Int. J. Electrochem. Sci., 12 **(2017)** 7931 – 7940, doi: 10.20964/2017.09.65

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Fabrication of an Immunosensor for Cardiac Troponin I Determination

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Received: 26 May 2017 / *Accepted:* 23 July 2017 / *Published:* 13 August 2017

Using the stripping voltammetry of platinum or palladium nanoparticles as the basis, this work proposes an emerging electrochemical technique to detect cardiac troponin I (cTnI). The amount of cTnI specifically adsorbed on an anti-cTnI-modified electrode surface was related to the PdNP (Pd) and PtNP (Pt) current responses. Plasma specimens from acute myocardial infarction (AMI) sufferers and healthy donors were used for cTnI surveillance. SPE/PdNP/anti-cTnI exhibits a wide detection range of 0.1–40 ng/ml, with a low detection limit (DL) of 0.1 ng/ml. SPE/PtNP/anti-cTnI exhibits a wide detection range of 0.1–55 ng/ml, with a low detection limit (DL) of 0.07 ng/ml.

Keywords: Cardiovascular diseases; Immunosensor; Cardiac troponin I; Platinum; Palladium

1. INTRODUCTION

Acute myocardial infarction (AMI) is one of the most common death-induced factors globally, and the sex- and age- adjusted occurrence rate is 200 cases per person-year. Many research enterprises and teams have devoted attention to developing tools for an accurate and fast AMI diagnosis and prognosis. Poor specificity has been exhibited in the isoforms of creatine kinase, lactate dehydrogenase, myoglobin (Mb) and many other biomarkers despite their identification as AMI diagnostic markers [1-3]. However, cTnI, together with the troponin complex containing cTnI, cardiac troponin T (cTnT) and cardiac troponin C (cTnC) in cardiac muscle tissue is released into the blood circulation just after an AMI, and they have been proposed as prominent biomarkers for AMI [4, 5].

The determination and surveillance of cTnI and the troponin complex are regarded as important techniques for early AMI diagnosis because of their significant sensitivity and specificity to AMI.

To propose a cTnI diagnosis technique with an enhanced specificity and sensitivity, a myriad of studies have been conducted. The radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are usually employed for the surveillance of cTnI levels in clinical cases because they are both based on a selective antibody-antigen interaction [6-11]. Nevertheless, such antibody-based techniques have some drawbacks, such as the costly generation of antibodies, instability at high temperatures, and difficulty in chemically modifying antibodies for biological determination. Thus, oligonucleic acids, peptide molecules or aptamers that feature binding to specific targets have been proposed to substitute antibodies in diagnostic and therapeutic applications because of their merits, which can supplement the drawbacks of the antibodies [12-16]. In diverse research fields, these aptamer-based diagnostic instruments are considered "rising stars".

For the target diseases to be accurately diagnosed, it is essential for a sufficiently sensitive detection system to be constructed. Due to the desirable sensitivity, rapidness, directness, simplicity and wide application for target protein quantification, the electrochemical technique excels over many other detection approaches [17-19]. Particularly, since Fc performs well in an electron shift reaction as a mediator, ferrocene (Fc)-modified nanoparticle-based electrochemical analysis has gained active utilization [20-22]. Fc-modified silica nanoparticles (Fc-SiNPs) have unique strengths, such as significant electroactivity, simple modification, and stability under severe conditions [23]. The target molecules are successfully quantified through a remarkable signal amplification in electrochemical detection with the use of Fc-SiNPs with an electron shift.

Bioelectrochemistry has witnessed the widespread use of metal nanoparticles [24-27]. As efficacious electrocatalysts for electrode surface nanostructuring, Au nanoparticles (AuNPs) function as tags in a "sandwich" stripping analysis [28-30]. The electrochemical reduction of P450scc (CYP11A1) is enhanced by alkanthiol-stabilized AuNPs [31-35]. Furthermore, an efficient catalysation of a straight electron shift between electroactive cytochromes and the electrode was achieved using AuNPs that were stabilized with a synthetic membrane-like material with a polycationic property, namely, didodecyldimethylammonium bromide (DDAB) [36, 37].

