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# High Specific Surface Gold Electrode on Polystyrene Substrate: Characterization and Application as DNA Biosensor

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ABSTRACT In the past decades, many efforts have been made to improve the sensitivity and specificity of electrochemical DNA biosensors. However, it is still strongly required to develop disposable and reliable DNA biosensors for wide and practical application. In this article, we reported superior electrochemical properties of an integrated plastic-gold electrode (PGE) fabricated in-house by chemical plating on polystyrene substrate. PGEs were found having extremely high capacity of DNA immobilization compared with gold electrodes fabricated by standard sputtering based photolithography. Unique nano structured surface was observed on PGEs through morphology techniques, which would to some extend give an explanation to higher capacity of DNA immobilization on PGEs. A probable mechanism of carboxylic acid produced on polystyrene substrate after exposure to UV irradiation was proposed and discussed for the first time. This biosensor was applied to detection and manipulate of DNA hybridization. Detection limit of $7.2\times10^{-11}$M and 1-500 nM of linearity range was obtained.

## 1. Introduction

DNA biosensors, based on nucleic acid recognition processes, are frequently being developed towards the goal of rapid, simple and inexpensive testing of infectious, inherited and genetic diseases [1, 2]. Compared with many existing techniques for nucleic acid detection such as fluorescence [3-5], surface plasmonresonance spectroscopy [6, 7] and quartz crystal microbalance [8], electrochemical technique affords a lot of advantages such as its simplicity, rapidness and low-cost [9]. A successful operation of a DNA/RNA biosensor requires high specificity and high sensitivity. However, nucleic acids are relatively simple molecules, finding the sequence that contains the desired information, and distinguishing between perfect matches and mismatches, are very challenging tasks. A conventional biosensor for nucleic acid detection usually comprises three parts: the sensitive element, the transducer and signal processor. Among the above parts, sensitive element is a crucial factor for good performance, because it commonly decides the specificity and has significant influence on sensitivity of biosensors.

Modern microfabrication techniques, especially the screen-printing and standard photolithography, have led to miniaturized and integrated electrochemical devices that address the need for rapid decentralized testing [10-12]. Single-use biosensors have several advantages, such as avoidance of contamination among samples, constant sensitivity and reproducibility and ease of use because no pretreatment is needed, especially avoid tedious polishing of traditional solid electrodes. In particular, screen-printed gold electrodes (SPEs) attracted considerable attention in the development of DNA biosensors [13, 14], because of their low-cost mass production along with minimal sample volume and easily manipulation for thiol modified nucleic acid immobilization through strong binding between sulfur and gold [15]. Compared with SPEs, integrated electrodes fabricated by sputtering based photolithography makes it feasible for further

miniaturized and subtle electrode structures. However, no matter SPEs or fine integrated gold electrodes, special and bull instruments such as photoetching machine and screen printer were more or less indispensible in the whole process. As for the cost, although SPEs were generally recognized much cheaper than sputtered gold electrodes, it would spend several US dollars for individual electrode. Therefore, it is always a compelling requirement to develop further simplified and low-cost gold electrodes as a disposable DNA/RNA biosensor for clinical applications and potential commercialization.

In the past decades, many efforts have been made to improve the sensitivity and specificity of electrochemical DNA biosensors [2, 16-18]. For example, enzymatic reaction [19-21] and gold nano-particle-based signal amplification [22, 23] were successfully employed for sensitive detection of nucleic acids. In addition, an alternate way is to increase the immobilization amount and accessibility of DNA probes by direct fabrication or coupling of nano particles [21, 24, 25], nanotubes [26] and other nano-scaled structures [27] on the surface of electrodes. Compared with enzymatic and nano-particle signal amplification, construction of nano-structured electrode is more essential and direct for achievement of higher specific surface area and larger amount of DNA immobilization. Moreover, it could be combined with signal amplification technique for further improvement of the sensitivity [21]. However, the existing techniques for fabrication of nanostructured electrode always need a separated secondary processing (e.g. electro-deposition) applied on a ready-made electrode which is subjected to cleanliness of the electrode and leads to variable reliability. Furthermore, it is a problem to selectively modify the working electrode from integrated three-electrode biosensor by electro-deposition because of the non-specific adsorption. Therefore, it is still strongly required to develop disposable and reliable DNA biosensors for widely and practical application [28].

