A Label-Free Cardiac Troponin T Electrochemiluminescence
Immunosensor Enhanced by Graphene Nanoplatelets

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Abstract

In this study, a direct and label-free immunosensor was designed and constructed by modifying the screen-printed electrode with graphene nanoplatelets (GNPs) for the detection of the cardiac troponin T (cTnT). Firstly, GNPs were drop-casted onto carbon working electrode. Monoclonal cTnT antibodies were then immobilised on the GNPs via physical adsorption and finally, BSA was introduced to block non-specific binding sites. The detection of cTnT was performed using electrochemiluminescence (ECL) technique with tris(bipyridine)ruthenium(II) chloride ([Ru(bpy)_3]Cl_2) used as a luminophore and TPrA (Tripropylamine) as a co-reactant. The ECL intensity was demonstrated to be directly proportional to cTnT concentration where a linear range from 100 pg mL\(^{-1}\) to 5 fg mL\(^{-1}\) of the cTnT detection was established. An extremely low limit of detection was achieved to be 0.05 fg mL\(^{-1}\) with an outstanding specificity. Additionally, this immunosensor showed excellent percentage recovery for real samples analyses in artificially spiked human serum.

**Keywords:** Graphene Nanoplatelets, Screen-printed electrode, Electrochemiluminescence, cTnT detection
Introduction

Cardiovascular diseases (CVD) are a group of various disorders that adversely affect the heart and blood vessels. According to World Health Organization, CVD is one of the dominant causes of global morbidity and loss of life. In 2015, 31% of the world’s population deaths were solely due to CVD, of which 7.4 million deaths were caused by coronary heart diseases while stroke was the cause of 6.7 million deaths worldwide. Acute myocardial infarction (AMI) or generally known as heart attack, is one of the most fatal categories of acute coronary syndromes, associated with the blockage of the coronary artery and it is elucidated as necrosis of cardiac myocytes following perpetuated ischemia.

Apart from medication, management of AMI plays an important role in screening suspected patients so that appropriate treatment can be provided immediately. Specific biomarkers could be used for AMI to be rapidly, accurately and thoroughly diagnosed in patients as heart impairment originated from AMI is irrevocable. Inflated level of cardiac troponin (cTn) is related to myocardial infarction and accordingly, cardiac troponin T (cTnT) can be used as a biomarker for AMI diagnosis. Typically, the troponin level in healthy adults is negligible and often, undetectable. On the other hand, the concentration of the troponin in patients with AMI elevates within 24 hours to 14 days after symptoms are first observed. Particularly, cTnT is instantaneously discharged into the bloodstream during the heart infarction and its level increases within 4 – 6 hours of the AMI symptoms. By taking advantage of this information, biosensors have been developed by researchers allowing accurate and rapid diagnosis to be performed within the healthy and unhealthy range of cTn.

Point-of-care (POC) biosensors have been gaining interest from researchers in the past decade. This is because of the tremendous benefits that can be offered by POC devices such as simplicity in operating the device, its portability, low sample volume requirement, prompt in producing results, economically viable and their high specificity and sensitivity. Moreover, the overall execution of biosensors can be significantly improved by incorporating nanomaterials. Ubiquitous methods that are commonly implemented as a POC device are electrochemical- and optical-based detection techniques.
The utilisation of nanomaterials in biosensors particularly those derived from carbon contributes to multitudinous benefits such as enlargement of working surface area for bioconjugation process and improvement in the electroconductivity of the particular biosensors.\(^7\) Carbon nanomaterials (CNMs) are known to possess remarkable structural and electrochemical attributes making them a fascinating nanomaterial to be exploited for the fabrication of a biosensor.\(^7\) Graphene, single-walled carbon nanotubes (SWCNTs) and other carbon allotropes have been elucidated to have improved the sensitivity of the overall biosensor.\(^8\)-\(^11\) Graphene being a 2-dimensional nanomaterial is made up of \(\text{sp}^2\) hybridised carbon possessing numerous merits that include expediting the electron transfer between the electrode’s surface and the bio-recognition molecules. Graphene nanoplatelets (GNPs) can be simply defined as a few layers (2 – 10 layers) of graphene sheets.\(^12\), \(^13\) Large surface area (2675 \(\text{m}^2/\text{g}\)) of GNPs is advantageous for biosensors as it can easily accommodate more bioreceptor molecules for better sensitivity with great mechanical adhesion, even \textit{via} a facile physioadsorption.\(^14\), \(^15\) GNPs also possess high thermal stability and conductivity and they are inexpensive nanomaterials.\(^16\)

