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Electrochemical aptasensing of human cardiac troponin I based on an array of gold nanodumbbells-Applied to early detection of myocardial infarction

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ABSTRACT

Recently, convergence of nanotechnology, medicine, biology and chemistry has provided novel solutions for efficient diagnosis systems for disease markers. A detection method of troponin I (TnI), as the gold standard of acute myocardial infarction biomarker, was investigated and a novel aptasensor was presented for this biomarker. An array of gold nanodumbbells was firstly synthesized using putrescine as the shape directing agent at a surface. It was then applied as a transducer for the immobilization of a 76-mer TnI aptamer to fabricate a simple electrochemical TnI aptasensor. Using the aptasensor, TnI was detected in a linear range of 0.05–500 ng mL^{-1} with a limit of detection of 8.0 pg mL^{-1}. The aptasensor showed a diagnostic sensitivity of 100% and a diagnostic specificity of 85% when challenged with blood serum samples of 89 individuals.

1. Introduction

Heart diseases are considered as the most common cause of death in most countries in the world and are the main leading cause of disability [1]. Blocked heart blood vessels can inhibit blood circulation in heart and can leading to blood clots in the heart's arteries and acute myocardial infarctions (AMIs) in peoples [2]. If medical aids do not timely found by these patients, the heart's muscles will undergo irreversible damages [3] due to inability of absorption of enough oxygen. AMI is occurred due to the lack of oxygen in different parts of the heart and all of the heart's areas will be in oxygen deficiency [4]. AMIs can cause to death (11.8% of all deaths in the world [5]), otherwise, the patients suffer from heart failure, shortness of breath, swelling of legs, early fatigue and other symptoms such as a rapid heartbeat during activity and rest [6]. Therefore, rapid and precise diagnosis of AMIs is very important [7]. On the other hand, the symptoms of AMI in most cases are confused with the stomach gastritis disease, and this issue is considered also as a challenge to accurate AMI identification. Due to the high mortality and importance of prevention for serious complications after an AMI, early detection and treatment is inevitable [8].

Recently the relationship between cardiac myocytes damages and increased levels of cardiac biomarkers has been discovered [9]. For example, after an AMI, a biomarker troponin is released from damaged cardiac myocytes into bloodstream [10], and the most important laboratory test for detecting AMI is based on the troponin assay as a preferred biomarker. Troponin complex consists three subunits of troponin C (TnC), troponin T (TnT) and troponin I (TnI). They along with tropomyosin are located on actin filaments and their existence is essential in heart and skeletal muscle contraction systems mediated by calcium [11]. Cardiac TnC is not specific for heart, and is not employed for the AMIs diagnosis. Cardiac TnI is more specific for heart muscle than TnT, is not observed in skeletal muscles, does not affect against kidney disease (false positive) [8,12], and is considered as the "gold standard" for the diagnosis of myocardial injury [13].

Up to now, detection of cardiac disease biomarkers has been performed by a variety of methods of surface plasmon resonance [14,15], capacitive sensing [16,17], fluorescence immunochromatography [18], field-effect transistor [19], electrochemistry [20,21], electrogenerated chemiluminescence [22–24] and immunoassay [21,25]. However, immunogenicity and high production costs, have limited their clinical utility [26]. Detection of biomarkers using biosensors (mainly aptasensors) has been followed in recent years [27,28]. Cardiac disease biomarkers of C-reactive protein [14–16,29], natriuretic peptides [30] and TnI [31]

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have also been detected using aptamers. Aptamers have diverse advantages over antibodies including easy modification and high stability in harsh physical and chemical environments, rapid and economical production, no batch-to-batch variation, low immunogenicity and high flexibility [32]. Up to now, there is a little report on the application of aptamer in the electrochemical detection of TnI biomarker [31].

Nanotechnology has provided materials with a high real surface area with quantum confinement and small-size effects resulted in the fabrication of sensors and biosensors with a higher sensitivity and selectivity [33–36]. A variety of nanomaterials including metal nanostructures, metal oxide nanostructures, silica nanoparticles, carbonaceous nanomaterials and their composites with the unique properties of controllable physicochemical properties, high surface area, stability and biocompatibility have been employed to fabricate biosensors [33–36]. Nanostructured materials have also been conjugated with aptamers to fabricate aptasensors with different transduction methods [37–39]. For the biomarkers, different nanostructured materials have been employed to fabricate aptasensors [40,41].

In the present study, a specific aptasensor was design on an array of gold nanodumbbells and electrochemical detection was applied for accurate detection of TnI, and a quick diagnosis method for AMI detection was developed. The aptasensor was compared with ELISA method in the assay of samples from healthy persons and patients.

