis of spinal cord schistosomiasis, in particular for cases with negative CSF serology [18]. However, further studies are needed to confirm its clinical use.

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http://dx.doi.org/10.1016/j.jpba.2017.01.031
0731-7085/

In this context, electrochemical genosensors have been used as an excellent alternative for the development of new methods for diagnosis [19,20]. Electrochemical techniques reveal changes in the electrical properties of the electrode surface such as resistance and capacitance [21]. Impedimetric nanosensors are promising due to their potential for rapid detection of DNA sequences with low cost and sensitivity [21,22].

On the other hand, biosensors have appeared as an alternative to easy-to-handle, sensitive and low-cost diagnostic technology [23,24]. The advantages of the biosensors include the use of small amount of samples, reduced cost, portability, multiplexing capability and scalable for high-throughput applications. However, there are some issues to overcome such as stability, immobilization of biomaterials and interfacing of the systems between laboratories and clinics [25].

The association of metal nanoparticles have yielded nanostructured materials with distinctive properties [26]. Of note, the improved electroanalytical performance of nanostructured sensors is attributed to their high conductivity, large surface area, stability and biocompatibility [27]. In particular, optical, magnetic and catalytic properties of solid surfaces can be improved using magnetite (Fe_3O_4_NPs) and gold nanoparticles (AuNPs) [28–30]. Fe_3O_4_NPs and AuNPs have been used as platforms for the development of biosensors due to the ability to provide a stable immobilization of large amount of biomolecules and improvement of the biocompatibility [31,32].

In this work, we reported the construction of new highly sensitive electrochemical genosensor based on self-assembled monolayers of mercaptobenzoic acid (AMB) for *S. mansoni* detection. Aminated-Fe_3O_4_NPs and AuNPs were immobilized on AMB layers (Fig. 1). To the best of our knowledge this is the first time that an electrochemical platform is developed for specific detection of *S. mansoni*.

2. Experimental

2.1. Materials

S. mansoni samples were provided by the Parasitology Laboratory Aggeu Magalhães Research Center (Recife, Brazil). All samples were previously characterized using RT-qPCR. Mercaptobenzoic acid (AMB), gold(III) chloride hydrate ($HAuCl_4 \bullet xH_2O$), (3-aminopropyl)triethoxysilane (APTES), trisodium citrate, bovine serum albumin (BSA), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (NH_4OH), potassium ferri- and ferrocyanide were obtained from VETEC (Brazil). All other chemicals used in this work were reagent-grade quality and used as received without further purification. Ultrapure water was obtained from a Millipore Milli-Q system.

2.2. Sample preparation

CSF samples (1 mL) were obtained by lumbar puncture. CSF analyses were based on cell count, dosage of glucose and protein. In addition, protein electrophoresis and immunological determinations for others infections were evaluated [10].

Schistosoma DNA and genomic material from cerebrospinal serum fluid were purified using the illustra tissue and blood genomic Prep Mini Spin Kit (GE Healthcare, UK) in according to the manufacturer's instructions. The thiolated primer was specific for *S. mansoni* with the sequence Schfo17 (5′-GTGCTGGT GGGTTGACGAGTTC-3′) [33].

2.3. Synthesis of nanoparticles

Gold nanoparticles were synthesized in according to Frens [34]. A mixture of $HAuCl_4$ and trisodium citrate was obtained. 100 mL of the first solution (0.01 wt% $HAuCl_4 \cdot H_2O$) was boiled under vigorous stirring in a flask. Furthermore, 2.5 mL of trisodium citrate solution (1% w/v) was added quickly to the boiling solution, resulting in a color change from yellow to red, indicating the formation of AuNPs. Then, the solution was maintained at boiling temperature for 10 min.

APTES-modified Fe_3O_4_NPs were obtained according to Gan et al. [35]. Initially, 0.2 g Fe_3O_4_NPs were added to 90 mL ethanol. Subsequently, 2 mL NH_4OH (25%) and 300 μL APTES were added to the previous solution, and stirred overnight. Then, the product was magnetically separated and washed three times with deionized water to obtain aminated-Fe_3O_4_NPs.

2.4. Electrode modification

The bare gold electrode (BGE) was polished with sandpaper and deionized water. Subsequently, BGE was immersed in a piranha solution for 5 min, dried at room temperature and incubated with 2 μL AMB after washing with deionized water. Then, 2 μL of equal parts (1:1) of 0.4 M EDC and 0.1 M NHS was added on the electrode surface for a period of 10 min in order to activate AMB carboxylic groups. After this, 2 μL aminated-Fe_3O_4_NPs was dropped on the MBA-EDC/NHS-modified electrode following incubation for 10 min. Then, the residual protonated amine groups of MBA-Fe_3O_4_NPs was modified with AuNPs to obtain (MBA-Fe_3O_4_NPs-AuNPs) using the same EDC/NHS method. Finally, thiolated DNA probe was immobilized on the AuNPs surface. Fig. 1 shows the manufacturing process of the MBA-Fe_3O_4_NPs-AuNPs-DNAprobe system. BSA was used in all experiments to avoid non-specific interactions.

2.5. Hybridization analysis

Target DNA was diluted in 10 mM phosphate-buffered saline (PBS) solution (pH 7.4) and heated (3 min at 40° C) before the experiments. The hybridization was carried out by simple drop-coating technique using 2 μL of the target DNA obtained from biological samples at different concentrations.

2.6. Electrochemical measurements

Electrochemical analyses were performed using a PGSTAT 128N potentiostat/galvanostat (Autolab, Netherlands) with a three electrode conventional electrochemical cell interfaced with an analyzer controlled by a computer. All experiments were performed in the presence of a 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ solution (1:1) used as a redox probe. BGE was used as work electrode. Platinum wire electrode and Ag/AgCl (saturated KCl) were used as auxiliary and reference electrodes, respectively. Impedimetric assays were performed at a frequency between 100 mHz and 100 kHz, with amplitude of the applied sine wave potential of 10 mV. CV analyses were obtained in the range of potentials between +0.7 V and −0.2 V at a scan rate of 50 mV s^{-1}. All electrochemical measurements were performed in triplicate using three different samples (n = 3).

2.7. Atomic force microscopy measurements

Topographic analysis of the system were performed through an atomic force microscope (SPM-9700, Shimadzu Corporation, Japan) in a noncontact mode in air at room temperature (approximately 25 °C) [36]. Cantilevers with a silicon AFM probe (Nanoworld, Japan, resonant frequency = 300 kHz, spring constant = 42 N m^{-1})