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Flexible Gold Electrode Array for Multiplexed Immunoelectrochemical Measurement of Three Protein Biomarkers for Prostate Cancer

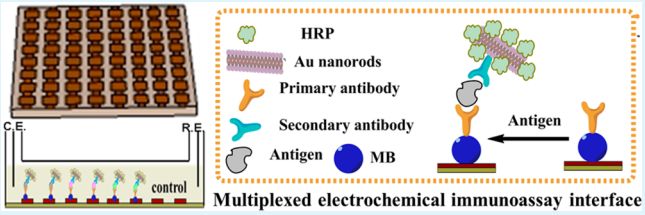
Jing Liu,[†,‡] Cai-Yu Lu,[†] Hong Zhou,[†,‡] Jing-Juan Xu,*[,†] and Hong-Yuan Chen*[,†]

[†]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P. R. China

[‡]School of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, P. R. China

Supporting Information

ABSTRACT: In this work, we report a simple and novel electrochemical multiplexed immunosensor on a flexible polydimethylsiloxane (PDMS) slice deposited with 8 × 8 nano-Au film electrodes for simultaneous detection of prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), and interleukin-6 (IL-6). Primary antibodies linked with magnetic beads (Ab_1-MBs) were modified on the nano-Au film electrodes via magnetic force. In the presence of corresponding antigen, horse radish peroxidase−secondary antibody-conjugated gold nanorods (HRP-Ab_2-gold NRs) were brought into the surface of electrodes, generating obvious electrochemical signals of H_2O_2 reduction reactions. Based on this, the designed immunosensor provide good performance in sensitivity and specificity during the detection of above three biomarkers for prostate cancer. The electrochemical multiplexed immunosensor was verified for selective and accurate detection of complex samples in human serum. Data suggested that the reported multiplexed immunosensing strategy holds great promise for applications in clinical assay and diseases diagnosis.



KEYWORDS: *electrochemical immunosensor, multiplexed measurement, flexible electrode, microchip array, prostate cancer*

1. INTRODUCTION

Since several decades ago, prostate specific antigen (PSA) has been selected to be the main clinical protein biomarker for prostate cancer (CaP).[1] However, the elevated PSA levels is not specific to CaP, because it is found that abnormal PSA values, specifically ranged from 4 ng mL^{-1} to 10 ng mL^{-1}, could also be found in men of benign prostate diseases including benign prostate hyperplasia and prostatitis.[2] Because of its low specificity, about 75% of men with danger zone of PSA levels suffered unnecessary biopsy.[3] Considering PSA detection as a single marker could not satisfy the requirements of accurate CaP diagnosis, multiple protein arrays in clinical samples has attracted more attention as a promising approach for reliable early detection of cancers.[4]

Recently, many approaches have sprung up for multiple protein biomarkers detection including electrochemiluminescence (ECL),[5,6] modern liquid chromatography−mass spectrometry (LC-MS) proteomics.[7,8] Among these approaches, electrochemical techniques, with many advantages such as simplified process, low-cost, convenience, and good sensitivity showed good performances in construction of biosensors for varied biomarkers including small molecules, nucleic acid, large proteins, and cancer cells.[9−12] Even so, detection of multiple protein biomarkers is still a challenge to electrochemical techniques, which has faced with much difficulty in employing large arrays on inexpensive electrode-based sensing platforms.[13−15] For example, the requirements of independently addressed electrical contacts corresponding to each sensing unit, as well as corresponding reference and counter electrodes, make the process of multiplexed arrays more complicated. Additionally, the complexity of the construction of integrated active electronics on microchip is another problem in multiple protein biomarkers detection via electrochemical techniques. Recently, Kelley[16] fabricated a microwell array based on an electrochemical biosensor for simultaneously multiplexed pathogen detection using glass substrates and electrodeposition of a Pd coating at −250 mV. Rigid glass substrates is relatively easy to create microwell channels and deposit electric conduction film on it; however, this expedience is followed with some problems such as easily damage of electrodes.

