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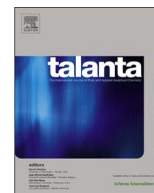

Highly sensitive amperometric detection of cardiac troponin I using sandwich aptamers and screen-printed carbon electrodes

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ABSTRACT

In this study, we developed a sandwich aptamer-based screen-printed carbon electrode (SPCE) using chronoamperometry for the detection of cardiac troponin I (cTnI), one of the promising biomarkers for acute myocardial infarction (AMI). Disposable three-electrode SPCEs were manufactured using a screen printer, and various modifications such as electrodeposition of gold nanoparticles and electropolymerization of conductive polymers were performed. From the bare electrode to the aptamer-immobilized SPCE, all processes were monitored and analyzed via various techniques such as cyclic voltammetry, electrochemical impedance spectroscopy, and X-ray photoelectron spectroscopy. The quantification of cTnI was conducted based on amperometric signals from the catalytic reaction between hydrazine and H_2O_2. The fabricated aptasensor in a buffer, as well as in a serum-added solution, exhibited great analytical performance with a dynamic range of 1–100 pM (0.024–2.4 ng/mL) and a detection limit of 1.0 pM (24 pg/mL), which is lower than the existing cutoff values (40–700 pg/mL). Furthermore, the developed sensor showed high sensitivity to cTnI over other proteins. It is anticipated that this potable SPCE aptasensor for cTnI will become an innovative diagnostic tool for AMI.

1. Introduction

Point-of-care testing (POCT) has been great attention because of several advantages over traditional diagnostic techniques, such as a broad availability to diagnosis, easy accessibility, rapid quantification, and minimal required sample volumes [1–3]. Among diverse POCT devices, screen-printed carbon electrodes (SPCEs), which are fabricated by printing several types of inks on a specific substrate have been considered superior because of the low cost of carbon, the good reproducibility of the results, the rapid responses to analytes, disposability, and surface functionalizations [4,5]. Such SPCEs have been recently employed as diagnostic tools for food poisoning, diseases, and environmental pollutants [6–8].

A number of investigations for the early diagnosis of acute myocardial infarction (AMI), one of the foremost causes of death, have been carried out based on specific biomarkers [9,10]. Since cardiac troponins (cTnI and cTnT) have shown the high sensitivity and selectivity toward AMI, they have been targeted to many diagnostic investigations. In particular, cTnI and cTnT are superior diagnostic markers for early AMI presenters and late presenters, respectively [11]. To this end, on-site POCT techniques and devices have been developed for AMI biomarkers including the isoform of creatine kinase, myoglobin, and the cardiac troponins (cTnI and cTnT) [3,12,13]. However, since all current assays are based on antibody-antigen interactions, there are some limitations related to the antibodies, such as poor stability, relatively high cost, and long incubation time.

Aptamers, oligonucleic acids or peptides with high sensitivity and selectivity toward target molecules have been considered as excellent substitutes for antibodies; they have also shown benefits compared to antibodies, such as easy functionalization, high stability in harsh conditions, and rapid production [14–16]. In particular, aptamer-based electrical biosensors have been used to many clinical applications because of lots of superiorities such as relative stability of electroactive labels, promising speed, and low cost [17–19]. We have recently screened cTnI-specific aptamers showing high selectivity and sensitivity toward only cTnI, which is a promising AMI biomarker among currently available markers [20]. Among various biomarkers for AMI such as the isoform of creatine kinase, myoglobin, and lactate dehydrogenase, cTnI have been shown to be a valuable biomarker for AMI because of its high specificity and long residence time [21,22]. Based on diverse advantages of cTnI-specific aptamer, the accurate and precise diagnosis for AMI could be achieved.

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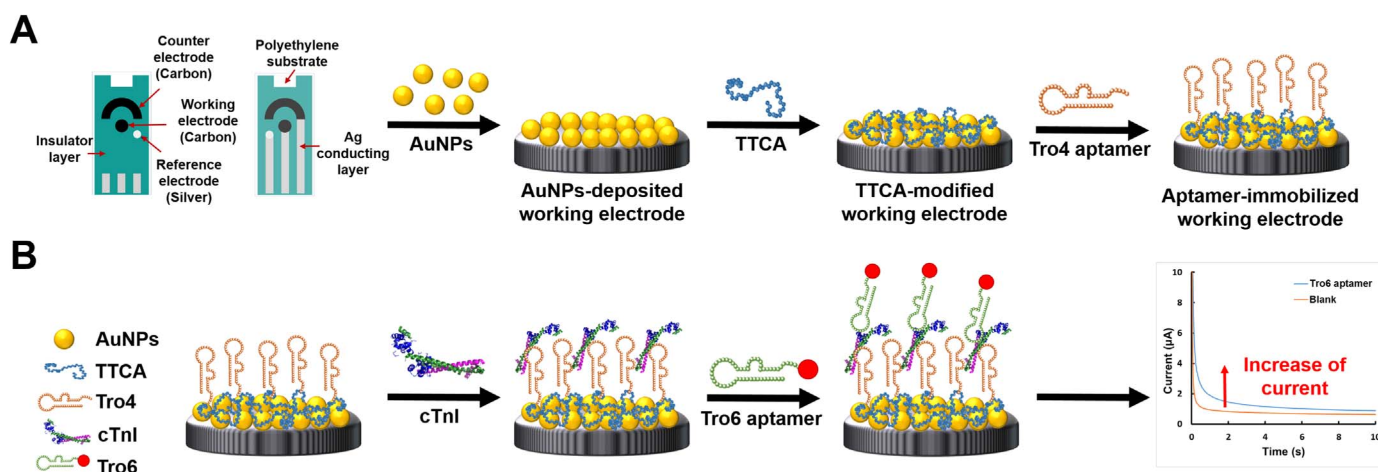