Chen and coworkers earlier reported a novel chemical plating method for fabrication gold electrode on plastic substrates [30-32]. However, in their work small chemicals were detected as a demonstration for application and sensitivity was not satisfied. In this manuscript, integrated plastic-gold electrodes (PGEs) demonstrated superior electrochemical properties as DNA biosensor and much higher specific surface compared with traditional sputtered gold electrode. Several conditions including UV exposure, $HAuCl_4$ treatment and chemical plating of the whole procedure were further optimized, which were essential for electrochemical characteristics of biosensor and DNA immobilization. Conventional characterization methods including Attenuated total reflectance(ATR)-FTIR Spectroscopy, Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM) were employed to investigate the morphology of PGE surface. In addition, a probable mechanism of carboxylic acid produced on polystyrene substrate after exposure to UV irradiation was proposed and discussed. Finally, this biosensor was applied to detect DNA molecules by hybridization, as well as the procedure of hybridization on PGE surface was also manipulated through chronocoulometry.

## 2. Experimental

### 2.1 Reagents and materials

DNA probes including thiolated probe1 (5'-SH-AAAAAAAAAAAAAAAAAAAAGACAACCACACTGCTTAGA ), its complementary sequence (5'- TGAGATGAAGCACTGTAGCTC) and a random sequence (5'-AATTTACGCTAAGTCTAAGAAATTTT) were purchased from Sangong (Shanghai, China)and purified by Ultra-PAGE or HPLC and quantified with Nanodrop 2000 spectrophotometer from Thermo scientific. All solutions were prepared with milli-Q water (Millipore, 18 mΩ•cm at 25ºC). The buffers involved in this work are as follows: buffer for

immobilization (I buffer) contained 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP (pH 7.4). Buffer for hybridization and electrochemical measurement (H buffer) composed of 10 mM Tris-HCl, (pH 7.4) and 50 μM $[Ru(NH_3)_6]^{3+}$. Hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich.

2.2 Fabrication of PGE by chemical plating

Integrated gold three-electrode biosensor was fabricated on polystyrene (PS) substrate through UV directed chemical plating technique [29]. In brief, a piece of PS sheet was exposed to UV-lights (254 nm) emitted by a low-pressure mercury lamp for 3-6 hours. The exposed PS sheet was then immersed into 100 mM of phosphate buffer (pH 7.0) containing 360 mM of ethylenediamine (Sigma, China) and 50 mM of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, Fluka, China) at room temperature for three hours. After rinsing with deionized water, the selectively aminated substrate was sequentially treated with 1 mM of $HAuCl_4$ (Sinopharm, China) aqueous solution for 2.5 hours and 0.1 M of $NaBH_4$ aqueous solution for 20 min. Then the substrate was sonicated in 0.5 M KSCN solution for 20 min to remove non-specifically adsorbed gold species on the surface, so as to prevent non-specific over-plating in the following plating process. Finally, the activated PS was placed into a gold plating bath containing 0.125 M of $Na_2SO_3$ (Sinopharm, China), 0.6 M of methanal (Sinopharm, China) and 8 mM of $Na_3Au(SO_3)_2$ (Changzhou Institute of Chemical Research, Changzhou, China) for about three hours. After rinsing with deionized water, the PGE substrate was baked at 80ºC for 1 hour and ready for usage. A digital multimeter was used to measure the resistivity of electrodes.