In this work, we report a novel 8 × 8-electrode array on a flexible polydimethylsiloxane (PDMS) microchip, which could be employed to simultaneously detect three different cancer biomarkers for prostate cancer via simple electrochemical techniques. The three cancer biomarkers are PSA, prostate specific membrane antigen (PSMA), and interleukin-6 (IL-6), which are elevated in prostate cancer patients.[17−19] The construction of flexible microchip array and the further sensing

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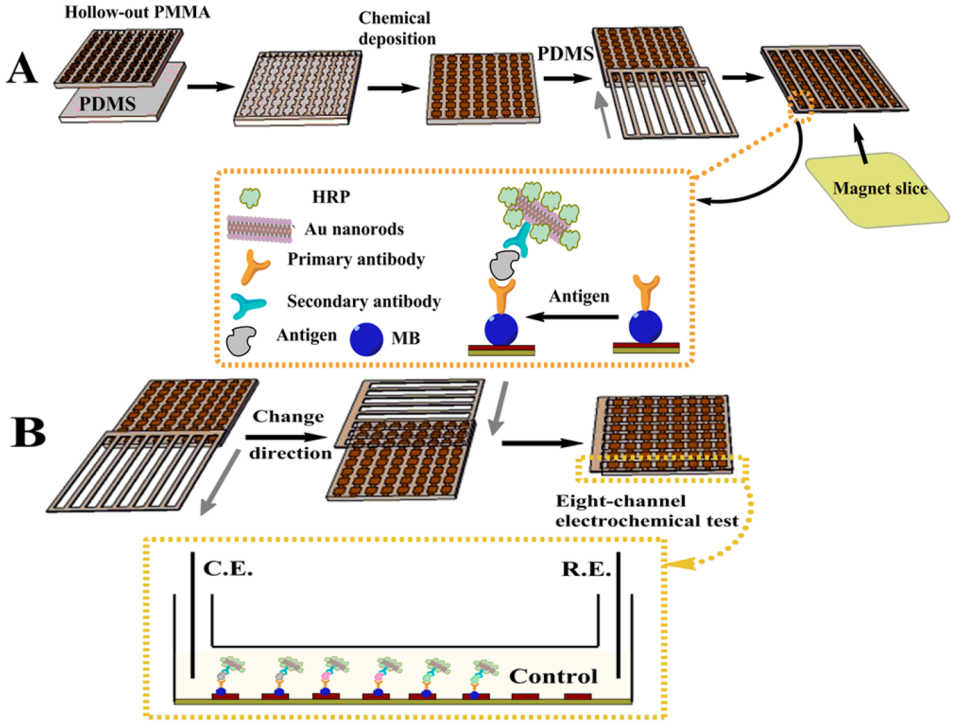
Scheme 1. (A) Preparation of the Flexible Microchip and the Construction of Multiplexed Immunoassay Interface; (B) Sketch Map of Eight Spots in One Microchannel in Detection of Three Antigens for Prostate Cancer



approach is shown in Scheme 1. The 8 × 8-electrode microarray platform is formed on PDMS with uniform nano-Au film on each electrode site using chemical deposition instead of electric deposition. Compared with electric deposition, chemical deposition is instrument-free, results-stable, and easy to operate, and what's more, it can widely applicable to many nonconducting substrate. Additionally, compared with the rigid glass substrates electrode, flexible PDMS microchip showed many advantages in microchip fabrication, store, and modification. For example, due to its flexible properties, PDMS microchip showed less probability of damage during the process of microchip fabrication and store. Additionally, benefited from the simple and effective depositing of nano-gold film on PDMS surface, the prepared PDMS microchip is very convenient for further modification of biomolecules and could be easily used for biosensing.

Here, three kinds of primary antibodies (Ab_1) were respectively preimmobilized on magnetic beads (MBs) and were modified on nano-Au film electrodes through the effect of magnet putting under the microarray platform. Gold nanorods were employed as carriers to decorate more secondary antibodies (Ab_2) and horseradish peroxidase (HRP).[20] Each channel includes eight electrodes, which were connected to the multichannel electrochemical workstation without mutual interference owing to the appropriate space between each electrode. Eight-channel ports were linked with eight-electrode CHI 1030 electrochemical workstation to realize fast multiplexed measurements. This designed sensing platform was further exploited to identify antigens mixture in real human serum based on the specific recognition of antigen−antibody. The results show that this microchip device has great potential for rapid and sensitive detection of multiple biomarkers, which could effectively avoid false positive result and provide a prospective testing approach for accurate cancer diagnosis.

