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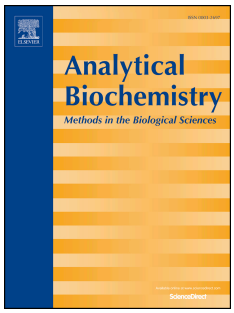

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Mintu Pal, Raju Khan

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Detection of prostate cancer risk factor immunosensor based deposition of graphene layer decorated gold nanoparticles

Mintu Pal[a, #], Raju Khan[b,#,*]

[a]Biotechnology Division, CSIR-North East Institute of Science & Technology, Academy of Scientific and Innovative Research, Jorhat-785006, Assam, India

[b]Analytical Chemistry Division, CSIR-North East Institute of Science & Technology, Academy of Scientific and Innovative Research, Jorhat- 785006, Assam, India.

# indicatesequal contribution to this paper

*Corresponding author

Senior Scientist, Analytical Chemistry Division, CSIR-North East Institute of Science & Technology, Jorhat- 785006, Assam, India. Tel: (+91) 376 2370806; E-mail: khan.raju@gmail.com (Raju Khan)

Abstract

In this work, we report a novel electrochemical immunosensorbased on gold nanoparticles deposited on the surface of graphene layers to detect prostate-specific antigen (PSA), a valuable biomarker for early detection of prostate cancer. The biosensor was fabricated using gold nanoparticles supported graphene oxide (Au-GrO) nanocomposite on platinum electrode and was used for immobilization of monoclonal anti-PSA antibody via EDC/NHS coupling method. To confirm the functionality of the antibody, we performed immunofluorescence staining using human normal prostate epithelial cells, RWPE-1. Scanning Electron Microscopy (SEM), cyclic voltammetry, and other electrochemical techniques were used to characterize the resulting electrode surface. We found that Au nanoparticles on the electrode surface with a spherical shape and their sizes of diameters were in the averagerange of 50-100 nm with a quite symmetric distribution on graphene layers.Unlike the previous research, this novel immunosensor functions very well with a low detection limit of 0.24 $fgmL^{-1}$ at signal to noise ratio of 3; furthermore, it exhibits a significantly increased electron transfer and high sensitivity of 5.4 $\mu A\ fgmL^{-1}$with regression coefficient (R2 = 0.99) toward PSA. The immunosensor was verified for selective and accurate detection of PSA in human serum with recovery of 97.67 %. Overall, data suggested that our developed biosensor holds great promise as auseful alternative diagnostic tool for the detection of different cancer biomarkers, in particular PSA present in biological samples.

Keywords: Prostate-specific antigen, Immunosensors, Electrochemical Impedance Spectroscopy, Gold nanoparticles and Graphene oxide.

1. Introduction

Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. The worldwide PCa burden is expected to increase to 1.7 million new cases and 499,000 new deaths merely due to the growth and aging of the global population [1], whereas the incidence rates of PCa vary by more than 25 fold across the world, particularly the highest rates in North America, Western and Northern Europe. The major concern for increasing the incidence as well as cancer related death due to not available any reliable diagnostic and detection technologies to detect indolent from aggressive PCa. Although there has been intense debate to use human prostate-specific antigen (PSA) test as a biomarker for the detection of PCa due to unacceptably high rates of false positive results, it is still widely used as screening tools.

PSA, also known as kallikrein 3 (hK3) is androgen-regulated serine proteases secreted by prostatic glandular epithelial cells. Malignant prostate epithelial cells secrete PSA (above the normal range 4-10 ng mL-1), which is detectable in serum and is widely used in routine analysis in medical practice is Enzyme-Linked Immunoabsorbent Assay (ELISA), as a screening tool for the early detection of prostate cancer as well as to monitor for recurrence of the disease [2].Despite the several analytical detection methods used for screening of PSA, ranging from optical, frequency to mass, widely used immunofluorometric assay for the detection of PSA in serum due to low detection limit, high selectivity, and accuracy, but it is quite expensive and time consuming [3].Recent studies also reported that the secretion of PSA is affected by a large number of factors, such as several physiological and pathological conditions. Moreover, several types of non-carcinoma prostate epithelial cells also can express PSA, depending on their microenvironment [4]. Therefore, due to its significant variability, it is important to have accurate, sensitive and reliable methods for detection of PSA within a broad range of protein concentrations. In this regard, biosensor may be the important rapid screening tools for early diagnosis of PSA.

Recently, electrochemical biosensor for screening of PSA has gained much attention due to its high sensitivity/selectivity, and longer stability. More precisely, gold nanoparticles (AuNPs) have received remarkable attention in different bio-affinity assays due to its unique physical and chemical properties, such as easily controllable size distribution, long-term stability, superior conductivity, large surface area, and biocompatibility [5]. In addition, the attachment of catalytic nanoparticles, such as gold, platinum, or silver, could further increase the electrocatalyticactivity of these graphite sheets [6]. Still, there is a significant challenge in controlling the uniformity of metal nanoparticle decoration on the graphene surface and especially the strength of the attachment between the organic and the inorganic nanostructure. Moreover, oxidized form of grapheme or graphene oxide (GO), 2D structured material with a large surface area [7],has also emerged as an alternative to graphene for selected applications, due to its low cost, production scalability, ease of processing and good compatibility. Therefore, most graphene-based composites are prepared from graphene oxide (GrO)

[8]. Electrochemical synthesis of gold nanoparticle-graphene composites has recently gained significant attention because of its simplicity and green characteristics. However, very recently reported an approaches whereby chloroauric acid and graphene oxide were co-reduced by cyclic voltammetry to prepare a gold nanoparticles-graphene composite [9]. Therefore, in order to provide large surface area with its high sensitivity as an improved effective immunosensors, it is important to use the nanocomposite of Au nanoparticles (NPs) decorated on graphene oxide (Au-GrO) as a sensor platform as shown in the schematic diagram of Figure 1.