2.3 Fabrication of gold electrodes by sputtered photolithography

Gold electrodes on glass substrate were fabricated by sputtering and standard photolithography. In brief, 150/2000Å Ti/Au were prepared onto a 4 inch glass substrate by physical vapor deposition (PVD, Sputtered Films Inc, SF II). Then, photolithography patterning and wet-etching were used to form Ti/Au electrodes. After a 5μm thick Parylene C deposition, photolithography and reactive ion etching(RIE) were applied sequentially to expose the electrode pads. Finally, the wafer was diced into chips after removing the remained photoresists.

2.4 DNA immobilization and hybridization assay

Before use, gold electrodes were cleaned by electrochemical oxidation and reduction. One hundred cycles of cyclic voltammetry in 100 μL of 0.1 M $H_2SO_4$ solution were applying on the electrode using the following parameters: potential range, -0.5 to 1.0V; scan rate, 0.3V/s. After being rinsed with Milli-Q water, the electrodes were dried with nitrogen. DNA modified surfaces were prepared by two-step immobilization procedure. In brief, the clean gold substrate was immersed in 1 μM solution of thiolated DNA probe 1 in I-Buffer for several hours at room temperature. Rinsed with Milli-Q water, the electrodes were immersed in 1 mM of 6-Mercapto-1-hexanol (MCH, Aldrich, China) solution for at least 2 hours. Excess MCH was removed by the rinsing with Milli-Q water and the electrodes were dried under nitrogen flow for usage.

For the hybridization assay, the electrode immobilized with probe1 was immersed into DNA H-buffer containing a desired concentration of target DNA and probe2 for an hour at room temperature. After hybridization, the electrode was extensively rinsed with washing buffer (10 mM Tris-HCl, pH 7.0) and dried under a stream of nitrogen prior to electrochemical characterization.

2.5 Electrochemical measurement

Chronocoulometry (CC) and cyclic voltammetry (CV) was performed with an electrochemical station (CHI830D, Chenhua, Shanghai, China). The following parameters were employed. For CC measurement, two-step potential was employed from -500 mV to 0 mV with 500 ms duration. CV was also performed from -0.5 V to 1 V at 0.3 V/s. The buffer for both CC and CV was 10 mM tris (pH 7.4) containing 50 μM of RuHex at room temperature. A home-made adapter was employed to connect the Au surface and potentiostate for electrochemical detection.

2.6 Characterization of gold electrodes

Scanning electron microscopy (SEM) images were performed on a KYKY-EM3200 apparatus. Atomic force microscope (AFM) images were achieved on a Bruker MULTIMODE 8 instrument. X-ray diffraction (XRD) patterns were obtained using a RIGAKU Ultimate IV diffractometer with Cu Kα radiation. The X-ray photoelectron spectra (XPS) were recorded using a Thermo ESCALAB 250 instrument with Al Kα X-ray radiation for the X-ray source.

3. Results and discussion

3.1 Mechanism of the photochemical surface modification

Electroless plating based on UV induced surface modification has been previously developed [29-31] and it was earlier reported that carboxylic acid was produced from ester groups of poly(methyl methacrylate) (PMMA) and polystyrene by UV radiation [29, 32]. However, it has been never discussed on the mechanism of this procedure. It is accepted that phenyl rings on polystyrene material remain high chemical activity. Low pressure mercury lamp more or less produces 184 nm of emission which release ozone, a high active oxide. ATR-FTIR spectra was subsequently employed to confirm the formation of carboxyl groups generated by UV exposure.

It was shown in figure 1b, strong absorption band centered at ~1721 $cm^{-1}$ and a broaden absorption peak around 3500 $cm^{-1}$ appealed by photo-chemical treatment. After introduction of ethylenediamine, IR absorption reduced caused by amination reaction. Therefore, we propose a probable mechanism (figure 1a) on carboxylic acid modified surface of polystyrene substrate. With high-energy UV irradiation, scission of the phenyl ring dominated instead of that of alkyl side chain C-C and in the presence of ozone, two terminals of cracked phenyl ring were further oxidized into carboxylic acid groups for following electroless plating. Compared with the acrylic ester in PMMA, polystyrene would produce more carboxylic acid groups, indicating more active reaction centers. That is probably a main reason why PMMA substrate could not be modified with gold electrode in same conditions (data no shown) in our experiment.