2. EXPERIMENTAL SECTION

Bovine serum albumin (BSA), *N*-hydroxysuccinimide (NHS), N-(3-(dimethylamino)propyl)-N′-ethyl-carbodiimide hydrochloride (EDC), imidazole, and horse radish peroxidase (HRP) were purchased from Sigma-Aldrich, Inc. (U.S.A.). EDC and NHS were dissolved in water immediately before use. Human prostate-specific antigen (PSA), two mouse antihuman total PSA monoclonal antibodies (primary capture antibody, Ab_1 and secondary detection antibody, Ab_2) were purchased from Linc-Bio Science Co., Ltd. Monoclonal antihuman interleukin-6 (IL-6) antibody, secondary antihuman IL-6 antibody, and recombinant human IL-6were from ABclonal Biotech Co., Ltd. (U.S.A.). Human PSMA, monoclonal primary antihuman PSMA antibody, and secondary anti-PSMA antibody were obtained from Epitomics, Inc. (U.S.A.). ELISA kits for huaman PSA, IL-6, and PSMA were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). Sylgard 184 including polydimethylsiloxane (PDMS) monomer and curing agent was purchased from Dow Corning (Midland, MI, U.S.A.). PMMA plate was designed according to the needed shape of microchip electrode and drawn a picture with precise scales and proportion. Then, the designed PMMA plate was produced out by Beijing champions league ZhuoYi business center (Beijing, China). Human serum samples were obtained from cancer hospital of Jiangsu province without further treatment. All other reagents were of analytical reagent grade.

The procedure of gold NRs synthesis was according to a reported literature.[21] To prepare the seed solution for gold NRs, 5 mL of 0.5 mM $HAuCl_4$ was added to 5 mL of 0.2 M cetyltrimethylammonium bromide (CTAB) solution. Then, 1 mL of 6 mM $NaBH_4$ solution was mixed with the Au (III)-CTAB solution under conditions of vigorous stirring. The solution color could be found changed from yellow to brownish-yellow, and then, the solution remained still at room temperature and used soon.

For the preparation of the growth solution, 0.9 g CTAB and 0.08 g sodium salicylate were added in 25 mL of 50−70 °C warm water. The warm solution was cooled to 30 °C, then 0.6 mL $AgNO_3$ solution (4 mM) was injected and the mixture remained still for 15 min. Following this, 25 mL $HAuCl_4$ solution (1 mM) was added with gentle stirring for another 15 min. Then, with the addition of 0.064 M

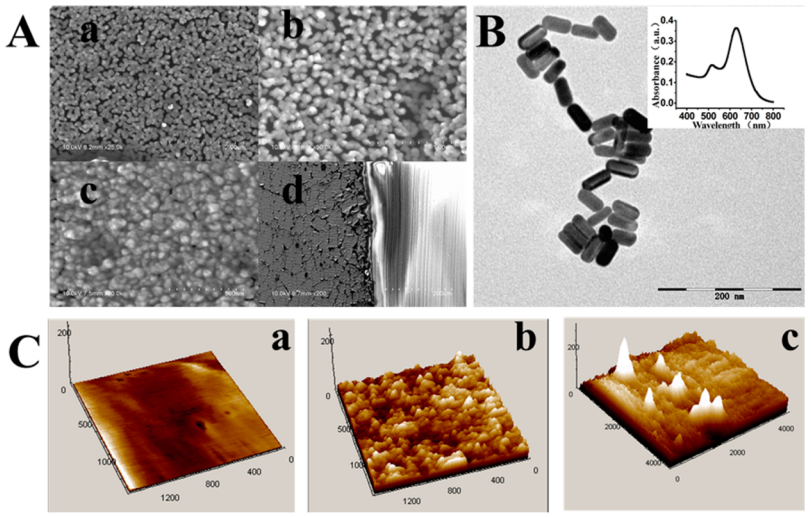


Figure 1. (A) SEM images of nano-Au film on PDMS microchip. (a–c) Platforms of nano-Au film structure in different amplificatory grade, (d) longitudinal section of nano-Au film; (B) TEM images of gold NRs. Insert is UV spectrum of gold NRs; (C) AFM images of bare PDMS slice (a), nano-Au film modified PDMS slice (b), and Ab_1-MBs modified PDMS slice (c).

ascorbic acid (AA) under the condition of vigorously stirring for 30 s, the solution became colorless.

The final step was conducted as follows. 0.08 mL of above prepared seed solution and growth solution was mixed under stirring for 30 s, the mixture remained still at 30 °C for 12 h making NR grow. Then, the mixed solution was centrifuged for 25 min (at 8500 rpm) and the obtained precipitates were redispersed by addition of 10 mL water.