Therefore, the aim of this study was to develop a low cost, and highly sensitive electrochemical immunosensorto detect PSA based on gold nanoparticles deposited on the surface of graphene layers platformusing an electrochemical process. In this work, Au-GrO electrode is functionalized with the monoclonal antibody of PSA and thentested the effectiveness of the electrochemicalbiosensor for the detection ofPSAin human serum samples and finally reproducibility of the electrodes shown promising results. Thus, graphene-based biosensor made by electrochemical process may provide many potential applications for the detection of different cancer biomarkers; in particular, PSA in clinical settings.

2. Experimental Section:

2.1. Materials and Methods:

Recombinant Prostate specific antigen (PSA) protein, monoclonal anti-PSA antibody (produced in rabbit), bovine serum albumin (BSA), and hydrogen tetrachloro-auratetrihydrate (HAuCl4.3H2O) were obtained from Sigma. Graphene oxide was purchased from TCI. All other chemicals unless mentioned were purchased from Sigma.All electrochemical measurements (CV, DPV and EIS) were recorded using a model Gamry-3000 potentiostat/galvanostat (USA). In our studies, Ag/AgCl (in saturated KCl) and a platinum (Pt) wire were used as reference and counter electrodes, respectively. Scanning electron microscopic (SEM) was used with amodel Carl Zeiss SIGMA.

2.2 Cell culture: Human prostate epithelial cells, RWPE-1was purchased from American Type Culture Collection (ATCC), USA. This cells were grown and maintained according to the guidelines of using appropriate culture medium supplemented with 10 % fetal bovine serum, and 1 % penicillin streptomycin antibiotic solution at 37 ºC in 5 % CO2 incubator. Medium in the stock flasks was changed every 48 hours and the cells were sub-cultured when they reached 80-90% of confluence. For our experiments, cells at a concentration of $5x10^3$cells/well were seeded in 8-well chamber slidesand performed the immunofluorescence staining as previously used [10].

2.3. Preparation of the Gold (Au) and Au-GrOelectrodes:

The Pt electrode surface was freshly polished prior to use with 0.05 µm α-Al2O3 slurry, and rigorously rinsed with double distilled water. The electrode was successively rinsed with ultrasonicationin 98% H_2SO_4, 95% ethanol and distilled water for 10 mins each, followed by dried with nitrogen gas. 3mMH$AuCl_4$was prepared by dissolving into 0.5 M H_2SO_4 solution followed by sonication for 15 mins. The working platinum electrode was then immersed into the above prepared H$AuCl_4$solution as an electrolyte, and electrochemical deposition of gold nanoparticles was conducted by the electrochemical scanning rangesat potential of -0.3 V to 1.2 V (*vs* Ag/AgCl) for a selected number of 20 cycles at scan rate of 20 mV/s. The electrode surface showed a very visible yellowcolour Au filmon the surface of electrodes through electrochemical process; whereas 0.1 mL of 10.0 mg/mL GO suspension in 10 mL dH2O consisting of 3 mM H$AuCl_4$and 0.5 M H_2SO_4 followed by sonication for 15 mins for the preparation of Au-GrO electrodes.

2.4 Fabrication of the PSA immunosensor

For activation of the electrode, 50µL of freshly prepared 400 mM EDC and 100 mM NHS in water were placed onto the Au-GrO electrode and washed off after 10 mins. After activation of the electrode surface, 5 µL of monoclonal PSA-antibody (300 μgmL^{-1}) in 100 mM phosphate buffer (pH 7.0) containing 0.05% Tween-20 was incubated for 5 h at 25 ºC for attachment onto the electrode surface. The electrode was then thoroughly washed with 100 mM phosphate buffer (pH 7.0). The Au-GrO-antiPSA antibody was then immersed for 3 minutes in 1% BSA+0.05% Tween-20to avoid non-specific binding of analyte onto the electrode and again washed thoroughly with100 mM phosphate buffer (pH 7.0)forsubsequent experimental studies.

3. Result and Discussion

3.1. Cyclic voltammetry studies

The cyclic voltammetry studies of bare platinum (Pt), modified gold (Au) and gold graphene oxide (Au-GrO) electrodes in 100mMPBS (pH 7.4) with 5mM$[Fe(CN)_6]^{-3/4}$ solution at different scan rate ranging from 5 to 50 mVs^{-1} were studied (Figure 2). Our results revealed a quasi-reversible diffusion-controlled behaviour with an electron transfer process. However, in Pt electrode, peaks were observed at 0.27 V and reverse peak at 0.16 V; butafter the modification of Pt electrode with gold nanoparticlessimilar peaks as Pt electrode were observed on 0.25 V and reverse peak at 0.17 V, in addition to another oxidation peak at 0.86 V due to Au (0) to Au (III), and a reduction peak potential at 0.42 V due to Au (III) to Au (0) as shown in Figure 3 (I, curve b). We also observed the current characteristic of GrO in Au matrix (Au-GrO) was more than 3 times of Au as observed in Figure 3 (I, curve c), suggesting that GrO sheets supported to Au nanoparticles have higher conductive properties, as expected. This enhancement of current may bedue to the electrocatalytic effect of anAu-GrO modified electrode may be attributed to π-π stacking interaction and the sp2 hybridized carbon in