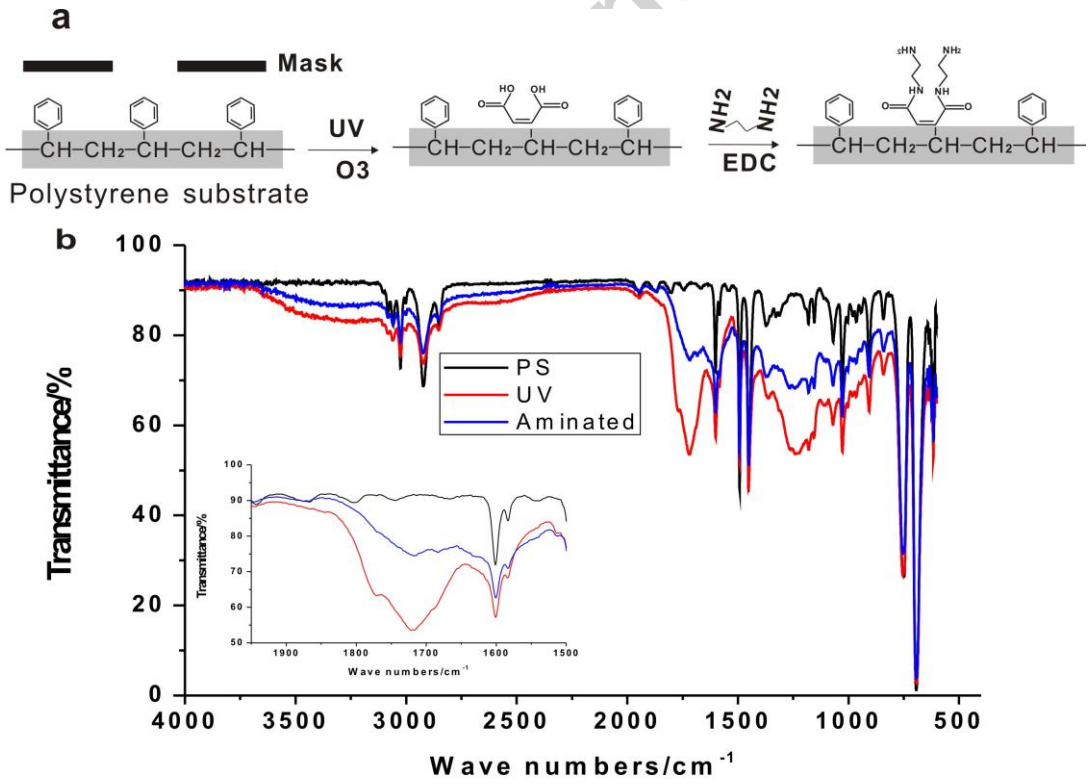

Figure 1. The procedure of UV surface activation and amination (a); ATR-FTIR spectra of PS (polystyrene substrate) UV (PS treated with UV exposure) and Aminated (treated with ethylenediamine after UV exposure)(b).

3.2 Optimization of conditions in fabrication of plastic gold electrode

In order to achieve high sensitivity and reliability, it is essential to optimize the conditions including UV exposure time, $HAuCl_4$ treatment and electroless plating （figure2） which probably affect the surface topography of gold electrode greatly, afterwards the performance of biosensor. Electrical resistance was measured to evaluate different conditions based on the strategy that more gold atoms produced on the surface of PS substrate leads to reduced resistance (increased conductivity) of the gold electrodes. Conditions were optimized to 5-6 hours for UV exposure, 2.5 hours for $HAuCl_4$ treatment and 15 hours for chemical plating and average resistance of 5-10 ohm were obtained. Indeed, gold electrode formed on PS substrate far earlier (~1 hour) since immerged into chemical plating solution. Longer exposure time to UV light was helpful to activate more carboxylic acid groups, as well as $HAuCl_4$ and chemical plating time, leading to lower resistance. However, it was found that longer time of UV exposure or plating time would easily result in the expansion of gold electrodes (figure S1, ESI†) caused by morphological changes during UV exposure and non-specific adsorption of gold atoms in $HAuCl_4$ and chemical plating steps.