The preparing procedure of HRP-Ab_2-labeled Gold NRs was according to a previous literature.[22] The steps of conjugation process was described as follows: 1.5 mL gold NRs in triplicate were centrifuged at 8500 rpm for 10 min and the soft sediment were kept. 200 μL 1.2 mg mL^{-1} HRP and above gold NRs sediment were added to 1 mL PBS (pH 8.0), which contained 1 μg mL^{-1} Ab_2. After stirring for 1.5 h at room temperature, the conjugate was further centrifuged at 15 000 rpm for 10 min and then washed with PBS. Following this, the soft sediment was resuspended in 1 mL 1% BSA solution and stored in refrigerator at 4 °C.

The Ab_1 antibody for three respective antigens was decorated on the surface of MBs according to a reported method with a slight modification.[23] Briefly, 100 μL MBs solution was magnetically separated from the solution, followed by washing three times with 0.01 M imidazol–HCl buffer (3 × 200 μL). Then, it was resuspended to a final volume of 100 μL using the same buffer solution. A 200 μL solution containing 0.06 mM NHS and 0.08 mM EDC were mixed in a tube for incubation for 30 min at room temperature in order to activate the carboxylate groups on the MBs. The activated carboxylate group-modified MBs were then washed carefully using above washing buffer solution and resuspended again to obtain a 100 μL solution. Related 1.0 μg mL^{-1} Ab_1 antibody aqueous solution (100 μL) was then added to the solution and kept overnight at 25 °C, allowing the modification of corresponding Ab_1 onto MBs surface. At this point, in order to eliminate the rate of unspecific binding, the prepared Ab-MBs were further magnetically separated and resuspended in 100 μL 1% BSA solution for 2 h. After washed twice using 100 μL washing buffer, the resulting products were carefully kept for use.

To fabricate the flexible multiplexed immunoassay microchip, a predesigned hollow-out poly(methyl methacrylate) plate (PMMA plate) was putted on the PDMS slice, then a chemical deposition solution[24] concluding 2:1:1 (volumetric ratio) 1% $HAuCl_4$, 200 g L^{-1} $KHCO_3$ and 2% glucose was added into the hollows of PMMA for 3h in dark. The uniform nano-Au films were obtained on PDMS slice as electrodes, which were demonstrated in Scheme 1. The spatial separation between every electrode site brought an individual immunoassay for different antigens at each spot without interference resulting from diffusion crosstalk. Microchannels were prepared using PDMS to replicate the predesigned shape of channels on glass with photoresist technique.[25] The presented microchip contained 8 microchannels (each microchannel containing eight nano-Au spots) and each nano-Au spot was employed as a working electrode. Then, 64 individual testing samples could be constructed on one chip and results of eight electrodes were obtained at one time owing to the eight-channel CHI 1030 electrochemical workstation, which was connected with eight designed nano-Au spots to realize a multiple-detection.

In order to fabricate a multiplexed immunoassay interface on nano-Au film electrodes, a magnet slice lay under the microchip to trap Ab_1-immobilized MBs on the nano-Au electrode array surface to fabricate the immunoassay interfaces. A sandwich immunoassay was adopted for the detection of PSA, IL-6, and PSMA antigen. The sensing surface (Ab_1-MBs-Au) was first incubated with 80 μL (for one channel) different concentrations of antigen solutions of PSA, IL-6, and PSMA for 40 min at 37 °C; then, it was washed thoroughly with washing buffer. Second, the electrodes of antigen-Ab_1-MBs-Au was further incubated with 80 μL (for one channel) of prepared solution of HRP-Ab_2-gold NR conjugate for 2 h at 37 °C with further careful washing to remove unreacted residue. The electrochemical immunoassay was then conducted in10 mM H_2O_2 solution. Ag/AgCl and Pt wire acting as reference and auxiliary electrodes, respectively, were placed at the two ends of each microchannel in all electrochemical measurements. The above-mentioned procedures were also used for measurement of subsequent human serum samples without further treatments.