graphene, which makes the electron transfer feasible (REF). Then we were interested to immobilize anti-PSA monoclonal antibody onto the electrode surface. After immobilization of monoclonal anti-PSA antibody onto the Au-GrO electrode, current slop was decreased from 14.07 μAmVs-1 to 1.51 μAmVs-1 with regression coefficient (R2 = 0.99) for linear fit due to slow redox process during the bio-electrochemical reaction.The anodic and cathodic peak currents both are linearly proportional to the square root of the relevant scan rate (Figure 3 III and IV), and ΔE increases with increase in scan rate (data are not shown).We confirmed the functionality of the antibody by performing immunofluorescence staining using normal prostate epithelial cells, RWPE-1 (Figure 4). To further confirm the effective immobilization of antibody onto the electrode surface, we accomplisheddifferential pulse voltammetry (DPV) studies.DPV is a very useful technique for measuring trace levels of organic and inorganic species in which the height of the measured current is directly proportional to the conductivity of electrode and concentration of the corresponding analytes [11,12].In the observed current-voltage response, the peak potential (Ep) can be used to identify the species as E_p = E1/2 - ΔE/2, where E1/2 is the polarographic half-wave potential and ΔE is the pulse amplitude used in the DPV measurements. Results indicate that the addition of GrO in Au matrix increased the current corresponding to the peak potential as compared with that in Au matrix. For all DPV measurements, we have used the potential ranges from -0.3 to 1.2V with 5 mV pulse height, 85 mV amplitude, and 60 Hz frequency.DPV measurements of bare Pt, Au, Au-GrO and Au-GrO-anti-PSA were also characterized with varying potential scans from -0.2 to 1.2 V as shown in Figure 3 II. Overall, these two independent studies confirmed the effective immobilization of PSA antibody onto the Au-GrO electrode surface.

3.2. Electrochemical impedance spectroscopic studies

To get the information about the stepwise assembly of the immunosensor from the view of impedance changes of the electrode surface, we performed electrochemical impedance spectroscopy (EIS) studies, whereas the semicircle diameter equals the electron-transfer resistance (Ret). To re-confirm the proper capturing of antibodies onto the Au-GrO electrode surface, we carried out EIS studies, have shown Nyquist plots of bare Pt and modified electrodes in the presence of 100mM (pH 7.4) with 5mM[Fe(CN)6]-3/4 solution as shown in Figure 5 I. The electron transfer resistance of bare Pt, Au, Au-GrOwere Ret = 1.49±0.11 kΩ, Ret = 0.343±0.03 kΩ, andRet = 0.132 kΩ±0.01 as shown in Figure 5, curve a-c respectively. However, immobilization of monoclonal anti-PSA antibody onto the Au-GrO electrode surface shown a drastic difference of electron transfer resistance, Ret = 2.89±1 kΩ (Figure 5, curve d). Thisdifference of Ret may be due to the immunocomplex layer on the electrode acts as the electron communication and mass-transfer blocking layer, further insulating the conductive support and hindering the access of redox probe toward the electrode surface. The gradually EIS changes of the modified process prove that monoclonal anti-PSA antibody are successfully

immobilized on the surface of Au-GrO electrode, which is in good agreement with the CV and DPV results as shown in Figure 3.

3.3. Validation of anti-PSA antibody immobilization on the Au-GrO surface by Scanning electron microscopyand immunofluorescence studies

The surface morphology of the electrodes was examined by SEM, as illustrated in Figure 6. Au-GrOshowsnumerous spherical nanoparticles on the surface of the electrodes.Gold nanoparticles of different sizes with diameters varying from 50 to 100 nm were deposited on the surface of graphene layers and made a large accessible surface area for the subsequent electro-catalytic reaction of analytes.Moreover, transformation to highly accessible larger surface area with bright streaks morphology is observed after immobilization of a monoclonal anti-PSA antibody onAu-GrO surface (Figure 6, II), indicating high loading of the monoclonal anti-PSA antibodycan efficiently interact with the analytes in the samples. To reconfirm the immobilization of anti-PSA antibody on the surface of Au-GrO, we performed immunofluorescence staining using secondary antibody of goat anti-rabbit Alexa-fluor 594. Results shown that the dotted pattern of immobilization of anti-PSA antibody onto the electrode surface with red fluorescenceas shown in Figure 6, III, whereas there is no detectable signal in two negative controls of only bare electrode with secondary antibody of goat anti-rabbit Alexa-fluor 594(Figure 6, IV) and only anti-PSA immobilized antibody on Au-GrO(Figure 6, V), suggesting the confirmation of the immobilization of anti-PSA antibody onto the Au-GrO surfaces consistent with our SEM images. This validated working electrode was used for subsequent experiments.

