Electro-immunosensor for ultra-sensitive determination of cardiac troponin I based on reduced graphene oxide and polytyramine

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ABSTRACT

This work reports the construction of a novel nanostructured immunosensor for detection of the troponin I biomarker (cTnI). Anti troponin I antibody was anchored on the modified graphite electrode with reduced graphene oxide and polytyramine for detection of troponin I in serum samples. The performance of the electro-immunosensor was evaluated by

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differential pulse voltammetry. The immunosensor presented a wide work range, from 4 ng mL^{-1} to 4 pg mL^{-1}, whose detection limit (4 pg mL^{-1}) is significantly lower than the basal level in human serum, and maintained 100% of response after 30 days of storage. Moreover, the immunosensor showed good selectivity for detection of cTnI in real sample containing interfering substances and specificity of response to cTnI. in the serum of healthy and sick patients, and demonstrated the possibility of reuse for 2 consecutive analyses, in addition to using a simplified and inexpensive platform when compared to other devices, demonstrating them excellent potential for application in diagnosis in the early stages of acute myocardial infarction.

Keywords: *bioelectrode; biosensor; acute myocardial infarction; nanocomposite; cardiac troponin I; reduced graphene oxide; polytyramine.*

1. Introduction

Cardiovascular diseases (CVD) affect a large part of the world population and are considered a serious public health problem, being the number one cause of death worldwide [1]. Some risk factors, such as obesity, physical inactivity, hypertension, stressful routine, age between 40 and 50 years, hypercholesterolemia and family history can be found in individuals affected by such diseases [2]. The most relevant CVD are: systemic arterial hypertension, acute coronary syndrome, heart failure, arrhythmias, valvular diseases and acute myocardial infarction (AMI), which occurs due to ischemia (partial or total interruption of blood supply) in the muscle. resulting in decreased contractile capacity, cardiac output, blood pressure and stroke volume [3].

Different cardiac biomarkers can be used for diagnosis or prognosis of AMI cases, and troponins are considered gold standard biomarkers. Troponins (Tn) are myocardial proteins found in a complex with actin, tropomyosin and myosin, species responsible for

cardiac contractile motion and are subdivided into three units: C (cTnC), I (cTnI) and T (cTnT), which in turn are expressed in both cardiac and skeletal muscle. For the diagnosis of AMI, only the cTnI and cTnT are used, observing the greater specificity [4,5]. Troponin I (cTnI) is a protein of molecular weight corresponding to 23,500 Da, rarely found in free form, being mostly complexed with cTnC, cTnT or both, so its definition as an isolated analyte is complex [6], nevertheless, its detection in biological fluids is a great tool in the diagnosis or prognosis of AMI [7–9].

Standard troponin detection methods are radioimmunoassay and enzyme-based immunoassay. Such tests, although selective, take time, require qualified labor, laboratory environment and expensive reagents [9]. Biosensors are a great tool capable of replacing traditional tests for more quick and cheap point-of-care devices. In particular, the use of electrochemical immunosensors as an analytical tool option capable of detecting cTnI, in biological samples of patients with AMI, is a promising field of study [10–12].

There is a significant improvement in the performance of electrochemical immunosensors by modifying the surface of their working electrodes, achieving a more efficient immobilization of the biomolecule (probe) and resulting in a more sensitive and selective device [13–15]. The modification of electrodes with conductive polymers via electrochemical polymerization is a widely explored methodology, as these materials have network of π electrons that facilitate current conduction, standing-out the simplicity of the methodology, reproducibility, stability, film thickness control and biocompatibility [16]. Tyramine (4-hydroxyphenethylamine) is a molecule widely used to obtain polymeric films due to the reproducibility and stability of the obtained films [17,18]. These characteristics are linked to its molecular structure, which has a hydroxyl group, making it soluble in polar compounds, a protonated amine group that facilitates the anchorage of bioreceptors by

physical adsorption (via electrostatic interaction) and aromatic rings configuring a delocalization of the π cloud of electrons [19].

Carbon-derived nanomaterials can be used in combination with organic polymers to improve their electronic properties [20,21]. Graphene oxide (GO) is a nanomaterial that stands out for being easy to produce, and its reduced form (rGO) is an excellent conductor, making it ideal for electrode modification [22,23].

The aim of this work was the development of an ultra-sensitive immunosensor for the detection of troponin I in serum based on graphite electrodes/reduced graphene oxide/polytyramine (GE/rGO/Ptyr) platform to use in point of care for cardiac troponin I detection. It is worth notice to the platform based on GE/rGO/Ptyr is an easy and cheap to produce, besides their robust chemical characteristics, which makes it an excellent option to construct an immunosensor. The device demonstrated important analytic parameters, such as: high sensibility with a wide working ranging encompassing patients in early stages of AMI, reusability for 2 times which reduce costs, stability maintained in 100% during 30 days, selectivity from different analytes, being able to discriminate biological samples from healthy to AMI patients without further purification of serum samples, a really complex biological sample due their rich composition. These characteristics listed above, combined, highlight this immunosensor for its novelty and importance.

2. Experimental

2.1 Materials and instrumentation

All the reagents were analytical grade and all solutions used were prepared with ultrapure deionized water (resistivity = 18.2 MΩ cm^{-1}, Gehaka, Brazil), deoxygenated with nitrogen gas (N_2) and all the experiments were performed at room temperature. Cardiac troponin I monoclonal antibody (anti-cTnI), cardiac troponin I (cTnI) and bovine serum