Fig. 1. Schematic illustration for the fabrication processes of the sensor and the detection of cTnI. (A) A three-electrode SPCE was manufactured using a typical printing system. AuNPs were electrically deposited on the working electrode, and then, a TTCA monomer was electrically polymerized. After EDC/NHS activation, the amine-modified aptamer was covalently immobilized on the SPCE. (B) Various concentrations of cTnI were incubated with the SPCE, followed by washing with DW. The hydrazine-modified aptamer was then incubated with the electrode, and the amperometric signals were recorded in a 10 mM H_2O_2 solution (AuNPs: gold nanoparticles; TTCA: 5,2′:5′2′′-terthiophene-3′-carboxylic acid; Tro4 aptamer: capture probe; Tro6 aptamer: detecting probe).

For the accurate monitoring of target molecules, the detection techniques are exceedingly important. Numerous detection systems based on colorimetry, fluorometry, and electrochemistry have been carried out to measure target-probe interaction. In particular, chronoamperometry have been received great attention and applied in many fields because of various superiorities like high-speed, simplicity, and high signal to noise ratio [23–25]. In the present study, we designed aptamer-based SPCEs for the early diagnosis of AMI using chronoamperometry and aptamer sandwich assays, for the first time (Fig. 1). These SPCE sensors exhibit high sensitivity and selectivity toward cTnI in buffer conditions as well as in a serum-supplemented solution.

2. Materials and methods

2.1. Materials

Gold (III) chloride trihydrate, human serum albumin (HSA), human serum (human male AB plasma), bovine serum albumin (BSA), sodium borohydride, and adipic acid dihydrazide were bought from Sigma-Aldrich (St. Louis, MO, USA). Trisodium citrate dehydrate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Carbon ink, silver ink, and insulation paint were obtained from Jujo Chemical (Tokyo, Japan). The 5′-amine modified Tro4 aptamer (5′-amine-CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA-3′) and 5′-phosphate modified Tro6 aptamer (5′-phosphate-CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC) were synthesized by Cosmo Genetech (Seoul, Korea). The 5,2′:5′2′′-terthiophene-3′-carboxylic acid (TTCA) was newly synthesized by Shim's group following their previous report [26]. Interleukin 13 receptor (IL-13R), interleukin 5 receptor (IL-5R), cluster of differentiation 4 (CD4), and CD166 were acquired from Sino Biological (Beijing, China). Lysozyme was bought from Bio Basic (Markham, Ontario, Canada). The BL21 (DE3) *Escherichia coli* strain was purchased from Invitrogen (USA). Luria Bertani (LB) was obtained from Merck (Kenilworth, NJ, USA). Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was purchased from Calbiochem (San Diego, CA, USA).

2.2. Preparation of gold nanoparticles

Prior to the synthesis, all of the glassware was washed with a 3:1 mixture of HCl:HNO_3 and rinsed with deionized water (DW). For the synthesis of gold nanoparticles (AuNPs) having a size 5 nm, 1 mL of 1% (w/v) gold(III) chloride solution was added to 90 mL of DW under vigorous stirring [27]. After the stirring for 1 min, 2 mL of 38.8 mM trisodium citrate was added, and the solution was incubated for 1 min. And then, 1 mL of 0.075% (w/v) sodium borohydride was slowly supplemented to the mixture. The concentration of the AuNPs was measured via UV–VIS spectroscopy (Libra S22, Biochrom). The spectroscopic and morphological characteristics were analyzed by UV–VIS spectroscopy and transmission electron microscopy (TEM) imaging with a JEM-1011 instrument (JEOL, Tokyo, Japan), respectively.

2.3. Expression and purification of cTnI

The pET-28a plasmid containing *Troponin I* was transformed into *E. coli* strain BL21 (DE3), and one positive clone was obtained from independent plaques. The transformed *E. coli* cells were grown in LB broth at 37 °C until the absorbance at 600 nm reached 0.6. The expression of cTnI was induced by an addition of IPTG at a final concentration of 0.2 mM. After incubating the cells at 18 °C overnight, they were harvested by centrifugation at 5000 rpm at 4 °C for 20 min, and washed once with phosphate-buffered saline (PBS). The cTnI-expressing cells were resuspended in a lysis buffer (20 mM Tris, pH 8.0, 500 mM NaCl, 0.5 mM β-mercaptoethanol, and 0.1% Tween 20) and disrupted by sonication on ice. After the centrifugation of the cell lysate at 15,000 rpm for 30 min, the supernatant was filtered through a 0.45 µm membrane filter. The supernatant was applied to a Ni-NTA column pre-equilibrated with the lysis buffer. After binding, the protein was eluted by increasing the concentration of imidazole from 0 to 300 mM with an elution buffer (20 mM Tris, pH 8.0, 500 mM NaCl, 0.5 mM β-mercaptoethanol, 0.1% Tween 20, and 300 mM imidazole). In addition, the eluted protein was applied to a desalting column to remove imidazole and was further purified using a Superdex peptide gel filtration column (GE Healthcare, USA). The purified cTnI was stored in a final buffer (20 mM Tris, pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol, and 0.1% Tween 20) at a concentration of 20 µM.

2.4. Fabrication of SPCEs

All experiments were carried out in a three-electrode cell utilizing an all-in-one SPCE. The SPCEs were manufactured on the polyethylene-based film using a screen printer (BANDO industrial, Korea). First of all, silver was coated on the film as conductor, and then carbon was printed as working and counter electrodes. Finally, insulator was also covered on the top of the film. The cleaning using 0.1 M HNO_3 was

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