4. ElectroanalyticalImmunosensor performance

In order to determine the concentration of PSA levels, differential pulse voltammetry studieswere adopted. Our results shown that the current enhancement in terms of oxidation peak at 0.15 V (Figure 7, I) in presence of lower to higher concentrations of PSA using developed immunosensor. The sensitivity calculated with the calibration curve of current *vs* PSA concentrationsranging from 0.001 $fgmL^{-1}$ to 0.02 μgmL^{-1} (Figure 7, II). The detection limit for PSA was found to be0.24 $fgmL^{-1}$. We have compared our experimental data with the previously reported literatures as shown in Table 1

5. Validation and Reproducibility characteristics of PSAimmunosensor in serum samples:

We performed with a standard method of recovery percentage (97.67 %) to check for the existence of interferences of PSA in serums as shown in Table 2, indicating that there was no significant cross-reaction of the components of the serum with the immunosensor. In this study, we obtained RSD values for PSA in PBS and in serum were 2.43 % and 2.91 % respectively (Table 2). The Relative Standard Deviation (RSD) of the five measurements (n=5) for the electrode was 2.43 %, suggesting

the precision and reproducibility of the immunosensor was quite good.Moreover, it has shown considerable repeatability and reproducibility data (Figure 5, II), confirming a promising alternative approach for the direct determination of PSA in biological fluids with minimum interferences.In order to test the stability, we stored theimmunosensorat 4 ^{o}C for two weeks. After a storage period, the immunosensor still retained 85% of its initial current response, indicating satisfactory stability.

Conclusion:

In summary, an easy to fabricate and sensitiveAu-GrOimmunosensorwas developed to detect PSA with an Au-GrO based PSA immunosensor employing immobilized anti-PSA monoclonal antibody strategy. The signal amplification was achieved through the generation of large surface area by electrochemical process on the electrode surface. The biosensor showed excellent selectivity and satisfactory stability and also shown a low detection limit of 0.24 fgmL^{-1} at signal to noise ratio of 3.The performance and effectiveness of the PSA immunosensor were evaluated in the serum. We also found satisfactory selectivity, reproducibility, and stability of this immunosensor.We anticipate that this high sensitive and selective biosensor has potential to be applied in clinical applications. Moreover, this biosensor may serve as a useful diagnostic tool for accurate quantification of other proteins by simply changing the respective antibodies and has the potential future for reliable point-of-care cancer diagnostics.

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Notes and references

1. S. Jain, S. Saxena, A. Kumar, Meta Gene.2(2014) 596-605.
2. J. D.Wulfkuhle, L.A. Liotta, E.F. Petricoin, Nat Rev Cancer.3 (2003) 267-75.
3. V. Vaisanen, S. Eriksson, K.K. Ivaska, H. Lilja, M. Nurmi, K. Pettersson, Clin Chem.50(2004) 1607-17.
4. S. H. Lang, F. M. Frame, A.T. Collins, J Pathol.217(2009) 299-306.
5. M. C. Daniel, D. Astruc, Chem Rev.104 **(**2004) 293-346.
6. M. Du, T.Yang, K. J. Jiao, Mater. Chem.20(2010) 9253-9260.
7. H. P. Cong, J. F. Chen, S. H. Yu, ChemSoc Rev.7 **(**2014) 7295-7325.
8. S. Chandrasekaran,S. H. Hur, E. J. Kim, B. Rajagopalan, K. F. Babu,V. Senthilkumar,J. S. Chung,W. M. Choi, Y. S. Kim, RSC Adv.5(2015) 29159-29166
9. Z. Zhao, M. Zhang, X. Chen, Y. Li, J.Wang, Sensors15 **(**2015) 16614-16631.
10. M. Pal, M. J. Tan, R. L. Huang, Y. Y. Goh, X. L. Wang, M. B. Tang, N. S Tan, PLoS One.6,(2011) 25377.
11. K. Radhapyari, P. Kotoky, M.R. Das, R. Khan, Talanta111 (2013) 47-53.
12. R. Khan, N.C. Dey, A.K. Hazarika, K.K. Saini, M. DhayalAnal. Biochem.410(2011) 185-190.
13. Y. He, S. Xie, X.Yang, R.Yuan, Y. Chai, ACS Appl. Mater. Interfaces7 (2015) 13360-13366.
14. R. Akter, M. A. Rahman, C. K. Rhee, Anal. Chem.84 (2012) 6407-6415.
15. H. Pei, S. Zhu, M. Yang, R. Kong, Y. Zheng, F. Qu. Biosens. Bioelectron.74 (2015) 909-914.
16. X. Shoujiang, L.Yang, W. Taihong, L. Jinghong, Anal. Chem.83(2011) 3817-3823.
17. M. Hongmin, L. Xiaojian, Y. Tao, L.Yan, Z. Yong, W. Dan, W. Qin, D. Bin, Biosens. Bioelectron. 79(2016) 379-385.
18. L. Tian, L. Liu, Y. Li, Q. Wei, W. Cao, New J. Chem.39(2015) 5522.
19. D. Wu, R. Li, H. Wang, S. Liu, H. Wang, Q. Wei, B. Du, Analyst137(2012) 608.
20. J. H. Lin, L. J. Zhang, H. Zhang, S. S. Zhang, Chinese Journal of Chemistry,26 (2008) 480-484.
21. Z. Wu, Q. Fu, S. Yu , L. Sheng , M. Xu , C. Yao , W. Xiao, X. Li, Y. Tang, BiosensBioelectron.85(2016) 657-663.
22. Z. GaO, M, Xu, M. Lu, G. Chen, D. Tang, Biosens. Bioelectron.70(2015) 194-201.
23. Y. D. Zhu, J. Peng, L.P. Jiang, J.J. Zhu, Analyst139 (2014) 649-55.

Figure captions:

Figure 1. Schematic illustration of the fabrication process for an electrochemical sensor (Au-GrO) based on Monoclonal*anti*-PSA antibody immobilized on AuGrO electrode.