albumin (BSA) were purchased from Invitrogen and Sigma Aldrich (USA). Potassium hexacyanoferrate III {$K_3[Fe(CN_6)]$}, Potassium hexacyanoferrate II {$K_4[Fe(CN)_6]\cdot 3H_2O$}, potassium chloride (KCl) and sodium dodecyl sulphate ($CH_3(CH_2)11OSO_3Na$) were purchased from Vetec, Reagen and Neon, (Brazil) respectively. Disodium hydrogen phosphate (Na_2HPO_4) and monosodium dihydrogen phosphate (NaH_2PO_4) were obtained from Synth (Brazil). Tyramine hydrochloride ($C_8H_{12}ClNO$, 98%) was obtained from Acros Organics (USA). Graphite disk (Ø = 6 mm, 99.99% purity, Alfa Aesar, USA) was used as working electrode, and as reference electrode and counter electrode was used Ag/AgCl (KCl 3 mol L^{-1}) and platinum plate, respectively. The graphite electrodes (GE) were submitted to mechanical polishing, using sandpaper of different grains (400 and 2000). Afterwards, the electrodes were polished for 60 seconds on felt moistened with a suspension of alumina, and rinsed with deionized water. After cleaning, the GE in distilled water with ultrasonic washing for 3 minutes, the electrodes were electrochemically cycled from 0.3 to + 1.1 V (vs. $Ag/AgCl/Cl^-$) varying the scan rate from 500 to 50 mV s^{-1} performing several cycles at each speed, in 0.1 mol L^{-1} H_2SO_4, followed by rinsing in distilled water.

Electrochemical tests were performed in a potentiostat (CHI Instruments, model 760 C, USA) with a three electrode electrochemical cell. Morphological analyses were performed by a scanning electron microscope (SEM) model Vega3 LMU (TESCAN, Czech Republic), operated at 5.00 kV, and scanning probe microscopy (SPM) model 5100 (Hitachi, Japan), performed in dynamic force microscopy (DFM) mode operated at intermittent contact mode with silicon cantilever of molar constant 1.0 N m^{-1} and 0.38 Hz frequency. Fourier transform infrared (FT-IR) spectrum was recorded using a spectrophotometer FT-IR Frontier Single Range– MIR (Perkin Elmer, USA). The FT-IR analyzes were performed directly on the surface of graphite electrodes modified with the nanocomposite (GE/rGO/Ptyr) and with its individual components (rGO and Ptyr). Human serum samples from sick (infarcted patients)

and healthy (non infarcted patients) were obtained from Sabin Medicina Diagnóstica Laboratory in Uberlândia, Brazil. All the analyses performed in this work were made using this samples.

2.2 Immunosensor preparation

The GE/rGO/PTyr platform was prepared according to Moço et al., 2019 [22]. After modification of the graphite surface, the electrodes were rinsed with deionized water. Then 10 µL of the anti-troponin I antibody (anti-cTnI) was dripped onto the modified graphite electrode. Antibody concentrations of 1 µg mL^{-1} and 10 µg mL^{-1} were tested. The electrode was maintained in a sealed container for 30 minutes, and 30 µL of a blocking solution (BSA, 0.5% v/v in phosphate buffer pH 7.4) was added and washed with 50 µL of phosphate buffer for 3 times. Indirect electrochemical analysis were carried out using the differential pulse voltammetry (DPV) in KCl solution (0.1 mol L^{-1}) containing $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (5 mmol L^{-1}). Figure 1 shows a scheme of the immunosensor construction.

<Figure 1>

2.3 Determination of the cTnI in human serum samples and calibration curve

For the formation of the immunocomplex (anti TnIc: cTnI), 10 µL of a 1:1 solution dilution containing healthy patient serum enriched with the analyte (troponin I antigen - cTnI) in different concentrations ($4x10^{-3}$ ng mL^{-1}, $8x10^{-3}$ ng mL^{-1}, $8x10^{-2}$ ng mL^{-1}, 1 ng mL^{-1}, 2 ng mL^{-1}, 4 ng mL^{-1}) was used and the previously specified conditioning, washing and drying were repeated. The incubation time between antibody and antigen was based in works developed by our research group [24, 25, 26]. Limit of detection was reported as the lowest concentration detected.

2.4 Evaluation of the specificity, stability, regeneration and reproducibility

The specificity of the immunosensor was evaluated using serum of healthy patients enriched with Troponin T, C-reactive protein and free triiodothyronine (T_3), all in the same concentration of 4 ng mL^{-1}. Shelf life was tested maintaining the immunosensor packed at 8° C in an airtight container with nitrogen atmosphere and measurements were taken every 10 days at 25° C during 40 days. Regeneration test was conducted applying 30 µL of sodium dodecyl sulphate (SDS, 1% w/v) during 3 minutes onto the immunosensor, washing with phosphate buffer (0.1 mmol L-1, pH 7.4) and drying with N2. It is known that the SDS disrupts the interaction between antigen and antibody. For reproducibility analysis, all experiments were performed in triplicate and numeric results are presented as arithmetic mean ± standard deviation.

3. Results and discussion

3.1 Immunosensor construction

3.1.1 Characterization of GE/rGO/PTyr nanocomposite

The rGO/Ptyr nanocomposite has interesting characteristics, which justify its deposition onto graphite electrode, rGO shows good conductivity and produces increase in surface area of the electrode surface, due to increase in like-globule structures, favoring deposition of the polymeric material, when compared to bare EG [22],[26]. Amino groups present in the Ptyr structure favor the interaction with biomolecules by covalent bonds or hydrogen bonds. rGO and Ptyr act synergistically in the composite material, favoring the incorporation of increased amount of bioreceptor units (antibodies).

The literature shows that, generally, nanomaterials improve the performance of biosensors, increasing sensitivity and reducing detection limits by several orders of magnitude [27], and are responsible for enhance biomolecules in the surface of the device.

About this particular nanomaterial, based in GE/rGO/Ptyr, was related the immobilization of the probe biomolecule on the platform modified with the rGO/Ptyr nanocomposite showed an increase of 17% in the peak current response, reinforcing that this nanocomposite improves the electrochemical properties of the surface [22].

Figure 2 presents analysis by FT-IR of Ptyr (A), rGO (B) and GE/rGO/Ptyr nanocomposite (C).