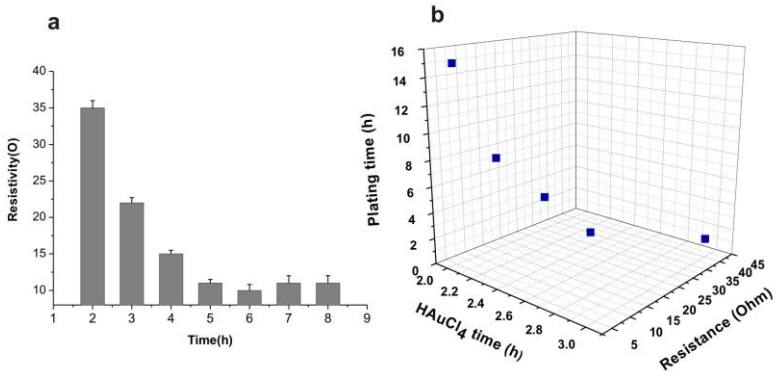


Figure 2. Optimization of the conditions for fabrication of PGE, including UV exposure time(a), $HAuCl_4$ treatment and electroless plating time (b). During (a), the other conditions were fixed (treated with EDC for 3 hours, $HAuCl_4$ for 2.5 hours and plating for 15 hours). While during (b), UV exposure time was fixed at 5 hours.

3.3 Determine by electrochemical methods the electrochemical surfaces for sputering and proposed electrodes

Freshly fabricated PGEs under optimized condition were investigated on electrochemical characteristics and immobilization capacity of thiolated DNA probe and the performance was compared with gold electrode fabricated on glass substrate by traditional photolithography. As demonstrated in figure3a, PGE showed increased CV redox peak indicating larger area of gold electrode, while other conditions remained same. However, actually the apparent area of both electrode were the same (~0.03 $cm^2$). The specific surface area of electrode could be estimated with Randkes-Sevcik equation ($ip = 0.4463nFAC(nFvD/RT)^{1/2}$) [33]. Since other parameters except A (area) are constant and same for both PGE and sputtered GE, we could conclude PGE possiblly had a higher specific surface than sputtered-GE (~ 10 fold). That means more electrical active centers exist on electrode surface and would lead to higher capacity of thiolated DNA

probe immobilization. This assumption came true when we measured and calculated DNA probe density immobilized on electrode surface through chronocoulometry (figure3b, detailed methodology was provided in ESI†).