Using the stripping voltammetry of platinum or palladium nanoparticles as the basis, cTnI determination in plasma specimens was achieved via an emerging electrochemical technique proposed in this contribution. Nanoparticles (AgNPs or AuNPs) were used for a label-free cTnI determination, and the immobilization of the antibodies was performed on Pt or Pd-modified electrode surfaces.

2. EXPERIMENTS

2.1. Chemicals

Sigma–Aldrich was the material source for sodium borohydride, $NaBH_4$, $Pd(O_2CCF_3)_2$, tetrachloroaurate, $PtCl_2$, troponin T, mouse antihuman troponin T (T anti-cTnI: 2.37 mg/ml) and mouse antihuman troponin I (anti-cTnI: 3.6 mg/ml). Note that all the chemicals were

of analytical grade. Double-distilled water was employed for the preparation of the phosphate buffer solutions (PBS) (pH 7.4) produced using a mixture of NaCl (50 mM) and KH_2PO_4 (0.1 M).

2.2. Synthesis of Pd nanoparticles

A mixture of 12.5 mL water with 16.6 mg $Pd(O_2CCF_3)_2$ (0.05 mmol) and 10.6 mg neocuproine (0.05 mmol) was stirred for 1 h with an added cosolvent agent (12.5 mL) for stabilization. After 3 min, 15 min of violent stirring was performed on the mixture for its reduction with H_2. The resulting product was denoted Pd NPs.

2.3. Synthesis of Pt nanoparticles

A beaker containing H_2O (18 mL) was employed for a representative Pt NP synthesis via the simple combination of 30 μL NaOH (5 M), 0.2 mL ascorbic acid (1 M) and 2 mL H_2PtCl_6 (18.9 mM). A brown suspension containing well-defined and monodispersed DPNs was attained after the as-prepared mixture was heated for 10 min in a water bath at a temperature of 60 ℃. The consistent total solution volume was 20.23 mL during the diverse control experiments. The resultant product was denoted Pt NPs.

2.4. Immunosensor fabrication

The obtained Pd or Pt (2 μl) was employed for the modification of a screen-printed electrode (SPE) surface (denoted SPE/PdNP and SPE/PtNP) where anti-cTnI (1 μl, 120 ng/μl) was subsequently placed (denoted SPE/PdNP/anti-cTnI and SPE/PtNP/anti-cTnI). To prevent complete drying, a humid chamber was employed, and the electrodes were kept at a temperature of over 4 °C for 12 h. After being placed on the surface of the electrode, 1 μl of the plasma solution was dried at 37 °C for 45 min. The cell with PBS provided a platform for the immunosensors to be maintained at a temperature of 37 °C for 0.5 h to achieve the reduction of the unspecific binding.

2.5. Voltammetry measurements

A standard triple-electrode system was used for all the electrochemical measurements, and the roles of the counter, reference and modified working electrode were performed by a platinum wire, a Ag/AgCl electrode and a CHI 760D potentiostat, respectively. Moreover, square wave voltammograms were attained with cTnI at diverse concentrations using 25 mV, 4 mV and 15 Hz for the amplitude, step potential and frequency, respectively. The cTnI calibration curve was obtained along with the oxidation peak currents (I_{pa}) that were monitored at 0.39 V (versus Ag/AgCl).

3. RESULTS AND DISCUSSION

As proteomic studies and methods are increasingly used, bioaffinity interactions are significantly important for clinical diagnostics at an early stage [38]. In studies concerning pharmacology, human metabolism and diseases, biorecognition is of vital significance. Target DNA-supplementary DNA/aptamer, enzyme-substrate/inhibitor, protein-protein, and antibody-antigen are regarded as popular and functionally significant couples. The electron shift is not involved in such interactions from an electrochemical aspect, and, thus, this work presents the tag-free detection of an antibody-antigen interaction on the basis of variations in the capacitance [24], mass changes [39-41], electromotive force measurements [38] and other physical parameters. The design trend for the prominent and forthcoming sensor is combining metal nanoparticles with a biorecognition material to electrochemically register an affinity interaction. For the antibody/cardiac troponin I pair (anti-cTnI/cTnI) interaction and other bioaffinity recognition incidents, Pd or Pt nanoparticles on screen-printed electrode surfaces take on the roles of the sensor elements, as proposed in this contribution. Possibly, the use of formaldehyde, sodium citrate and sodium borohydride as reducing agents contributes to the synthesis of the metal nanoparticles from their ions.