<Figure 2>

The Ptyr spectrum (Fig. 2A) presents bands at 3532cm^{-1}, 3200 cm^{-1}, 1615 cm^{-1} and 1482 cm^{-1} are characteristics of the O-H stretch, asymmetrical stretch of N-H, stretching of the bond between carbons (-C=C-) of aromatic rings and deformation angle of N-H. The bands present in 1213 cm^{-1} and 1072 cm^{-1} show that the tyramine electropolymerization occurred by the phenolic group with the carbon present in the aromatic ring of another monomer, forming the ether function (C–O–C). The rGO spectrum (Fig.2B) shows bands at 3367 cm^{-1} (O-H groups), 1646 cm^{-1} (C=O groups), 1558 cm^{-1} (C=C groups within the aromatic rings), 1205 cm $^{-1}$ (C-O-C groups of epoxides), 1070 cm $^{-1}$ (C-O groups of α-unsaturated secondary alcohols) and 855 cm $^{-1}$ (C-H groups of aromatic hydrocarbons).

The nanocomposite spectrum (Fig. 2C) shows bands in 2919 cm^{-1} and 2845 cm^{-1}, which are axial deformation characteristics of C - H aliphatic and an increase in bands 1213 cm^{-1} and 1072 cm^{-1}, characteristic of C–O–C stretch in addition to the bands shown in the A and B spectra of Figure 2.

3.1.2 Biofunctionalization of rGO/polytyramine nanocomposite

The morphology of the immunosensor was evaluated by SEM and DFM before and after interaction with sick and health patient serum (Figure 3).

<Figure 3>

The graphite electrode modified with the nanocomposite and the incorporated antibody probe presented an root mean square roughness coefficient (Rq) of 643.8 nm in the DFM analysis (Fig. 3E). With the blocking step with BSA, the Rq value decreased to 212.2 nm (Fig. 3F), and it is possible to observe disposed granules in the SEM images (Fig. 3B) which are not present in the previous step (Fig. 3A), suggesting that the BSA molecules occupy empty spaces between the nanocomposite and the immobilized antibodies. Analyzing the immunosensor images after contact with healthy serum (Fig. 3C and 3G, Rq = 373.8 nm) and sick patient serum (Fig 3D and 3H, Rq = 202.4 nm) a smoothing of the surface was observed. This difference in roughness and morphology can be explained due to the immunocomplex formation, by the presence of high concentration of cTnI in the sick patients, onto the surface of the electrode, due to the high molecular weight of these species.

3.1.3 Anti-cTnI concentration optimization

Figure 4 indicates the bar chart of current values as a function of the optimization of the cTnI antibody concentration. It is known that antibody concentration is an important parameter for improving performance of the immunosensor [28].

<Figure 4>

The bar chart shows an inverse relationship between antibody concentration and current response, where high concentrations of antibody resulted in lower current values. Human cTnI is a 24 kDa protein of 210 amino acids with isoelectric point (pI) around 5.4 [29], therefore in pH 7.4 has negative net charge, which causes blocking in the electronic transfer

process with the anionic probe $[Fe(CN)_6]^{-4}$. This obtained result also suggests surface saturation with probe antibody, resulting in a possible steric impediment making it difficult the immunocomplex formation. In contrast, the use of lower concentrations of antibody indicates a more homogeneous distribution along the surface, facilitating the interaction probe (anti-cTnI) with the target (cTnI), in accordance with literature [30].

3.2 Performance of the immunosensor

3.2.1 Calibration curve

The validation of a biosensor is an essential step to demonstrate that the results obtained will be close enough to the unknown reference value used in conventional assays for the analyte under study [31,32]. For testing the performance of a biosensor, parameters such selectivity, sensitivity, linear range, limit of detection (LOD) and stability were evaluated (Figure 5).

<Figure 5>

It is important to mention that healthy people present low troponin I concentrations in their plasma [33], however, in AMI episodes, there is a rapid release of this biomarker and a delay until its levels are normalized. In order to evaluate the immunosensor performance, the calibration curve was constructed to encompass the usual concentrations of troponin I in the serum of a healthy (<0.04 ng mL^{-1}) and sick individuals (>0.40 ng mL^{-1}) [34]. For this assay, healthy patient serum was enriched with cTnI for the calibration curve construction, from 4 pg mL^{-1} to 4 ng mL^{-1} as shown in Figure 5A. There was possible to obtain an linear regression between the cTnI concentration and the peak current values, fitted by the equation $I\ (\mu A) = 319_{*}\text{-}\log[cTnI] - 2157.4$, r = 0.996, which sensitivity and limit of detection (LOD) equal to 319 µA.mL ng^{-1} and 4 pg mL^{-1}, respectively. Cardiac troponin I quantification is

usually performed using commercial enzyme-linked immunosorbent assays (ELISA) or radioimmunoassays [9] although, some commercial ELISA tests have a lower working range than that obtained in this work[35,36] and radioimmunoassays utilizes high complexity reagents, such as radioisotopes used as markers, making the process more expensive and slower to obtain results[37]. All the results mentionated above indicate that the developed immunosensor was able to detect cTnI in human serum samples in concentrations even below of the reference value [34].

Fig 5B demonstrated there is an inverse relationship between the current response values as a function of the increase in cTnI concentration. The decrease in current response value suggests that the oxidation of the ferrocyanide redox pair is being hampered by the immunocomplex formation, since it causes a steric hindrance onto the electrode surface due the size of the antigen and antibody molecules, which difficult the electron transfer thus decreasing the current. There is also an influence of the isoelectric point: the isoelectric point of troponin I in human plasma vary from 5.2 to 6 [28].Considering that the cTnI solution were diluted in sodium phosphate buffer, which pH is 7.4, the protein is mostly negatively charged, intensifying the repulsion effect of the $[Fe(CN_6)]^{-4}$ redox pair, justifying the decrease in current values with the increase in concentration. Figure 5C shows the bar chart of the oxidation peak current values as a function of human serum samples from healthy patients (H1-H2) and patients with AMI (S1-S9), indicating a differentiation of the current response between the two types of patients, as healthy patients presented higher current values when compared to infarcted patients, corroborating to the results presented above, where higher current values are observed when there is no formation of the immunocomplex.

3.2.2 Selectivity analysis, regeneration and storage stability

Figure 6 shows the behavior of the developed immunosensor using others possible targets, reuse and shelf time.