Organised and thorough optimisation studies render a vital role when designing a biosensor as a means to ensure its reliability especially in practical situations. Experimental variables such as the type, structure and concentration of nanomaterials used, the concentration of the antibody and blocking agent that are selected for the fabrication on the electrode’s surface as well as the other parameters such as the immobilisation time of each component, have influence over the overall performance of a particular biosensor.\(^17\) Wherefore, orderly optimisation in developing a biosensor is pivotal as it can help in improving the accuracy, sensitivity, specificity, stability, and reproducibility. In addition, by performing systematic optimisation, it can also reduce the amount of the reagents required and subsequently, lessen the total cost of the biosensor.\(^18\)

To date, many works have been done but employing complicated and extensive procedures with the need of utilising bulk and expensive instruments. On that account, for this study, our strategy mainly focused to employ a simple and straightforward method and, yet sensitive, to detect our target analyte. To achieve this, we used GNPs which is a novel material in the development of ECL immunosensor for cTnT detection. We have chosen to work on SPE chip due to its small dimension as
this could pave ways for this biosensor to be integrated into a point-of-care (POC) device. In this work we also ensured that every aspect of experiments is thoroughly optimised for sensitivity and selectivity and this approach is not seen in other publications. Herein, we are reporting the development of a straightforward and label-free cTnT biosensor with high specificity and stability through a systematic optimisation.

**Experimental**

**Reagents and Materials**

The details on reagents and materials required for this study are stated in the Electronic Supporting Information (ESI).

**Fabrication of cTnT Electrochemiluminescence Immunosensor**

The fabrication of cTnT immunosensor was initialised by drop-casting 3 µL of 20 µg mL⁻¹ of GNPs (dispersed in ultra-pure water) onto the carbon working electrode of the SPE for 1 hour. Then, 10 mM PBS (pH 7.4) was used to wash off the excess solution. Next, 5 µL of 1 µg mL⁻¹ anti-cTnT (cTnT antibody) was spiked onto the GNP-modified working electrode, also for 1 hour. The surface was washed with PBS (pH 7.4) to remove unbound anti-cTnT. 5 µL of 1% BSA dissolved in 0.1% NaN₃ was subsequently immobilised onto the working electrode and incubated for 45 min. PBS (pH 7.4) was then used for the final washing step for the preparation of immunosensor. All of the steps were done and dried at room temperature (20 °C ± 1 °C). The fabricated immunosensor was then stored at 4°C until needed. The schematic diagram of the fabrication is depicted in the Figure 1 below.

[Figure 1 is in here]

**Electrochemiluminescence (ECL) and Electrochemical (EC)-based Measurement**

The electrochemiluminescence (ECL) signal of the samples was examined by an ECL-monitoring instrument (BDTeCLP100, Biodevice technology Ltd., Kanazawa, Japan), which consists of a USB-powered portable potentiostat manufactured by Biodevice technology Ltd. (Kanazawa, Japan)
and a photon-sensor unit (C9692-12) obtained from Hamamatsu Photonics K.K., Hamamatsu, Japan. For the counting time for photon detection was set to 500 msec and the measurement point was set to 60 points. A carbon-based disposable screen-printed electrode (SPE) was opted for the detection of the cTnT. The reference electrode of the SPE chip (Biodevice technology Ltd., Kanazawa, Japan) is made up of Ag/AgCl whereas for the working and auxiliary electrodes are made up of carbon. The working diameter of the electrode is 2.64 mm². Prior to analysis, 5 µL of sample was spiked onto the fabricated immunosensor at room temperature to permit the complete interaction between anti-cTnT and the sample. For all of the optimisation studies that requires the interaction between anti-cTnT and cTnT-Ag, 100 pg mL⁻¹ of cTnT-Ag (cTnT antigen) was chosen and spiked onto the SPE.