As indicated in Fig. 1, Au was electrochemically oxidized under polarization to electrochemically confirm the PdNPs on the SPE surface.

In each case, DDAB was used as the stabilizing agent for the nanoparticles and as a biomembrane for the antibody insertion. In the presence of cTnI, the specific interaction between cTnI and the aptamers prevents the approach [42, 43]. The SPE/PdNP was later modified with antibody molecules, and cTnI binding was indicated by the oxidation peak of Pd. The oxidation peak height was used to detect Pt. The immobilization of the antibodies was performed on the PtNP or PdNP-modified screen-printed electrodes using thiol groups at their termini.

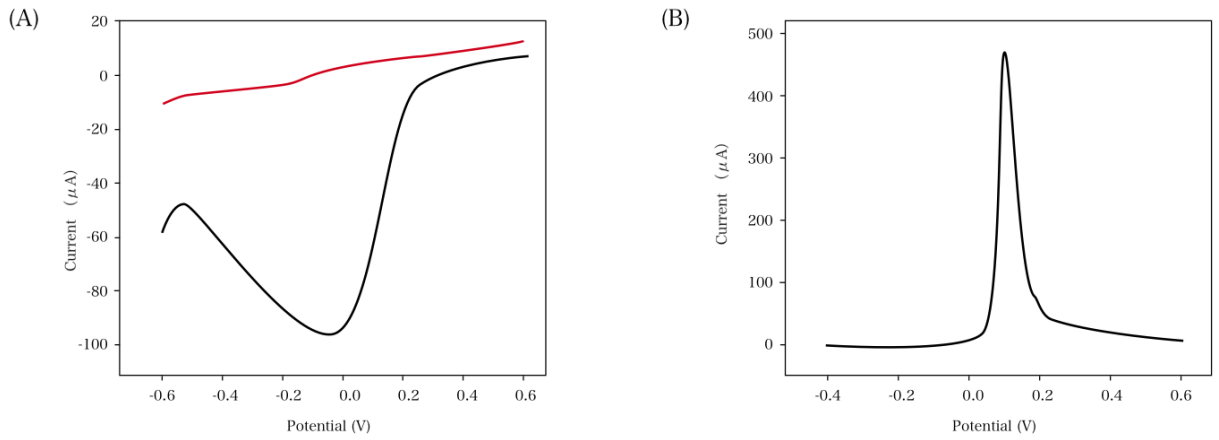

Figure 1. Stripping voltammograms of (A) SPE/$PdNP_{el}$ and (B) SPE/$PtNP_{el}$ preceding and succeeding the oxidation stage at a scan rate of 50 mV/s with PBS (pH 7.4).

CV was used for the characterization of the as-prepared sensor before cTnI was quantitatively determined. A chemically reversible reaction, a one-electron reaction and other representative electrochemical performances concerning ferrocene were exhibited by the as-prepared SPE/$PtNP_{el}$ and SPE/$PdNP_{el}$, as indicated by the prominent cathodic and anodic potential peaks that were monitored without cTnI (Fig. 2). Moreover, additional cyclic voltammograms were attained after the application of different concentrations of cTnI in the system, and together with the incubation in the binding buffer with the working electrode, the voltammograms confirmed the as-prepared sensor's effectiveness. The reason for this was the generally lower stability of the adsorbed cTnl after the large number of washing steps required for the immunoassay preparation and over the long-term [44, 45]. The reduction and oxidation peak currents (respectively, I_{pc} and I_{pa}) were observed to obtain the cTnI calibration curves. The reliability of the oxidation current herein was more desirable, and the signal deviation of the I_{pa} calibration curve was less than that of the I_{pc} curve despite the absence of an ideal linear relationship between the cTnI concentrations and the currents for the two calibration plots.