<Figure 6>

Figure 6A presented a differentiated response between the species analyzed, since they showed a higher current value when compared to the current generated by the formation of the immunocomplex with cTnI, indicating that there was no link between the antibody and the antigens used as a negative control, neither with a solution without cTnI. This result corroborates with the calibration curve, where higher current values were observed in small or none concentrations of cTnI, showing the reduced formation of the immune complex with exposure of the Fab portion of the antibody.

Immunosensor regeneration protocols aim to disrupt the immune complex, releasing the antigen and maintaining the integrity of the antibody, aiming cost reduction and envisioning the possibility of commercialization. The regenerated electrodes maintained the response until the second consecutive detection (Fig 6B), with a low response variation between (from 793 ± 11.3 μA to 757.5 ± 20.5 μA). The immunosensor response decreased 100% in the third regeneration probably due antibody leaching or the action loss of antibody function due to SDS [38].

Figure 6C presents a 40-day evaluation of the shelf time of the immunosensor and shows a maintenance of the current response of 100% at 30 days and 87% at 40 days. It is known that the biosensors can be susceptible to a complex mechanism of aging, affecting their biomolecules overall performance [39]. In this work, the method of physical adsorption was used for immobilization of the anti-cTnI molecule, which is based on the electrostatic interaction and Van der Waals interaction of the functional groups at the end of the Fc portion of the antibody (specially carboxylate groups) with the modified surface (amino groups from polythyramine). During the time of stability analysis, the antibodies probably maintained

their "end-on" orientation, enabling the subsequent formation of the immunocomplex, due to its interaction with the protonated amines on the surface of the polythyramine.

There are several immunosensors reported in the literature for Troponin I detection and listed in Table 1.

Table 1. Analytical parameters of different electrochemical immunosensors recently described for cardiac Troponin I.

Platform	Linear range	LOD	Stability	Regeneration	Detection Time	Ref.
Carbon Screen Printed Electrode/ AuNP	0.1-32 ng mL^{-1}	0.1 ng mL^{-1}	15 days	N.A.	30 min	[40]
AuNp/ graphene oxide	0.05-3 ng mL^{-1}	0.05 ng mL^{-1}	30 days	N.A.	5 min	[41]
Glassy Carbon Electrode/ anode-oxidation amidation	0.05-5 ng mL^{-1}	0.05 ng mL^{-1}	N.A.	N.A.	N.A.	[42]
Citrate-capped AuNP/ Carbon Screen Printed	0.2-12.5 ng mL^{-1}	0.2 ng mL^{-1}	N.A.	N.A.	N.A.	[43]
Graphene oxide nanostructure	0.1-10 ng mL^{-1}	0.07 ng mL^{-1}	N.A.	N.A.	N.A.	[44]
Glassy carbon electrodes/ biosynthesized	$1.25x10^{-5}$-8.25 µg mL^{-1}	$2.61x10^{-5}$ µg mL^{-1}	20 days	N.A.	N.A.	[45]

ZnONPs						
GE/rGO/PTyr/anti-cTnI platform	4 pg - 4 ng mL^{-1}	4 pg mL^{-1}	40 days	2 times	20 min	This work

(N.A. not available)

The listed biosensors in Table 1 lacks some important commercial features of biosensors that were highlighted in this work, such as: very low detection limit, possibility of reuse, stability studies, operation in the clinical demanding range and short response time. Besides that, the immunosensor developed in this work presents specificity, sensitivity and was able to diferentiate human serum samples of healthy patients from infarcted patients.

4. Conclusions

In this work we developed an ultrasensitive electrochemical immunosensor for cTnI cardiac, able to discriminate between healthy and sick patients, corroborated by the images of DFM and SEM. In optimized conditions, the platform can provide a low detection limit of 4 pg mL^{-1} for cTnI in serum samples, significantly lower than the level of health people, and maintained 100% and 87% of current response in 30 days and 40 days of storage, respectively. Thus, this biosensor demonstrated excellent potential for application in diagnosis in the early stages of acute myocardial infarction.

Credit Author Statement

Jéssica G. Brussasco: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing**. Pedro H. G. Guedes:** Methodology, Investigation, Validation**,** Conceptualization, Writing. **Dayane D. de Moraes:** Methodology, Investigation, Validation**. Anna C.R. Moço:** Methodology, Investigation, Validation. **José M. R. Flauzino:**

Methodology, Validation, Formal Analysis. **Heliane S. Silva:** Methodology, Validation, Formal Analysis. **Atair C. Silva:** Validation, Formal Analysis. **Jonathan P. Silva:** Human serum sample supply**,** Validation. **João M. Madurro:** Supervision, Writing - Reviewing and Editing, Funding acquisition. **Ana G. Brito-Madurro:** Supervision, Writing- Reviewing and Editing, Funding acquisition.

Conflict of interest statement

The authors declare no conflict of interest.

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Figure Captions

Fig. 1 - Scheme of the steps of immunosensor construction.

Fig. 2 - FT-IR spectrum of Ptyr (A), rGO (B) and GE/rGO/Ptyr (C).