3. RESULTS AND DISCUSSION

The nano-Au film structure prepared on PDMS microchip using chemical depositing method was first characterized using scanning electron microscopy (SEM). Figure 1A (a–c) show the nano-Au film structure in different amplificatory grade, while (d) presents the longitudinal section of nano-Au film which revealed the uniform size and the compact structure of the prepared nano-Au film on PDMS. The compact nano-Au film provides a good conductive interface for further modification of antibodies or antigens and electrochemical test. From Figure 1B, which showed the TEM images of the obtained gold NRs, we could find that the synthesized gold NRs were relatively homogeneous with an average diameter of 18.0 ± 1.0 nm and a length of 40.0 ± 2.5 nm. The aspect ratio is about 2.4, resulting in a longitudinal surface plasmon resonance (LSPR) band around 630 nm (inset of Figure 1B).

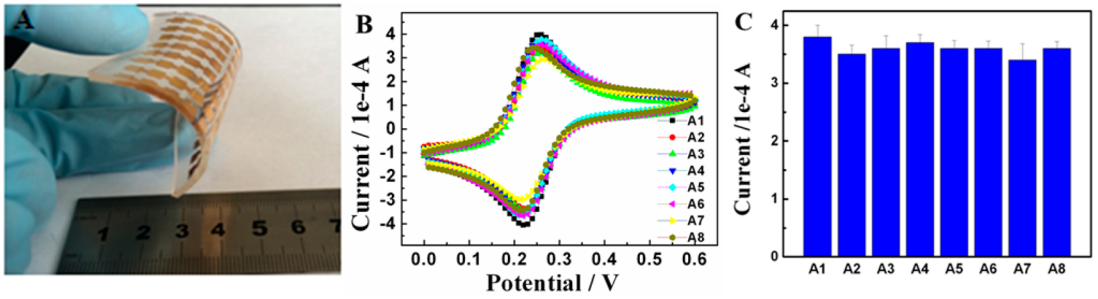


Figure 2. (A) Photograph of the prepared flexible microchip containing 8 × 8 electrodes; (B) CV responses of 8 electrodes (in one channel) in 1 mM $[Fe(CN)_6^{3-/4-}]$ containing 0.1 M KCl solution at a scan rate of 100 mV s^{-1}; (C) CV currents of nano-Au electrodes of one PDMS strip (each strip with eight electrodes). Error bars are standard deviations from eight electrodes on the same column of PDMS slice.

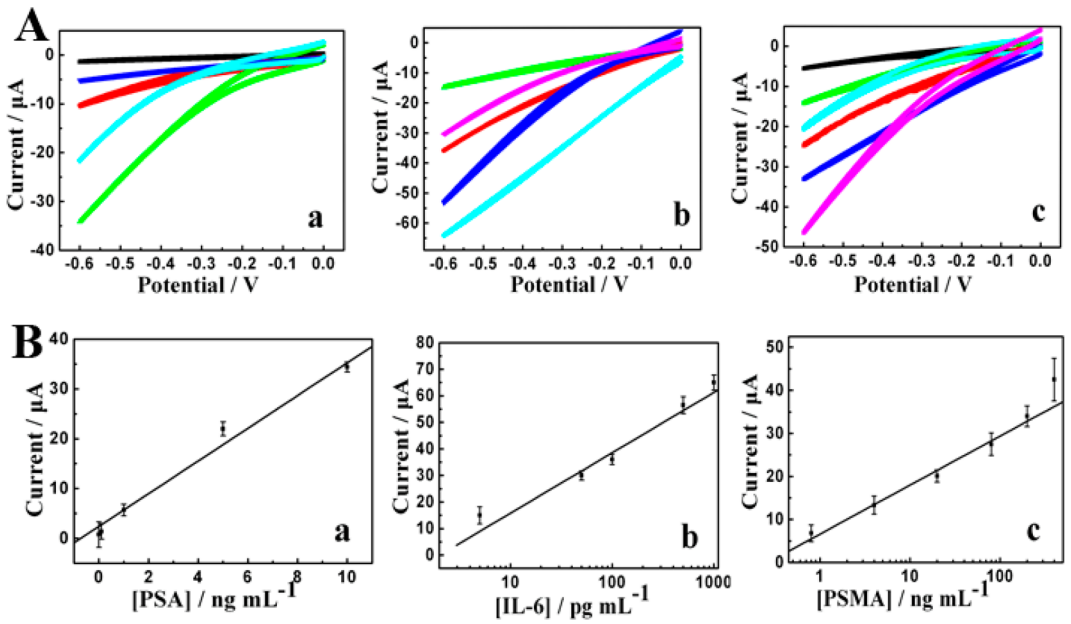


Figure 3. (A) CV of the multiple immunosensors with increased concentrations of PSA (0, 0.1, 1, 5, 10 ng mL^{-1}, respectively) (a); IL-6 (5, 10, 100, 500, 1000 pg mL^{-1}, respectively) (b); and PSMA (0.8, 4, 20, 80, 200, 400 ng mL^{-1}, respectively) (c). (B) The linear relationship between the current recorded at −0.6 V and the concentration of PSA (a), IL-6 concentration (b), and PSMA (c) (n = 3).