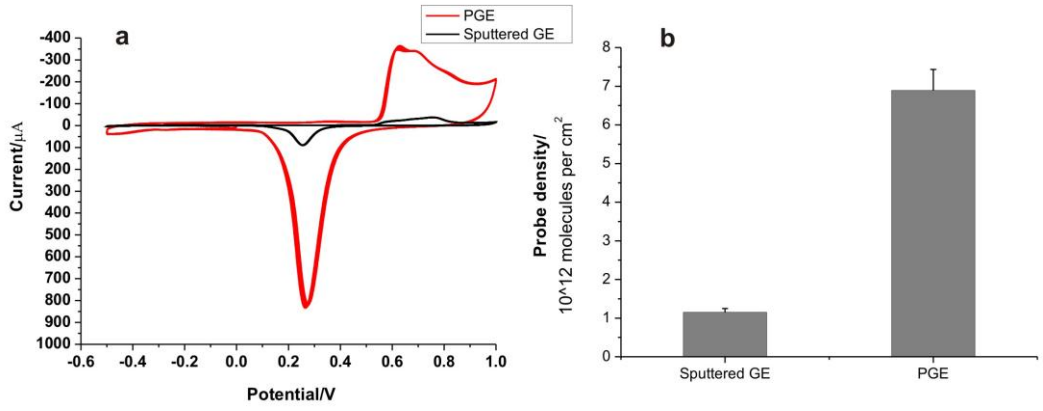


Figure 3. Cyclic voltammetry (CV) curves of both gold electrodes in 0.1 M of $H_2SO_4$ solution (a); Density of DNA immobilization on surface of electrodes were measured and calculated by chronocoulometry (b, detailed information was presented in ESI).

Subsequently we investigated the morphology of both gold electrodes through several commonly used tools for morphology characterization, including scanning electron microscope (SEM) and atomic force microscope (AFM). Combined with SEM and AFM images (figure4), we observed that gold nanoparticles on the electrode surface were large size (~100 nm diameter) for sputterd electrode on glass substrate, densely arranged and relatively smooth on surface (~0.8 nm). While gold nanoparticles were much smaller with irregular shape which resulted in larger interspace and rough surface (~ 28.3 nm). This morphological information could to some extend give an explanation to higher capacity of DNA immobilization on PGEs. Moreover, X-ray photoelectron spectroscopy (XPS) and X-Ray Diffraction (XRD) were also employed for elemental and crystal analysis ( figure S2 and S3, ESI†). The Au4f peaks in the XPS spectra of

the gold electrode fabricated by different methods were similar, both indicating metallic gold( $Au^0$) [34]. XRD patterns of the gold electrode fabricated by electroless plating exhibited well-resolved characteristic peaks associated with the face-centered cubic metallic gold. While, the XRD patterns of sputtered gold electrode showed a typical characteristic of single crystal nanoplates, indicating highly consistent orientation, which results in the smooth and compact structure. It is consistent with the results of SEM.

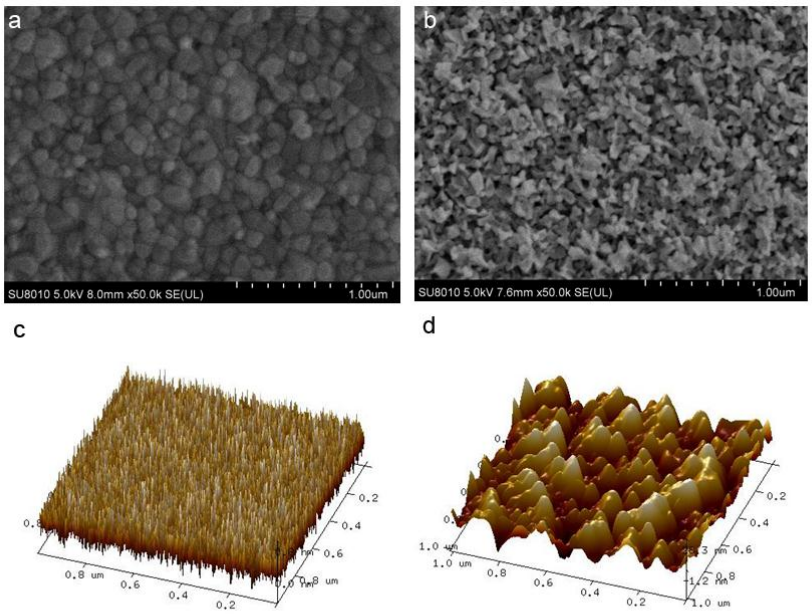


Figure 4. Morphology characterization of PGE and gold electrode fabricated by photolithography. SEM images of gold electrode fabricated by photolithography (a) and PGE (b); AFM images of the surface of gold electrode fabricated by photolithography (c) and PGE (d).