Autolab PGSTAT101 III (Metrohm, Netherlands) combined with its accompanying software, Nova 1.10 was chosen as the analyser for chronocoulometry. For the investigataion of the layer-by-layer modification of the immunosensor, field-emission electron microscopy (FE-SEM) JEOL, JSM-7610F (Tokyo, Japan) was utilised. The same type of SPE chip was selected as the platform for the electrochemical-based detections. 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (potassium ferricyanide/potassium ferrocyanide) prepared in 10 mM PBS, pH 7.4 were used as the redox probe for electrochemical (EC)-based analyses and all EC-based studies were performed at room temperature of 20 °C ± 1 °C.

Results and Discussion

Optimisation studies of the fabricated immunosensor

In this work, an immunosensor was developed by modifying carbon SPE chip with GNPs for the detection of cTnT. Before starting the process of immunosensor fabrication, there were several parameters needed to be optimised to achieve immunosensor with high sensitivity. The parameters included concentration of [Ru(bpy)₃]²⁺ and TPrA, the volume ratio between of luminophore to co-reactant, concentration of nanomaterial, incubation time for each layer and also pH of the system’s buffer.
Optimisation of ECL system.

The optimisation of these parameters was commenced by firstly determining the concentrations of luminophore and co-reactant in separate trials. The concentrations of [Ru(bpy)₃]²⁺ and TPrA were studied ranging from 200 – 1000 µM and 10 – 50 µM, respectively. From Fig. S1A and S1B (Supporting Information), it could be confirmed that 800 µM [Ru(bpy)₃]²⁺, in the presence of 20 µM TPrA, exhibited the highest and most stable ECL signal and these two concentrations were therefore selected for [Ru(bpy)₃]²⁺ and TPrA, respectively. The volume ratio of 3:1 (800 µM [Ru(bpy)₃]²⁺ : 20 µM TPrA) was opted for further study as the highest ECL signal was observed amongst the other tested volume ratios (Fig. S1C (Supporting Information)).

Selection of the Materials for the Biosensor Development

Nanomaterials acquire different inherent characteristics and this led us to investigate four different carbon-based nanomaterials that work as modifier on the working electrodes. The nanomaterials included GNPs, GNPs-COOH, SWCNTs and CNOs. Results shown in Fig. S2A (Supporting Information) suggested that GNPs as the best nanomaterial for use in the modification on working electrode since GNPs exhibited to be the best enhancement for the [Ru(bpy)₃]²⁺/TPrA ECL signal among these carbon allotropes. The structure of the graphene layers with the edges being unoccupied with functional groups allows more of its defects to be involved for facilitating the transfer of electrons. Furthermore, it was reported that carbon nanotubes-based materials demonstrated substandard electrocatalysis as compared to graphene-based nanomaterials and consequently, slower electron transfer towards the working electrode than graphene.

To further improve ECL signal output, a range of concentration (0 – 60 µg mL⁻¹) of GNPs was also evaluated. As displayed in Fig. S2B (Supporting Information), 20 µg mL⁻¹ of GNPs was chosen as optimum concentration for analytical evaluation since it provided the highest and most stable ECL intensity. Besides, when the concentration of GNP was increased to 40 and 60 µg mL⁻¹ respectively, the ECL signal reduced to and the signal was comparable with the signal of bare SPE. This might be
because at low concentration of 20 µg mL⁻¹, GNPs dispersed better compared to that at higher concentrations and thus, the large surface area was able to be deployed for better ECL performance.²³

Another experimental parameter evaluated was the concentration of anti-cTnT. Four different anti-cTnT concentrations of 0.1, 1, 10, and 100 µg mL⁻¹ were analysed with and without the addition of antigen. This is to assess the most favourable concentration of anti-cTnT for the detection of cTnT. As it can be observed from Fig. S4B (Supporting Information), 1 µg mL⁻¹ anti-cTnT displayed the highest and significant ECL intensity difference in the absence and presence of cTnT antigens with superior S/N ratio (signal-to-noise ratio) amongst the other studied concentrations. Therefore, 1 µg mL⁻¹ anti-cTnT was decided as the working antibody concentration for immunosensor development.