Figure 2. Cyclic voltammograms of (I) Pt (II) Pt-Au; (III) Pt-Au-GrO(IV) Pt-Au-GrO-monoclonal anti PSA antibody electrodes in PBS (100 mM, pH 7.4) containing 5 mM [Fe(CN)6]3-/4- at scan rate ranges 05 mVs-1 to 50 mVs-1

Figure 3. CV of (I) & DPV of (II) for (a) Pt, (b) Pt-Au (c) Pt-Au-GrO and (d) Pt-Au-GrO-monoclonal anti PSA antibody in PBS (100mM, pH 7.4) containing 5 mM [Fe(CN)6]3-/4- and (III) and (IV) for the higher potential side the dependence of reduction and oxidation peak current with square root of scan rate.

Figure 4. Immunofluorescence staining of PSA (green fluorescence) using monoclonal anti-PSA antibody in human RWPE-1 cells.

Figure 5. (I) & (II) for the lower potential side the dependence of reduction and oxidation peak current with square root of scan rate; (III) EIS Nyquist plots of (a) Pt, (b) Pt-Au (c) Pt-Au-GrO and (d) Pt-Au-GrO-monoclonal anti PSA antibody in PBS (100mM, pH 7.4) containing 5mM [Fe(CN)6]3-/4-; and (IV) Reproducibility of immunosensor in presence of PSA concentration i.e. 5 x 10^{-12} ngmL-1.

Figure 6. Validation of anti-PSA antibody immobilization on the Au-GrO surface. SEM micrograph of (I) Au-GrO (II) Monoclonal anti PSA antobody on Au-GrO after immobilization. Immunofluorescence images of immunosensor (III) immobilized with monoclonal anti-PSA antibody on Au-GrO (red fluorescence), whereas two negative controls of (IV) only bare electrode with secondary antibody of goat anti-rabbit Alexa-fluor 594 and (V) only anti-PSA immobilized antibody on Au-GrO.

Figure 7. (I) DPV plots of Immunosensor with bare electrode and different concentrations of PSA ranges 0.001 fgmL-1 to 0.02 µgmL-1. (II) Calibration plots showing current for different concentrations of PSA 0.001 fgmL-1 to 0.02 µgmL-1 and inset showing the at higher magnification of DPV curve.