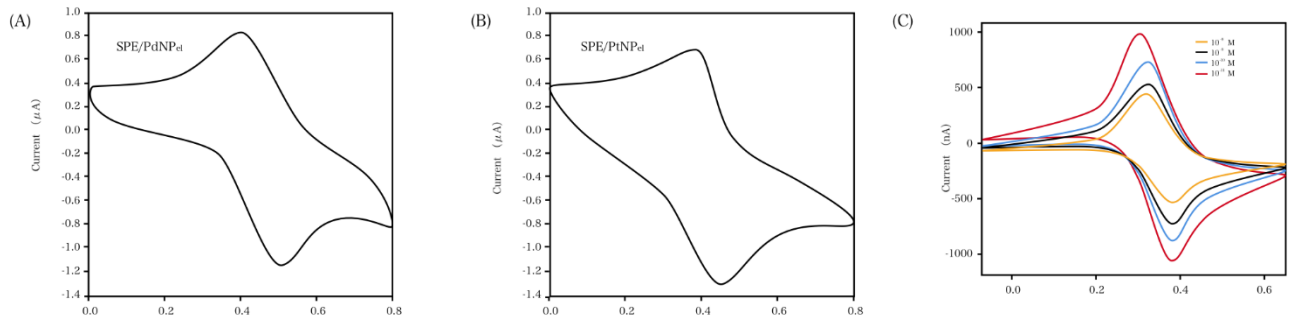

Figure 2. Cyclic voltammograms of a PBS solution (pH 7.0, 10 mM NaCl) complemented by (A) SPE/$PdNP_{el}$ and (B) SPE/$PtNP_{el}$ with a scan rate of 100 mV/s. (C) Cyclic voltammograms with different concentrations of cTnI (10^{-11}-10^{-8} M).

SPE/$PdNP_{el}$ and SPE/$PtNP_{el}$ with immobilized anti-cTnI were characterized via stripping voltammetry after the immobilization of the specific anti-cTnI antibodies on the as-prepared electrode. The impact that the proteins exerted upon the conditions of the electrode surface was indicated by the disappearance of the Pt or Pd peak heights with antibody molecules for all situations. An undiluted plasma specimen of just 1 μL was applied onto the electrode surface. Nonspecific binding was present on the sensor surface, and this is one of the main issues concerning immunoanalysis and is due to the multicomponent trait of the biological liquid-plasma. However, the cTnI protein produced a noticeable reduction in the signal [46]. The cTnI DL decreased with the stripping voltammetry technique, and the metal signal intensity declined with the conventional adoption of bovine serum albumin (BSA) (0.1 % *w*/*v*) as a blocking buffer. The washing process introduced prior to the voltammetry assay could minimize the nonspecific binding.

To analyse the plasma specimens, the electrodes (SPE/$PdNP_{el}$/anti-cTnI) acted as sensors. Specimens from both AMI sufferers and healthy donors were studied in this work. The cathodic peak

current of the Pd_{el} stripping voltammetry is dependent upon the cTnI quantities in the plasma specimens (Fig. 5). As the antibody/cardiac troponin I pair (anti-cTnI/cTnI) declined in its surface density, there was a drop in the slope of the calibration curve. The DL was 0.1 ng/ml for SPE/$PdNP_{el}$/anti-cTnI. As indicated in Fig. 6, a concentration range of 0.1 to 40 ng/ml corresponded to the linear range of the cTnI response (0.98 as the correlation coefficient). The considerably improved sensitivity is mainly attributable to the effective immobilization of the antibodies on the SPE/$PdNP_{el}$/anti-cTnI and the excellent electron mobility of the devised immunosensor [47].