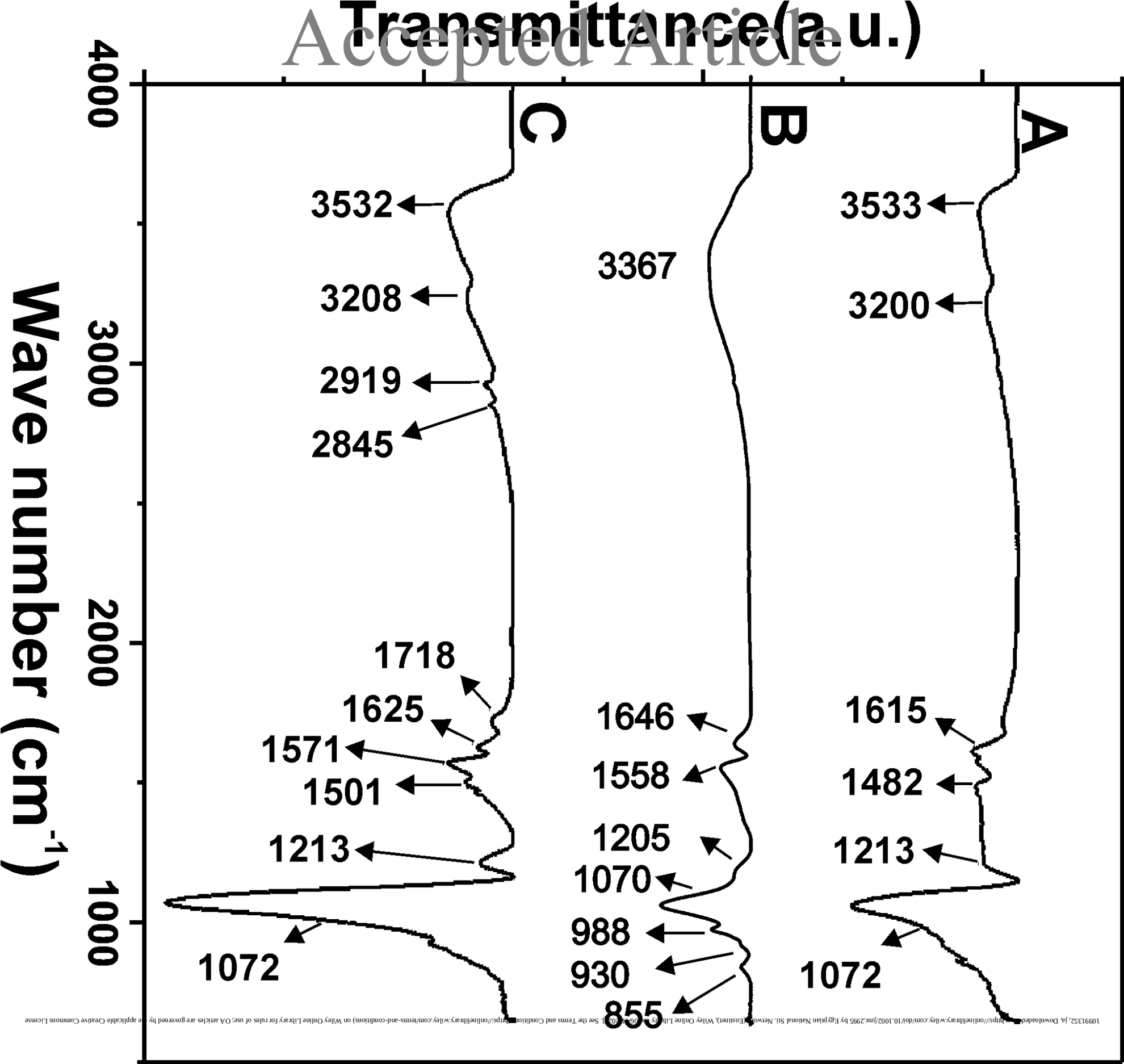
Fig. 3 - SEM and AFM images of GE/rGO/PTyr/anti-cTnI (A, E); GE/rGO/PTyr/anti-cTnI/BSA (B, F); GE/rGO/PTyr/anti-cTnI/BSA/health serum (C, G) E/rGO/PTyr/anti-cTnI/BSA/sick patient serum (D, H).

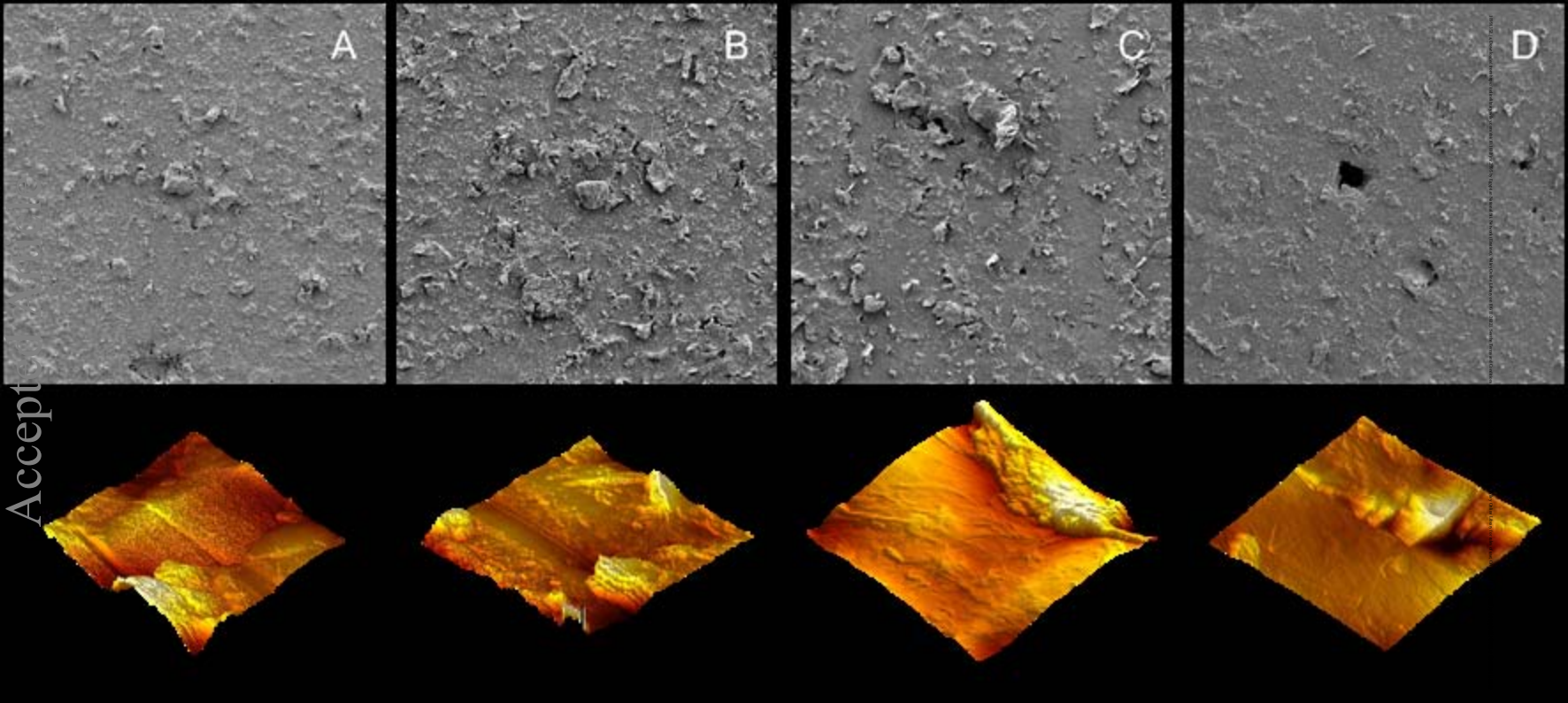
Fig. 4 – Bar chart obtained from current peak response of $[Fe(CN)_6]^{-4}$ oxidation in GE/rGO/PTyr/anti-cTnI/BSA/cTnI for the optimization of anti-cTnI concentration: (A) 1 µg mL^{-1} and (B) 10 µg mL^{-1} of anti–cTnI. Electrolyte: potassium ferrocyanide/ferricyanide (5 mmol L^{-1}) in KCl solution (0.1 mol L^{-1}). Modulation amplitude: 50 mV. Pulse interval: 0.2 s; Scan rate: 25 mV s^{-1}.