In order to confirm the immobilization of Ab_1-MBs on the nano-Au film electrode, the atomic force microscope (AFM) technique was employed to characterize the surface of the Ab_1 modified nano-Au film electrode on PDMS slice (Ab_1-MBs/nano-Au/PDMS) with comparative observations of nano-Au/PDMS and bare PDMS. The AFM images in Figure 1C further reveal that nano-Au film is uniformly deposited on PDMS slice and Ab_1-MBs were magnetically captured on nano-Au film surface.

A prototype device was fabricated in order to characterize the physical properties and the electrochemical behavior of the electrodes. The device, which were consisted of independent and parallel straight 8 channels with each channel containing eight 3 mm × 3 mm nano-Au electrode was totally molded out on PDMS. Figure 2A showed the flexibility of the nano-Au electrodes on PDMS slice which had an ability to withstand pressure and avoid the damage of the modified electrodes as no obvious change was found before and after bending course of the electrodes from SEM and electrochemical performance. Moreover, the nanomaterial processed higher surface area compared with traditional electrode materials such as bare gold electrode, which had good performances in those sensing or detections application. Here, the roughness of the prepared electrode was estimated to be 5.76 through cyclic voltammograms curve of electrode in 0.5 M H_2SO_4 based on the integral area of reduction peak and the practical area of electrode. The large electrode area and rough surface of nano-Au electrode can increase the amount of modified biomolecules, which brought benefit for antigen detection during the electrochemical biosensing.

As one microchip concluded 8 × 8 electrodes, which were prepared at one time for immune detection, the consistency and the repeatability of the prepared electrodes were investigated by observing the performances of eight electrodes in one channel in $Fe(CN)_6^{3-/4-}$ solution using eight-channel CHI 1030 electrochemical workstation. Figure 2B showed the CV responses of eight electrodes in one channel. From the results, we can see that stable and high electrochemical signals were obtained, which indicated that the constructed microchip array possessed excellent stability among individual electrode on it. The stable and consistent performance of individual electrode on microchip array ensured the reproducibility during the detection of three antigens carried through on individual electrode.

One of the advantages of a multiple-microchip is to provide convenience in face of a good deal of testing samples. The

designed 8 × 8 electrode array could simultaneously analyze 8 testing samples, which is timesaving compared with complicated processing procedure during conventional gold electrodes. Scheme S1 (in the Supporting Information) showed that a group of testing experiments in our experiment could be carried out in one piece of the multiple-microchip, including the following multiplexed detection of three important biomarkers for prostate cancer (shown in Figure 3) and the verification experiment for three kinds of antigens detection in human serum sample (shown in Figure 5).

Due to the high-throughput of the microchip, three important biomarkers for prostate cancer were tested on the different channels of microchip simultaneously. The sensitivity and quantitative range for three kinds of antigen biomarkers (PSA, IL-6, PSMA) were studied under the optimized conditions (incubation time, pH, and the ratio of HRP/antibody, Figure S1−S3 in the Supporting Information) with different antigen concentrations based on the designed strategy showed in Scheme 1. On the basis of the recognition reaction between antigen and corresponding antibody, HRP modified on the second antibody was introduced into the electrodes surface, resulting obvious catalytic current in H_2O_2 solution. The catalytic current was speculated to be influenced by the concentration of antigen (PSA, IL-6, and PSMA), and then, the catalytic current at −0.6 V was recorded in the quantitative detection of the three proteins. As expected, the current obtained at −0.6 V enhanced when the concentration levels of antigen increased in the sample solution (Figure 3A (a−c)). The results indicated good linear relationships between current and the antigens concentration (or logarithm of antigens concentration) in the testing ranges, which are shown in Figure 3B (a−c).