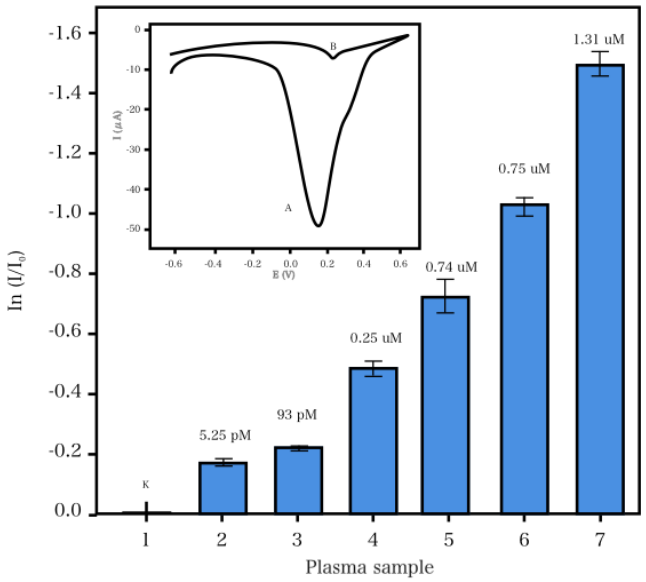

Figure 3. Dependence of the cathodic peak current of the $Pd_{el\ dep}$ stripping voltammetry on the cTnI quantity in the plasma specimens. I_o corresponds to the average cathodic peak current of the healthy donor plasma. *Inset:* stripping voltammograms of SPE/$PdNP_{el}$/anti-cTnI with plasma and SPE/$PdNP_{el}$/anti-cTnI with the plasma of an AMI.

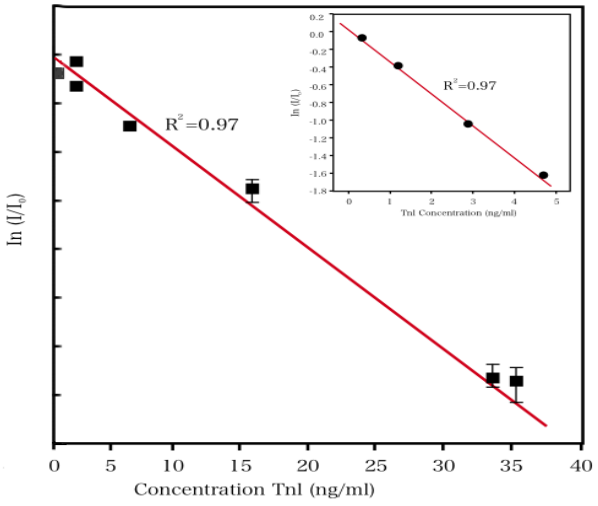

Figure 4. Responses of the screen-printed electrodes modified with $PdNP_e$, and immobilized anti-cTnI (120 ng/µl) on the troponin I interaction. *Inset:* responses for the standard cardiac troponin I in the electrolyte buffer (PBS) at pH 7.4. I_o corresponds to the average cathodic peak current of the healthy donors' plasma.

The plasma specimens were analysed using electrodes (SPE/$PtNP_{el}$/anti-cTnI) as sensors. Plasma specimens from AMI sufferers and healthy donors were studied in this study. The cathodic peak current of the Pt_{el} stripping voltammetry depends upon the cTnI quantity in the plasma specimens, as indicated in Fig. 3. As the antibody/cardiac troponin I pair (anti-cTnI/cTnI) declined in its surface density, there was a drop in the slope of the calibration curve. The DL of the SPE/$PtNP_{el}$/anti-cTnI was 0.07 ng/ml. As indicated in Fig. 4, a concentration range from 0.1 to 55 ng/ml corresponded to the linear range for cTnI with a 0.99 correlation coefficient. The sensing performance of the proposed sensor was compared with recently reported sensors, as shown in Table 1.

Interferents were analysed under the same conditions to assess the selectivity of our proposed electrochemical determination of cTnI. The response of the cTnI was compared in the presence of interferents such as galactose, fructose, glucose and inorganic ions, and their corresponding electrochemical responses are shown in Table 2.