For a specific system, various blocking solutions show different performances to prevent non-specific binding of proteins on electrodes. Hence, the effect of different blocking agents was studied to evaluate their blocking capacity of non-specific protein adsorption in this immunosensor. The blocking agents investigated were 50 mM glycine, 0.01% TWEEN® 20, 1% poly(ethylene) glycol (PEG), 1% bovine serum albumin (BSA) and 1% BSA/0.01% TWEEN® 20 (BSA-T). Although 1% PEG is showing the lowest ECL signal, which signifies successful immobilisation of blocking agents, 1% BSA was selected as the blocking agent for the development of our immunosensor as it has been shown to produce the most stable signal and excellent blocking ability (Fig. S2C (Supporting Information)). Besides that, the error bars of 1% PEG and 1% BSA are overlapping with each other, indicating that their individual blocking ability are comparable with one another. In addition, BSA as a blocking agent is well studied and widely used in developing biosensors evincing their credibility as a blocking agent.²⁴,²⁵

**pH Dependant Study of ECL System**

Following that, pH dependant study was then performed using 10 mM PBS with pH ranging from pH 5.4 to 9.4 in order to ascertain the best pH for the system as well as interacting environment between the antibodies with the cTnT. As illustrated in Fig. S4A (Supporting Information), as the pH increased from pH 5.4 to 9.4, the ECL signal in the presence of cTnT antigens also increased with the most prominent ECL intensity shown at pH 7.4. On the contrary, regardless of pH used, the ECL
intensity were comparatively similar in the absence of cTnT-Ag except for pH 7.4. The ECL signal was found to be the lowest at pH 7.4 signifying that the efficient blocking ability of BSA in contrast to other pH. This can be accounted to the active positively charged nitrogen-containing group present in serum albumin that is responsible for the binding sites in BSA.\textsuperscript{26, 27} Therefore, as $\text{[Ru(bpy)}_3\text{]}^{2+}$ molecules are positively charged, they were theoretically repelled, and resulting in the ECL signal decrease. After these analyses, the signal-to-noise (S/N) ratio was calculated and it was established that pH 7.4 has the superlative S/N ratio ($\approx 15.13$) amongst the other pH and hence, pH 7.4 was determined as the optimal working pH for the cTnT detection. This probably attributable to the optimum orientation and conformational stability of antibody-antigen binding sites (Fab) of the antibody structure at pH 7.4.\textsuperscript{28} Thus, in the presence of cTnT-Ag (pI value of 5.1, negatively-charged at pH 7.4), exceptional ECL signal was discerned.\textsuperscript{29}

\textit{Characterisation of the immunosensor}

The effect of the modification of working electrode with GNPs was examined along with the layer-by-layer immobilisation on the overall potential of the immunosensor for cTnT detection. GNPs possess an excellent electro-conductivity, which facilitates the electron transfer at their edges and hereafter, in the whole system.\textsuperscript{12} Henceforth, the layer-by-layer study was conducted in order to corroborate the enhancement made by modifying the electrode by GNPs.

As exhibited in Figure 2A, GNPs-modified SPE chip has doubled the ECL intensity compared to the bare (non-modified) carbon electrode confirming the ability of GNPs as a conducting material and henceforth, facilitating the electron transfer at their edges and hereafter, in the whole system.\textsuperscript{13, 30} In the presence of a blocking agent, the ECL signal once again decreased to the value at which is similar to the signal of unmodified electrode. This was expected since blocking agent prevented non-specific adsorption of proteins thus reducing background signal and subsequently increased the sensitivity of the immunosensor.\textsuperscript{31} This led to the observation of significant increase of ECL signal with the addition of 100 pg mL\textsuperscript{-1} cTnT-Ag onto the immunosensor. The increase in ECL intensity could be ascribed to the formation of immunocomplex between the cTnT-
Ag and anti-cTnT. The presence of immunocomplex allows more $[\text{Ru(bpy)}_3]^{2+}$ molecules to react TPrA in order to emit light signal.

![Figure 2 is here](image1)

The net charge of the system was also then studied to substantiate the role of GNP as an enhancement towards cTnT detection. Chronocoulometric analysis was performed at each step of fabrication process of the cTnT immunosensor (Fig. S5 (Supporting Information)). It was verified that the immobilisation of GNPs onto the working electrode improved the electron transfer as shown in Figure 2B. A significant increase in term of charge by almost twice in comparison to the charge of bare electrode was observed. However, upon introduction of blocking agent onto the GNP layer, the overall charge decreased by more than half as the BSA molecules were negatively charged at pH 7.4. The presence of cTnT-Ag further decreased the net charge as more repulsion occurred between the $[\text{Fe(CN)}_6]^{3-}/[\text{Fe(CN)}_6]^{4+}$ (redox probe) and the working electrode's surface. This repulsion was contributed by the negative charge of the antigens at pH 7.4. Surface morphology of the bare and modified working electrodes of SPE were scrutinised by comparing the images taken with SEM (scanning electron microscope). It is evident that GNPs (presence of cubic structures on the surface) were successfully immobilised onto the working electrode, as shown in Fig. S6 (Supporting Information).