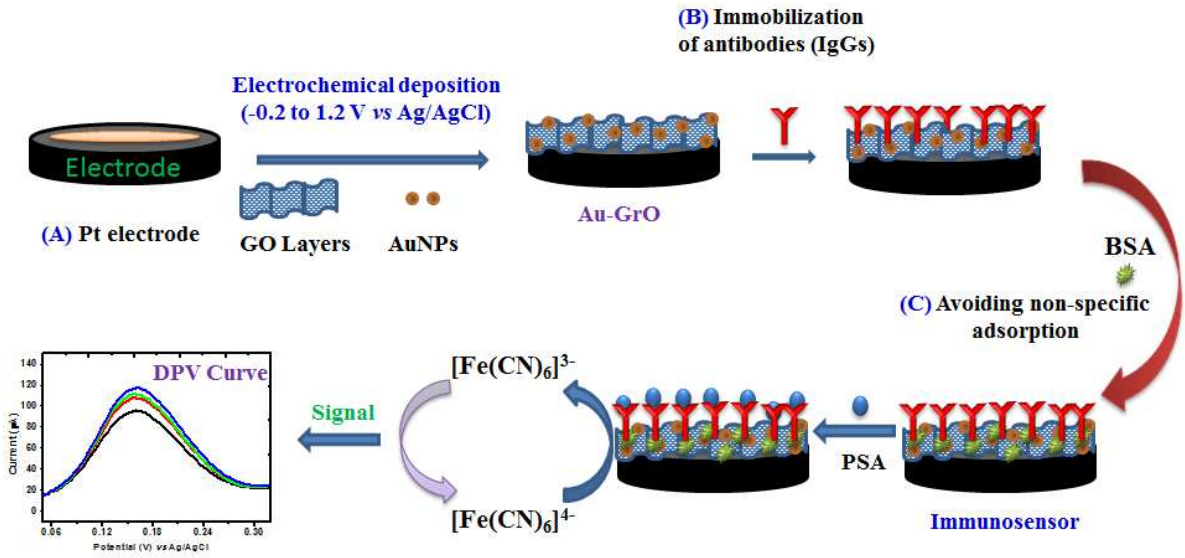

Figure 1

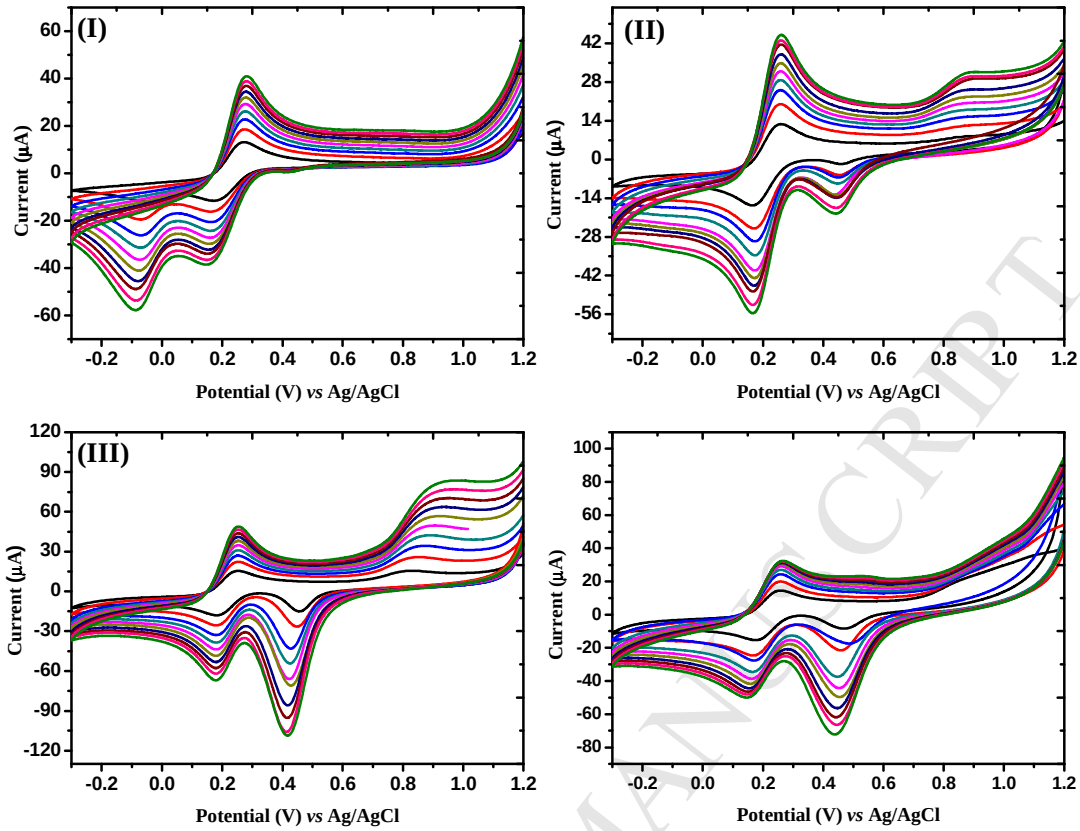

Figure -2

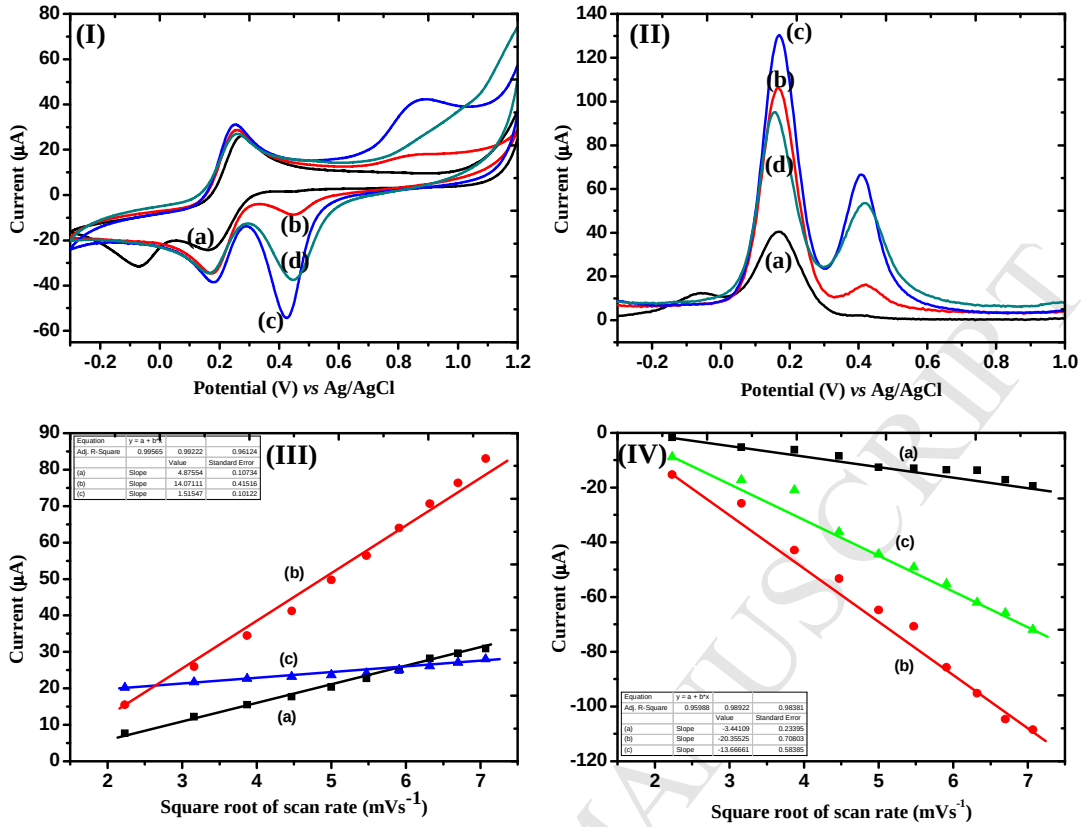

Figure 3

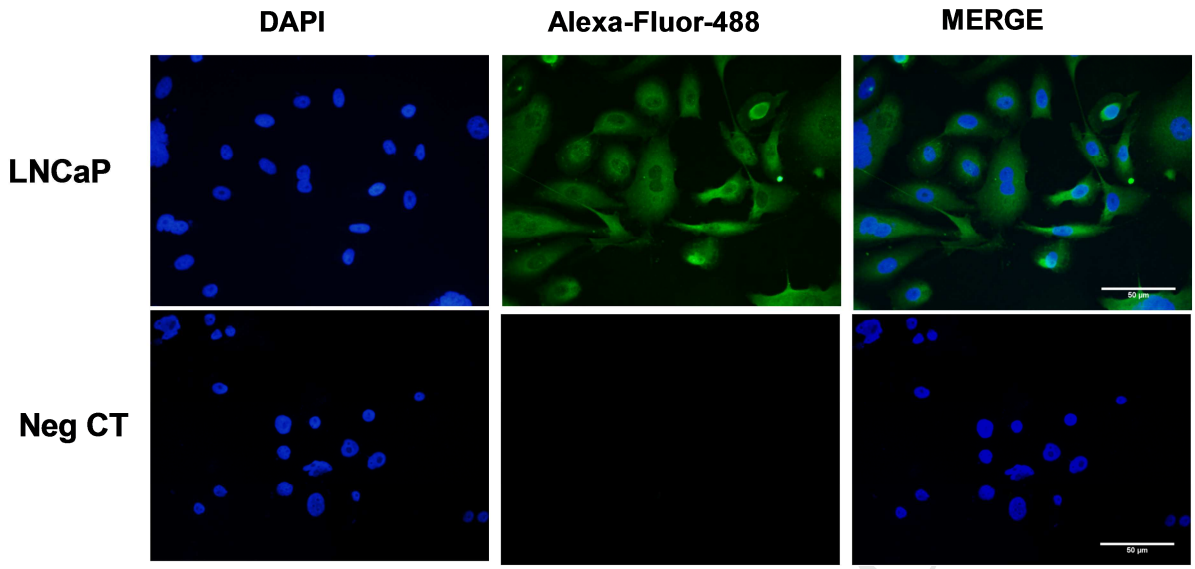

Figure 4

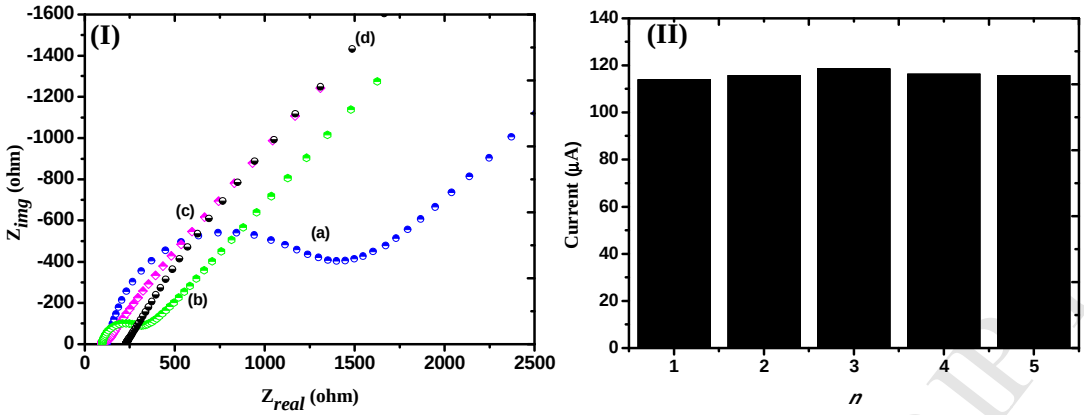

Figure 5

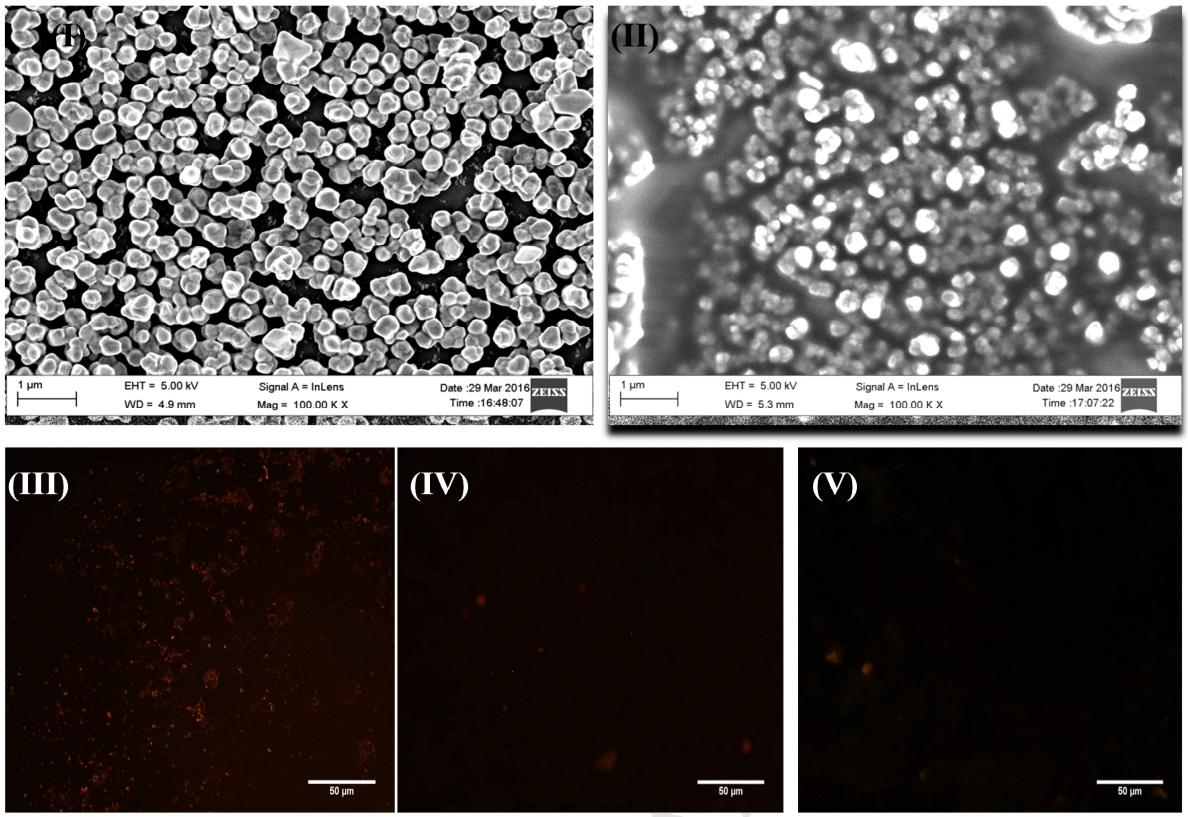

Figure 6

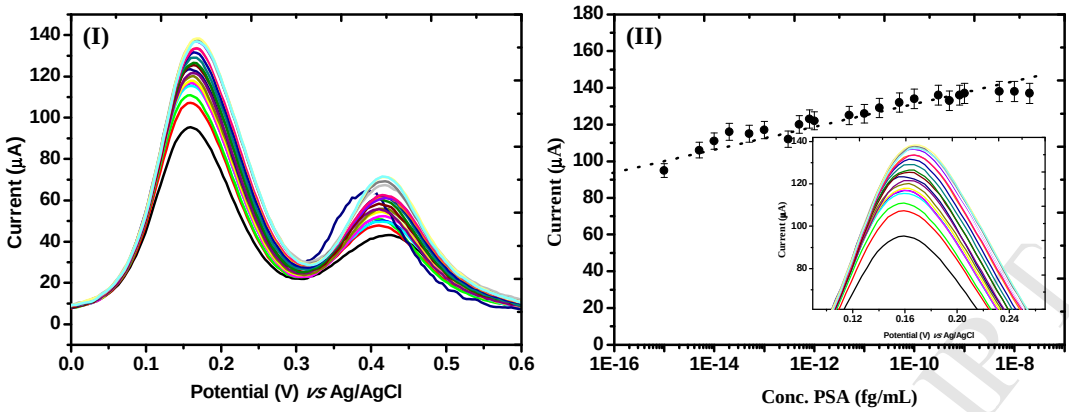

Figure 7

Table 1. Summary of the various metal/metal oxide nanoparticles and metal composites based PSA immunosensor reported in the literature, along with our experimental data.

SNo.	Electrode	Range	LOD	Ref.
1.	(MWCNTs-PAMAM)[a]	0.001 to 30 ng mL^{-1}	0.7 pg mL^{-1}	24
2.	MWCNTs-AuNPs[b]	1.0 $pgmL^{-1}$ to 10.0 ng mL^{-1}	0.40 ± 0.03 pg mL^{-1}	25
3.	GO-GQDs@Ag[c]	1 pg mL^{-1} to 20 ng mL^{-1}	0.3 pg mL^{-1}	26