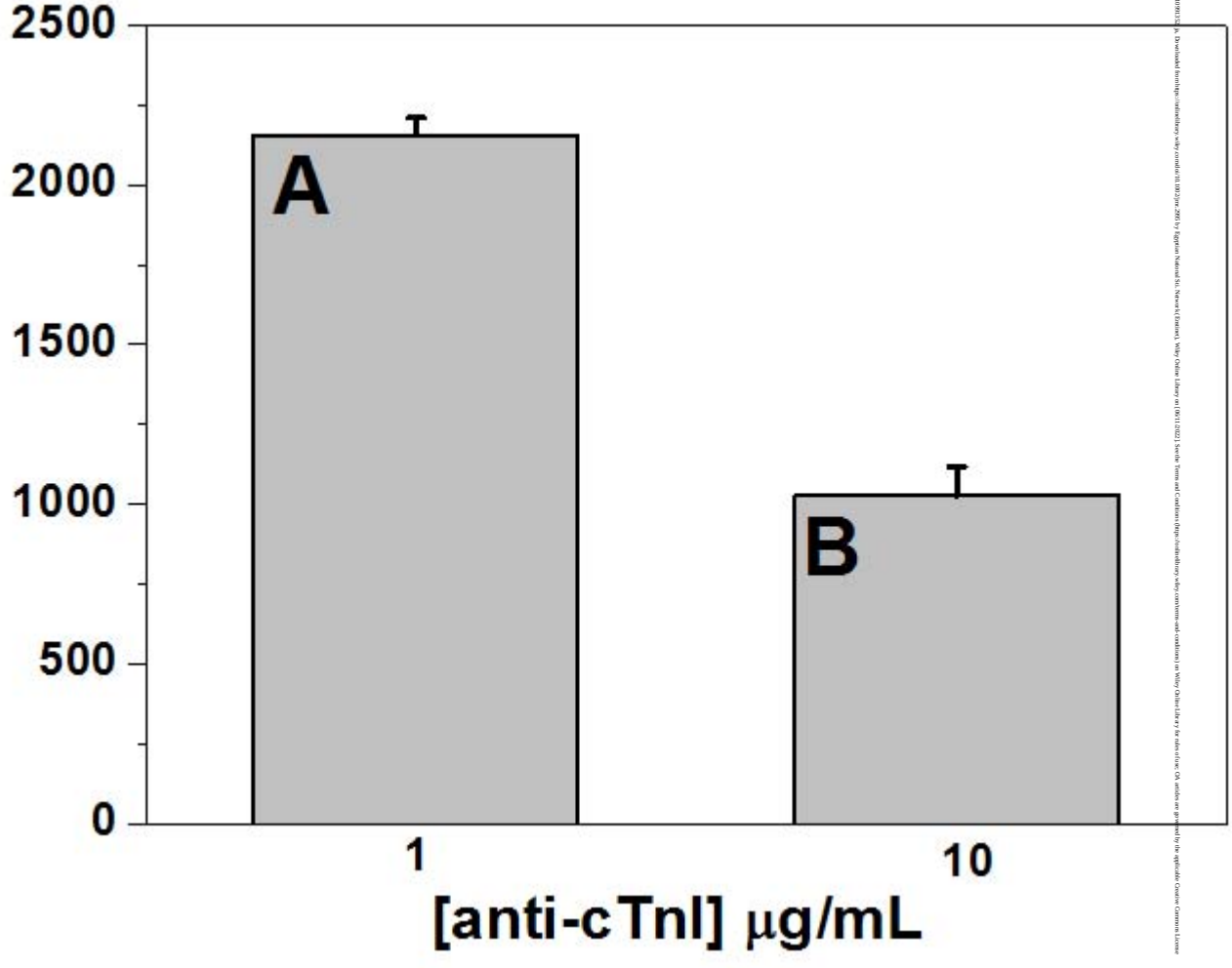
Fig. 5 - (A) Calibration curve of the immunosensor. (B) Differential pulse voltammograms of the immunosensor for different cTnI concentrations. Amplitude modulation: 25 mV; Pulse interval: 0.2 s; 50 mV s^{-1}. (C) Bar charts showing the current (I) for healthy (H1-H2) and sick (S1-S7) patients samples. Electrolyte: potassium ferrocyanide/ferricyanide (5 mmol L^{-1}) in

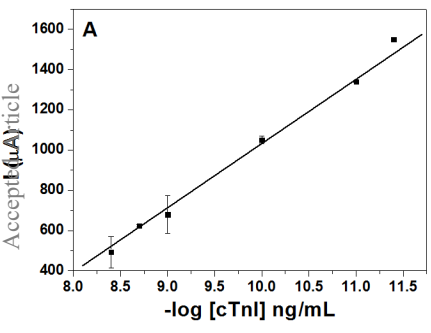
KCl solution (0.1 mol L^{-1}). Modulation amplitude: 50 mV. Pulse interval: 0.2 s; Scan rate: 25 mV s^{-1}.