Current increased linearly ranged from 0.1 to 10 ng mL^{-1} for PSA. The linear regression equation was i (μA) = 3.28 c + 2.42, where i was the current intensity recorded at −0.6 V and c was the concentration of PSA (ng mL^{-1}). As to IL-6 and PSMA, a linear dependence between the recorded currents and the logarithm of IL-6, PSMA concentration was found ranged from 5 pg mL^{-1} to 1000 pg mL^{-1} (for IL-6) and from 0.8 ng mL^{-1} to 400 ng mL^{-1} (for PSMA). The linear regression equations were i (μA) = −6.97 + 22.76 log c (IL-6, pg mL^{-1}) and i (μA) = −6.60 + 11.39 log c (PSMA, ng mL^{-1}), respectively.

The limit of detection (LOD) of the immunoarray for each biomarker was evaluated from the lowest antigen concentration in the linearity range and be presented in Table 1. As showed in Table 1, the danger levels of the three antigens in human serum (4−10 ng mL^{-1}, 0.02−1 ng mL^{-1}, and 300−650 ng mL^{-1} for PSA, IL-6, and PSMA) were completely contained or partly contained (for PSMA, danger zone 300−400 ng mL^{-1} was covered) in linear zone. As the dynamic ranges of these three protein biomarkers in real patients were within this danger range or higher than this range, it could be easily to find out the suspected patients from normal people (with PSA, IL-6, and PSMA lower than 4, 0.02, and 300 ng mL^{-1}, respectively) using this method. The detection sensitivity obtained as 0.1 ng mL^{-1}, 5 pg mL^{-1}, and 0.8 ng mL^{-1}, respectively, for PSA, IL-6, and PSMA is lower than or comparable with that of current multiple immunosensors (1.0 ng mL^{-1}, 30 pg mL^{-1}, and 10 ng mL^{-1} for PSA, IL-6, and PSMA, respectively).[4] The LODs are also lower enough to meet the requirements of clinical assay of the above three biomarkers for prostate diseases.

Table 1. Serum Ranges of Normal and Cancer Patient and Detection Limits (LOD) during the Immunosensing of PSA, IL-6, and PSMA

biomarker	normal range (ng mL^{-1})	cancer range (ng mL^{-1})	LOD (ng mL^{-1})
PSA	1−4	4−10	0.1
IL-6	0.006−0.020	0.02−1	0.005
PSMA	200−300	300−650	0.8

The selectivity of the presented biosensor was further evaluated. Here, potential interferents, which could be found in serum were used including bovine serum albumin (BSA) and another two marker proteins in serum (α-fetoprotein (AFP) and carcino-embryonic antigen (CEA), which indicated other diseases) to find its potential influence to the testing three proteins, respectively. As shown in Figure 4, the biosensor had good specificity toward target proteins (PSA, IL-6, and PSMA) even though high concentrations (200×) of BSA, AFP, and CEA were used in detecting solution. These tests denoted that the developed strategy could be used to identify target protein with high specificity.

The regeneration of the surface was tested through repeated utilization of electrode. The modified electrode was removed from the magnet following by washing totally, and the process was carried out four times. From Supporting Information Figure S4, it was found that the fabricated immunosensor for PSA, IL-6, and PSMA showed obvious signal resumption and similar result as first-time use after regenerated treatment by above washing process. The slight signal attenuation was considered to the result of the desquamation of modified gold film. The stability of the electrode was evaluated for long-time storage at 4 °C for 7 days and obtained no obvious change from initial current response, which indicated the good stability of the prepared electrode.

Finally, in order to find whether the proposed multiple-biosensing approach has potential clinical applications, three kinds of antigens detection in human serum sample at one time were demonstrated through the following experiments. We performed a verification experiment which could be served as spike and recovery experiments as well. Three serum samples were respectively spiked with 4 ng mL^{-1} PSA + 20 pg mL^{-1} IL-6, 4 ng mL PSA + 300 ng mL^{-1} PSMA, and 20 pg mL^{-1} IL-6 + 300 ng mL^{-1} PSMA. Above three samples were tested on the microchip as channel 1, channel 2, and channel 3. Figure 5 showed the CV intensity current values obtained in various antigens spiked serum samples. The results showed that a higher signal could be observed if the Ab_1-MBs were modified on the electrode and the corresponding antigen were present in the testing human serum sample. No obvious signal was obtained if no Ab_1 was premodified on sensing interface or the modified Ab_1 did not match the testing antigen. The above results clearly demonstrated a high selectivity of this designed simultaneous detecting method for three biomarkers of prostate diseases.