4.	Ag@Pb(II)-β-CD[d]	0.001–50 ng mL^{-1}	0.34 $pgmL^{-1}$	27
5.	KNbO3–Au nanoparticles@Bi2S3[e]	0.005–5 ng mL^{-1}	3 pg mL^{-1}	28
6.	rGO–MWCNT–Pd[f]	0.5 $pgmL^{-1}$ to 15 ng mL^{-1}	0.17 $pgmL^{-1}$	29
7.	NPG-HSMs[g]	0.01-10 ng mL^{-1}	6.00 $pgmL^{-1}$	30
8.	Au-chitosan[h]	5.0 to 30 ng mL^{-1}	1.0 ng mL^{-1}	31
9.	Pt@AuNPs[i]	0.02 to 2.5ng mL^{-1}	0.017ng mL^{-1}	32
10.	Au@PtNHs[j]	5-500 $pgmL^{-1}$	2.9 $pgmL^{-1}$	33
11.	CuSNPs[k]	0.5 $pgmL^{-1}$ to 50 ng mL^{-1}	0.1 $pgmL^{-1}$	34
12.	Au-GrO	0.001 $fgmL^{-1}$ to 0.02 μgmL^{-1}	5.4$fgmL^{-1}$	Present Work

[a]Multiwalled carbon nanotubes/poly(amidoamine) dendrimers nanohybrids
[b]Multiwall carbon nanotubes (MWCNTs)/gold nanoparticles
[c]Graphene oxide quantum dots@silver core-shell nanocrystals
[d]silver nanoparticles-doped Pb (II) metal-organic framework
[e]potassiumniobate-Au nanoparticles@bismuthsulfide (KNbO3–Au NPs@Bi2S3)
[f]reduced graphene oxide-multiwalled carbon nanotube-palladium nanoparticle (NP) nanocomposite
[g]nanoporous gold (NPG) and hollow mesoporous silica microspheres (HSMs)
[h]Nano-Au Modified Chitosan Membrane
[i]Platinum supported gold nanoparticles
[j](gold core)@(platinum shell) nanohybrids
[k]CuSnanoparticles

Table 2. Percentage recovery of PSA in serum samples based on DPV measurements (n = 5) using our developed immunosensor.

Concentration	Current (μA) (n = 5)	Average	SD	RSD %
PSA in PBS (5 x 10^{-12}fg mL^{-1})	113.8, 115.7, 118.5, 116.3, 115.7	116.0	1.68	2.43
PSA in Serum (5 x 10^{-12}fg mL^{-1})	112.1, 115.0, 115.1, 108.2, 116.5	113.3	3.30	2.91
Recovery = 97.67%				