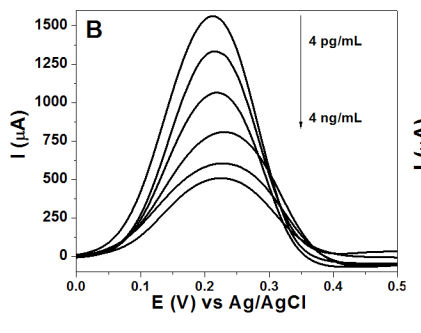
Fig. 6 - (A) Differential pulse voltammograms of the immunosensor for different types of antigens cTnI (I), free T_3(II), CRP (III), cTnT (IV) and solution without cTnI (V)**.** (B) Bar charts showing the current response for different types of antigens (cTnI, free T_3, CRP, cTnT and solution without cTnI). (C) Reuse capacity and (D) Storage stability. Electrolyte: potassium ferrocyanide/ferricyanide (5 mmol L^{-1}) in KCl solution (0.1 mol L^{-1}). Modulation amplitude: 50 mV. pulse interval: 0.2 s; scan rate: 25 mV s^{-1}.

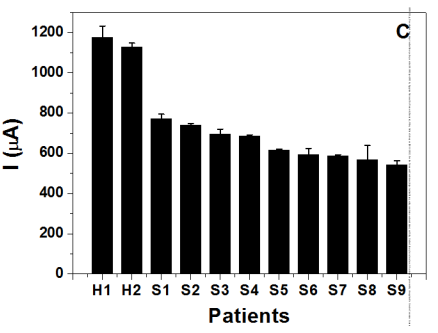


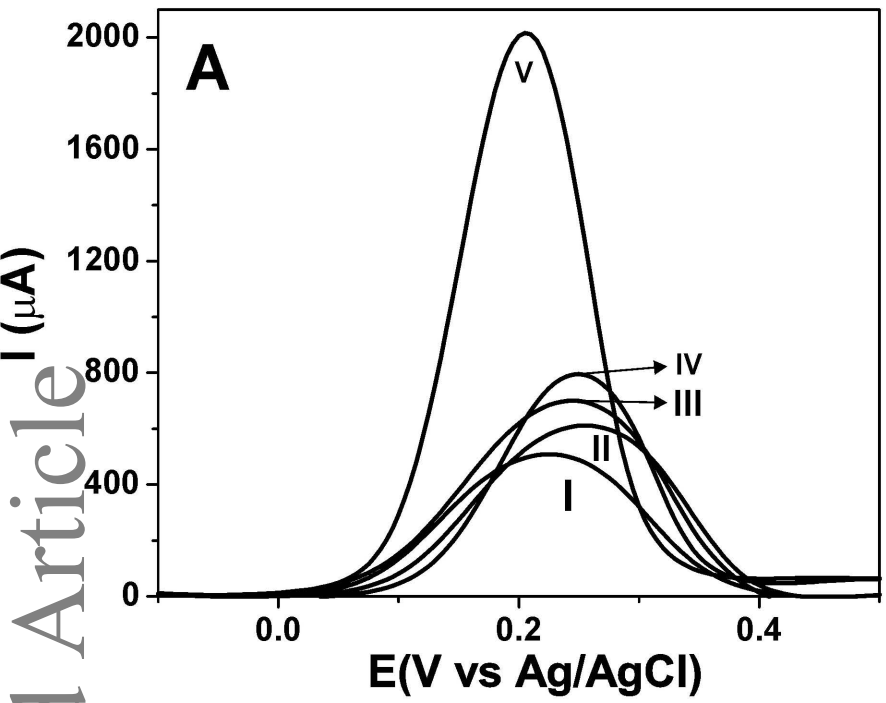


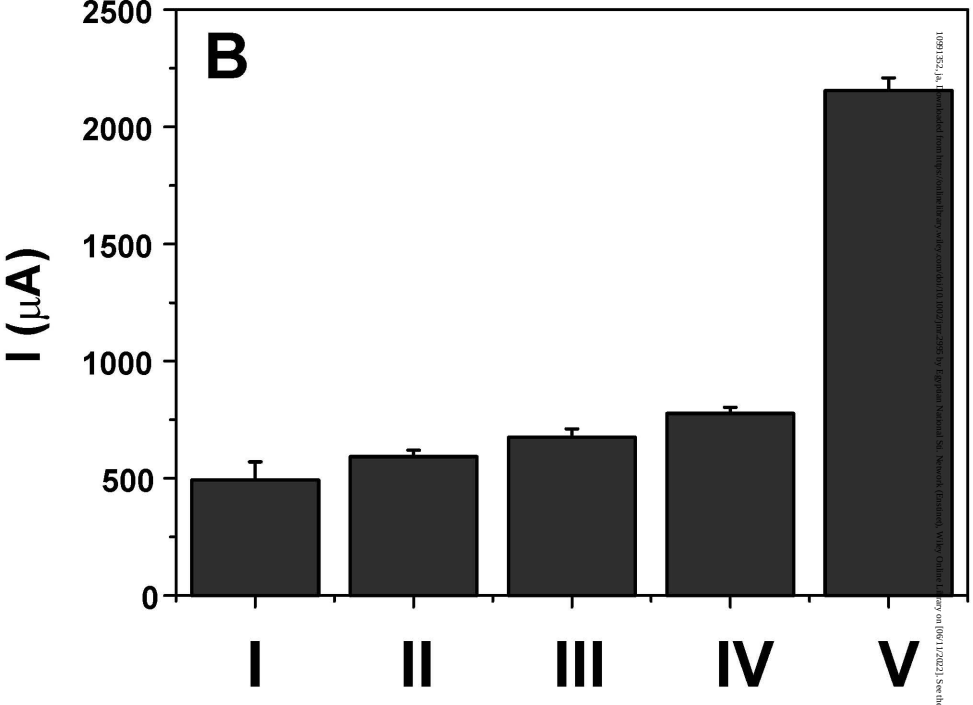


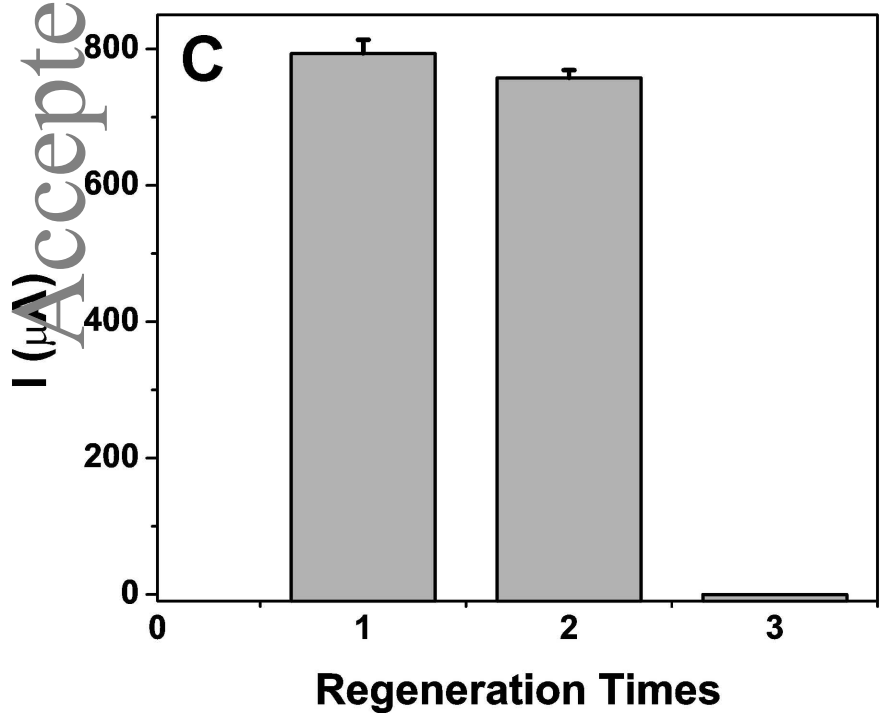












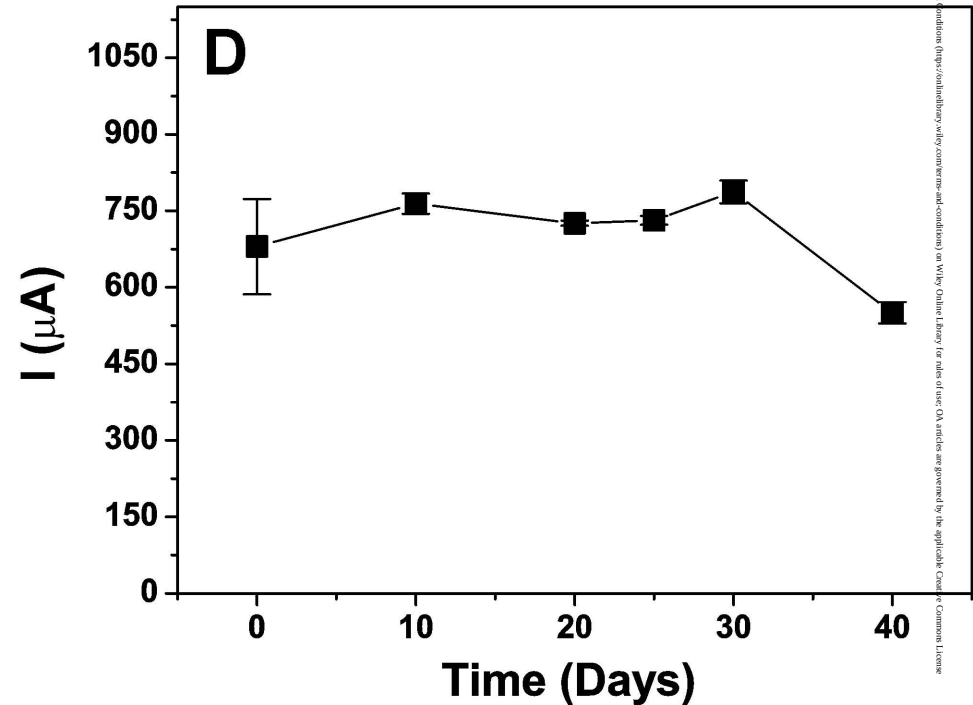