In order to verify the accuracy of the approach, ELISA kits for human PSA, IL-6, and PSMA were employed as a reference method, and relevant results provided in Table S1 (Supporting Information) showed the good accuracy of this approach. Additionally, the respective recovery of the three kinds of antigens was calculated based on the results in Figure 5 and respective linear regression equations. The recovery results were calculated to be 95% (PSA), 102% (IL-6), and 97%

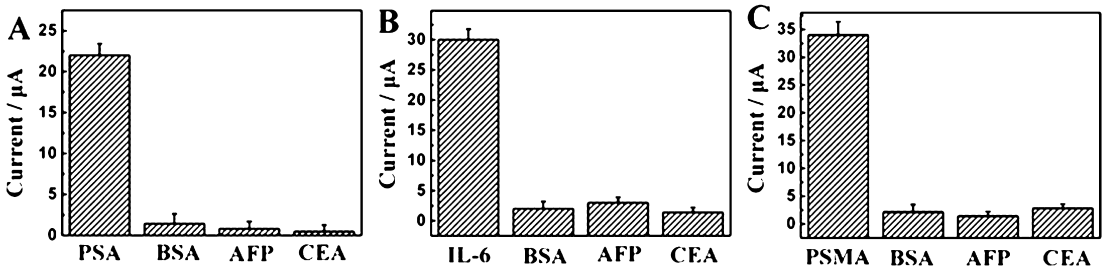


Figure 4. Specificity of the biosensor for (A) PSA detection with 0.5 μg mL^{-1} of BSA, 0.5 μg mL^{-1} of AFP, 0.5 μg mL^{-1} of CEA, and 5 ng mL^{-1} PSA; (B) IL-6 detection with 5 ng mL^{-1} of BSA, 5 ng mL^{-1} of AFP, 5 ng mL^{-1} of CEA, and 50 pg mL^{-1} IL-6; (C) 20 μg mL^{-1} of BSA, 20 μg mL^{-1} of AFP, 20 μg mL^{-1} of CEA, and 200 ng mL^{-1} PSMA.

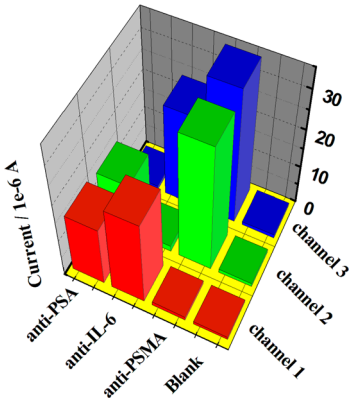


Figure 5. CV responses of three kinds of antigens added in human serum solutions in different combination on a complex matrix. Channel 1 was a human serum solution containing 4 ng mL^{-1} PSA and 20 pg mL^{-1} IL-6; channel 2 was a human serum solution containing 4 ng mL^{-1} PSA and 300 ng mL^{-1} PSMA; channel 3 was a human serum solution containing 20 pg mL^{-1} IL-6 and 200 ng mL^{-1} PSMA. Ab_1-MBs for PSA, IL-6, and PSMA were respectively modified on nano-Au electrodes of microchip as shown (from left to right were anti-PSA, anti-IL-6, and anti-PSMA). The rightmost three electrodes were used as a negative control.

(PSMA), respectively, which is acceptable and potential for multiple-antigens detection in real human serum samples.

4. CONCLUSIONS

In summary, a simple and novel method was presented for rapid assay of multiplexed cancer biomarkers using the flexible microchip array. The microchip array contains 8 × 8 nano-Au film electrodes which provide good conductive capability and high-throughput that can be employed to perform three important biomarkers detection simultaneously. Rapid and comprehensive information could be obtained for accurate diagnosis for a specific cancer disease such as prostate cancer. Additionally, flexible structure of the microchip avoids some problems following with rigid electrode such as easily damage of electrodes. The proposed multiplexed immunosensor with a suitable detection limit and good selectivity shows its extensive applications in clinical assay and diseases diagnosis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information on optimization of experimental conditions and detection experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Tel/Fax: +86-25-83597294. E-mail: xujj@nju.edu.cn.
*E-mail: hychen@nju.edu.cn.

Notes

The authors declare no competing financial interest.

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