**Analytical performance of the immunosensor**

As exhibited in Figure 2A, in the presence of antigen, the ECL signal of $[\text{Ru(bpy)}_3]^{2+}$ increased as more $[\text{Ru(bpy)}_3]^{3+}$ molecules were reduced by TPrA into $[\text{Ru(bpy)}_3]^{2+}$, which in turn emitted the light signal. $[\text{Ru(bpy)}_3]^{2+}$ functioned as an indicator to indicate the presence and absence of cTnT-Ag in the sample. This was achieved by the electrostatic interaction occurring between the positively-charged luminophore and the negatively charged cTnT-Ag at the physiological pH of 7.4. $[\text{Ru(bpy)}_3]^{2+}$ molecules, which in close proximity to working electrode and being electrostatically attached to cTnT-Ag, produced high light intensity even when only a small potential was applied leading to sensitive
detection of cTnT-Ag. One main advantage of such process is prior pre-treatment or labelling procedure on the anti-cTnT or the cTnT-Ag was not a requisite.

In order to investigate the analytical performance of the immunosensor, various concentrations of cTnT-Ag were selected and analysed. As it can be observed from Figure 3A, a linear range for quantitative detection of cTnT was determined to be from 100 pg mL\(^{-1}\) down to 5 fg mL\(^{-1}\) with the correlation coefficient value of 0.982. As stated in a report by Liyanage, Sangha and Sardar, the cTnT concentration perceived in patient with high risk of AMI is said to be > 10 ng L\(^{-1}\) (≈ > 10 pg mL\(^{-1}\)).\(^{32}\) The linear range of our developed immunosensor was included within the concentration range required for the detection of the unhealthy and healthy amount of cTnT in human serum for the diagnosis and prediction of AMI. As for the limit of detection of cTnT, it was visually verified and it was observed to be 0.05 fg mL\(^{-1}.\(^{33}\) This low LOD is attributable to the impressive electro-conductivity and large area of GNPs that facilitates the electron transfer between the electrode’s surface and the ECL system and also, providing large surface area for the immobilisation of abundant amount of anti-cTnT.\(^{34}\) Moreover, by executing elaborate and coherent optimisation studies, it ensures that all components in our biosensor work in a synergic manner. These factors further stimulate the performance of our biosensor and consequently, contributing to the low LOD.

[Figure 3 is here]

Specificity is an important criterion that need to be considered when designing this immunosensor due to the presence of numerous proteins and biological entities in human serum. Thereafter, specificity of the proposed assay was investigated with seven different protein biomarkers that can be found in human serum namely: cTnT (targeted biomarker), haptoglobin, β-2-microglobulin, carcinoembryonic antigen, leptin, human chorionic gonadotropin and α-fetoprotein. Result of the specificity study was obtained and depicted in Figure 3B. The fabricated immunoassay was evident to be highly specific towards cTnT. The ECL signals for all non-targeted protein biomarkers were similar to the ECL signal of the negative control thus confirming detection and specificity only for the targeted cTnT.
The other non-targeted proteins were then mixed with cTnT in 1:1 ratio with the final concentration of 100 pg mL\(^{-1}\) of each mixture as demonstrated in Figure 3C. The ECL signal of the individual mixture was determined in triplicates. It was substantiated that the existence of other proteins did not cause any significant interference towards the cTnT detection. Accordingly, this means the designed immunosensor was confirmed to be having an outstanding specificity towards cTnT-Ag. Afterwards, the repeatability of the obtained result with 100 pg mL\(^{-1}\) cTnT was justified with five independent fabricated immunosensors (Figure 3D). The calculated relative standard deviation percentage (%RSD) of the observed data was found to be 0.709% that implied our proposed immunosensor was able to repeat the data using different samples with high precision. Table S1 (Supporting Information) comprises the comparison study between published cTnT electrochemical- and ECL-based detection methods and this reported work in terms of their respective detection range and the limit of detection.
Real samples analyses