Table 1. Analytical parameters of different electrochemical immunosensors recently described for cardiac Troponin I.

Platform	Linear range	LOD	Stability	Regeneration	Detection Time	Ref.
Carbon Screen Printed Electrode/ AuNP	0.1-32 ng mL^{-1}	0.1 ng mL^{-1}	15 days	N.A.	30 min	[37]
AuNp/ graphene oxide	0.05-3 ng mL^{-1}	0.05 ng mL^{-1}	30 days	N.A.	5 min	[38]
Glassy Carbon Electrode/ anode-oxidation amidation	0.05-5 ng mL^{-1}	0.05 ng mL^{-1}	N.A.	N.A.	N.A.	[39]
Citrate-capped AuNP/ Carbon Screen Printed	0.2-12.5 ng mL^{-1}	0.2 ng mL^{-1}	N.A.	N.A.	N.A.	[40]
Graphene oxide nanostructure	0.1-10 ng mL^{-1}	0.07 ng mL^{-1}	N.A.	N.A.	N.A.	[41]
Glassy carbon electrodes/ biosynthesized ZnONPs	$1.25x10^{-5}$-8.25 µg mL^{-1}	$2.61x10^{-5}$ µg mL^{-1}	20 days	N.A.	N.A.	[42]
GE/rGO/PTyr/anti-cTnI platform	4 pg - 4 ng mL^{-1}	4 pg mL^{-1}	40 days	2 times	20 min	This work

(N.A. not available)