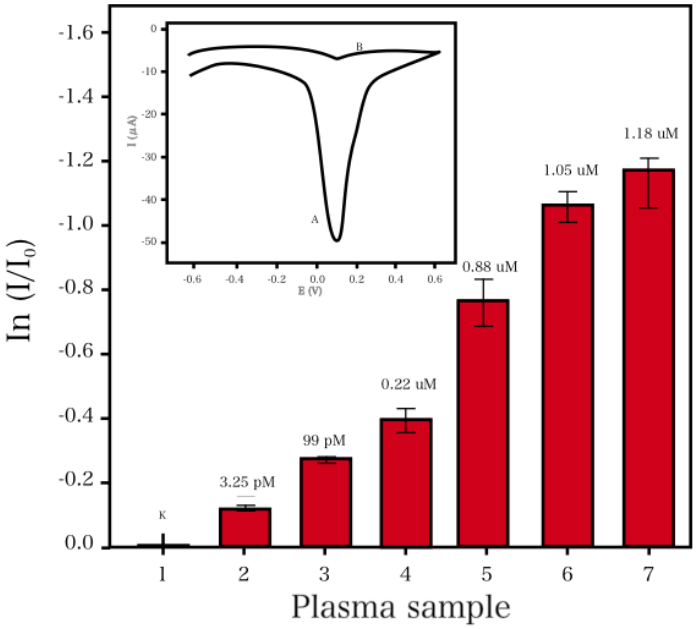

Figure 5. Dependence of the cathodic peak current of the $Pt_{el\ dep}$ stripping voltammetry on the cTnI quantity in the plasma specimens. I_o corresponds to the average cathodic peak current of healthy donors' plasma. *Inset:* stripping voltammograms of SPE/$PtNP_{el}$/anti-cTnI plus plasma and SPE/$PtNP_{el}$/anti-cTnI plus plasma from an AMI donor.

Table 1. Performance comparison of the SPE/$PtNP_{el}$/anti-cTnI plus plasma and other cTnI determination methods.

Method	Linear range	Detection limit	Reference
Carbon nanotube-based immunosensor	0.1 to 10 ng/mL	0.033 ng/mL	[48]
Gold nanoparticle-modified ITO	1 to 100 ng/mL	—	[49]
Surface-functionalized poly(dimethylsiloxane) channel	0.1 to 30 μg/mL	148 pg/mL	[50]
SPE/$PtNP_{el}$/anti-cTnI plus plasma	0.1 to 55 ng/mL	0.07 ng/mL	This work

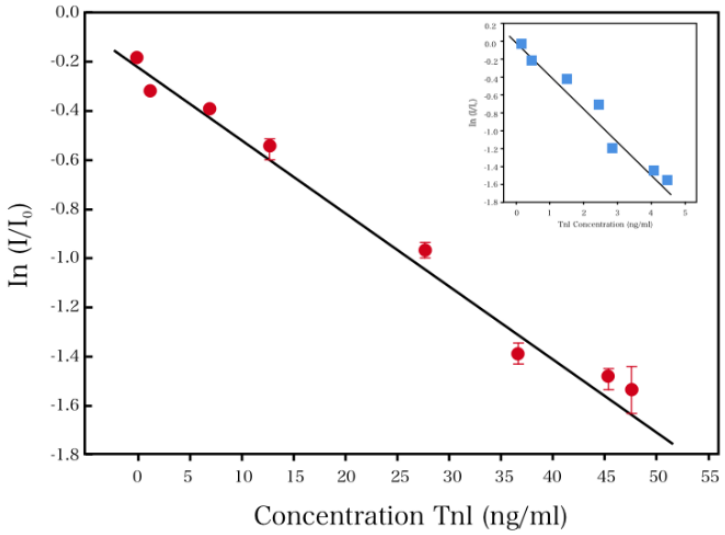

Figure 6. Responses of screen-printed electrodes modified with $PtNP_e$, and immobilized anti-cTnI (120 ng/µl) on troponin I interaction. *Inset* indicates responses for standard cardiac troponin I in electrolyte buffer (PBS, pH 7.4). I_o is corresponding to average cathodic peak current of healthy donors' plasma.

Table 2. Interference result of determination of cTnI with other common species.

Interference species	Current change (%)	Interference species	Current change (%)
Galactose	3.41	SO_4^{2-}	3.25
Fructose	1.92	Ca^{2+}	0.85
Glucose	2.40	Zn^{2+}	0.22

4. CONCLUSION

In this work, cTnI was selectively detected via a Pt NP and Pd NP-based stripping voltammetry technique without the need for secondary tagged antibodies. Industrially fabricated SPEs from several enterprises were used to conduct the electrochemical analysis, and such systems can potentially be utilized as cardiac marker on-site biosensors. The physiological level of cTnI in the plasma coincided with its dynamic ranges for the different modified electrodes. The real specimens from AMI sufferers and healthy donors were used for the cTnI surveillance.

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