2. Experimental section

2.1. Materials

All chemicals were of analytical grade from Merck (Germany) or Scharlau (Spain), and were used without further purification. TnI from human heart, bilirubin, hemoglobin, human serum albumin and heparin were purchased from Sigma (USA). TnI AccuBind ELISA Kit was purchased from Monobind (USA). A 5′ end thiol-modified DNA aptamer (the aptamer) was purchased from Bioneer (Korea) with the following sequence:

5′-(SH)-$(CH_2)_6$-AGTCTCCGCTGTCCTCCCGATGCACTTGACG TATGTCTCACTTTCTTTTCATTGACATGGGATGACGCCGTGACTG-3′

2.2. Apparatus

Electrochemical measurements were performed in a conventional three-electrode cell powered by a μ-Autolab potentiostat/galvanostat (the Netherlands). An Ag/AgCl, 3 mol L^{-1} KCl, a platinum wire, and a gold disk (Au electrode, 2 mm of diameter) or the Au electrode covered with an array of gold nanodumbbells (ND-Au electrode) were employed as the reference, counter and working electrodes, respectively. The system was run on a PC by GPES 4.9 software. Screen-printed electrodes were purchased from DropSens (Spain) and employed in some experiments. Time-dependent open circuit potential (OCP) measurements were carried out by a Mastech MS8340B digital multimeter (China) connected to a PC and its software.

Field emission scanning electron microscopy (FESEM) was performed using a Zeiss, Sigma-IGMA/VP (Germany) equipped with energy-dispersive X-ray spectroscopy (EDS). The samples were coated by a 2–5-nm thin film of gold by sputtering.

2.3. Preparation of the ND-Au electrode

Firstly, the Au electrode was polished on a sand paper and then a polishing pad with 50 nm-alumina powder lubricated by water. Polishing was continued to reach a mirror-like surface. The electrode was immersed in a 1:3 water/ethanol mixture and ultrasonicated for 8 min in an ultrasound bath. The Au electrode was then placed in the synthesis solution contained 500 mmol L^{-1} H_2SO_4, 20 mmol L^{-1} $HAuCl_4$ and 150 mmol L^{-1} putrescine. Electrodeposition was performed at 0 mV for 300 s. The ND-Au electrode was then rinsed thoroughly with distilled water.

2.4. Immobilization of the aptamer

A lyophilized aptamer sample was dissolved in appropriate distilled water. Then, 10 μL dithiothreitol (DTT) solution (containing 10 mmol L^{-1} sodium acetate, pH 5.2 and 500 mmol L^{-1} DTT) was added, mixed, and incubated at room temperature for 15 min. Excess DTT and unwanted thiol fragments were removed from the

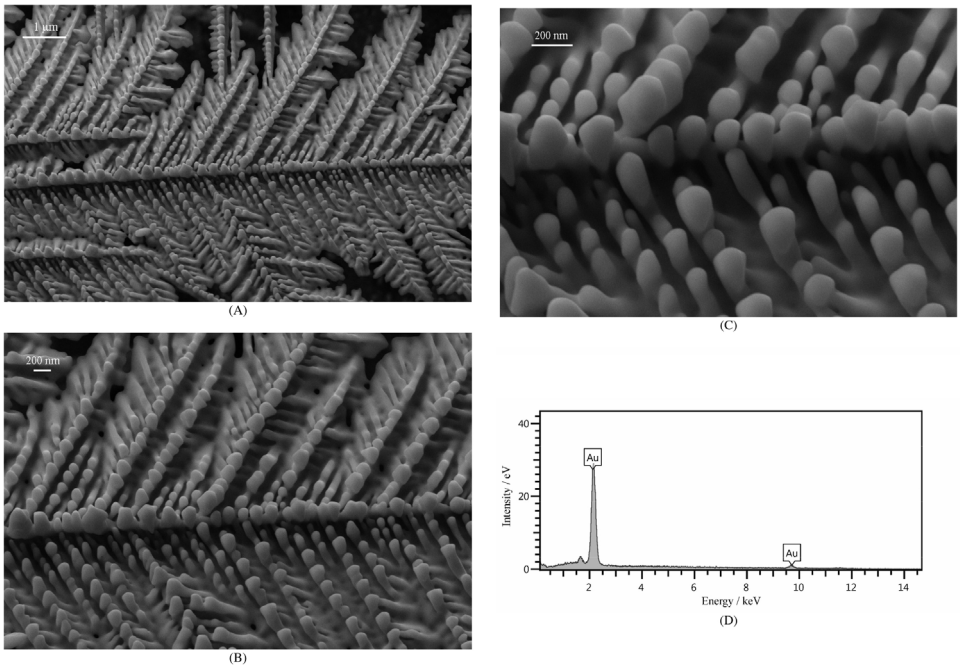

Fig 1. FESEM images (A-C) and an EDS spectrum (D) of the ND-Au electrode surface.