The applicability of this disposable GNP-modified SPE cTnT immuno-sensor for practical implementation was evaluated using human serum. First of all, the human serum was diluted into 10-fold, 100-fold, 1,000-fold and 10,000-fold with 10 mM PBS buffer (pH 7.4). This was to determine which dilution factor would provide the best reduction in signal as well as signal that would be closest to blank (negative control) signal. It was perceived that 10,000-fold dilution was adequate to emit ECL signal similar to the negative control leading 10,000-fold dilution to be chosen as the human serum dilution factor. This requirement of high dilution factor might be arising from the existence of other intricate proteins (such as serum albumin) in the human serum that could interfere with the response.35,36 Thereupon, three different cTnT concentrations (1, 10 and 100 pg mL⁻¹ cTnT-Ag) were formulated by artificially spiking the cTnT stock solution into the diluted human serum. The observed analytical data were tabulated in the Table 1. The percentage recovery of the simulation spiking was in the scope of 103.00% to 108.24% whereas % RSD obtained was extended from 5.89% to 9.87%. It is perspicuous from the analyses that our developed immunosensor is feasible for the application of cTnT detection in human serum.

[Table 1 is here]

Conclusion

The effect of the modification of carbon screen-printed electrode with GNPs for cTnT detection was scrutinised for the first time in this work. GNPs have been proven to enhance ECL signal and by employing GNPs, we have effectively, qualitatively and quantitatively detected cTnT antigen with our simple and direct immunosensor with high specificity and sensitivity (as low as 0.05 fg mL⁻¹). Since it involves facile protocol by performing initial systematic optimisation studies, our immunosensor only necessitates low reagent and sample volume for cTnT detection and hence, avoid wasting resources. In addition, the SPE was made of low-cost materials and hence by utilising these electrodes, this reported biosensor is disposable, inexpensive and offers opportunity for miniaturisation.37,38 Furthermore, this
immunosensor has been verified to have excellent opportunity and prospective to be integrated in a POC device for clinical diagnosis real applications.

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Table 1: Real sample analyses’ results with human serum.

| Samples | Concentration of cTnT-Ag added (pg mL⁻¹) | Concentration of cTnT-Ag Detected (pg mL⁻¹) | % RSD | % Recovery |
|---------|------------------------------------------|-------------------------------------------|-------|------------|
| 1       | 1                                        | 1.03                                      | 6.91  | 103.00     |
| 2       | 10                                       | 10.44                                     | 5.89  | 104.43     |
| 3       | 100                                      | 108.24                                    | 9.87  | 108.24     |
Figure Captions

Figure 1: (A) The preparation step of the cTnT immunosensor whereby (I) unmodified carbon SPE, (II) GNPs-modified carbon SPE, (III) anti-cTnT/GNPs/SPE, (IV) BSA/anti-cTnT/GNPs/SPE. Meanwhile, (B) illustrates the principle of detection of this work as (V) is BSA/anti-cTnT/GNPs/SPE and (VI) is cTnT-Ag/BSA/anti-cTnT/GNPs/SPE, both with spiked luminophore/co-reactant mixture solution on the electrodes.

Figure 2: (A) Layer-by-layer study with ECL technique with [Ru(bpy)3]2+/TPrA system at pH 7.4, (B) Bar graph depicting the differences in charge (from chronocoulometry analyses with [Fe(CN)6]3-/4-) of bare electrode, after the addition 20 µg mL⁻¹ GNPs, after the immobilisation of 1 µg mL⁻¹ anti-cTnT and 1% BSA as the blocking agent, and after the incubation of 100 pg mL⁻¹ cTnT-Ag.

Figure 3: (A) Determination of linearity for quantitative cTnT detection ranging from 5 fg mL⁻¹ to 100 pg mL⁻¹ (B) Specificity study with seven different protein (100 pg mL⁻¹) where NC is negative control, cTnT (cardiac troponin T), HP (haptoglobin), B-2-M (β-2-microglobulin), CEA (carcinoembryonic antigen), leptin, HCG (human chorionic gonadotropin) and AFP (α-fetoprotein) whereas (C) Comparison of ECL signal with cTnT-Ag and the ECL signal of the mixture of cTnT and other proteins in 1:1 ratio. (D) ECL signal from five individual fabricated immunosensors for repeatability study.
Figure 1
Figure 2
Figure 3