3.4 Detection and manipulation of DNA hybridization on PGE

In order to investigate the performance of the integrated PGEs, we detected DNA molecules through hybridization, which is the most basic and direct way for various detection methods for DNA and RNA [35-37]. Rapid and high efficient hybridization is essential for DNA detection and would be timesaving and helpful for improving sensitivity. Based on the improved sensitivity obtained by PGEs, the procedure and efficiency of DNA hybridization on electrode was manipulated and studied in our work by measurement of chronocoulometry every several minutes. Rapid increase of electrochemical signal was observed when ssDNA modified PGEs were immersed into 500 nM of complementary DNA molecules (figure 5a and b), while neither hybridization buffer nor independent sequence had any contributions to the electrochemical signal. Furthermore, it was observed that hybridization based on molecular diffusion reached equilibrium within 15 minutes at this concentration. The process of hybridization delayed at lower concentration and longer time was required to reach the plateau (Figure5c). Based on the plateau signal of stepwise diluted target DNAs after 30-minute hybridization, a standard curve was obtained linearly ranging from 1 nM to 500 nM. The detection limit was $7.2\times10^{-11}$M using $3\sigma/S$.

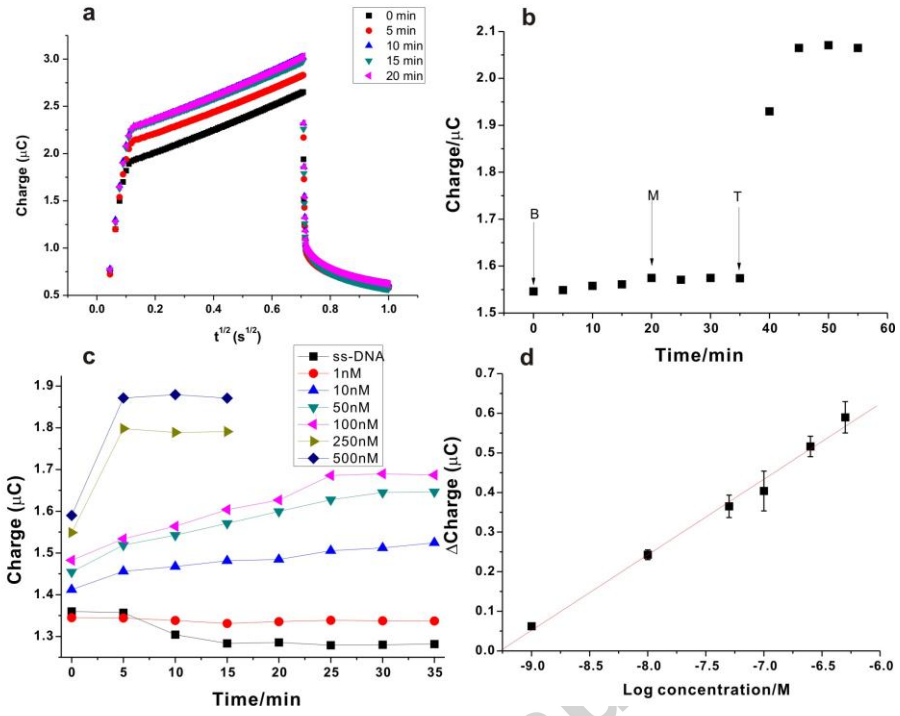


Figure 5 DNA hybridization assay by chronocoulometry. (a) Chronocoulometry of hybridization process at 500 nM DNA concentration; (b) Electrochemical signal plot of hybridization solution containing buffer only (B), 500 nM of random DNA sequence (M) and 500 nM of complementary DNA (T); (c) Hybridization plot of complementary DNA at different concentration; (d) Standard curve of complementary DNA ranging from 1 nM to 500 nM.



## 4. Conclusions

Although large number of articles relating to DNA biosensor using gold electrodes was reported in the literature [21, 23-25], our work demonstrated PGEs have superior electrochemical properties and are promising for DNA biosensing. Compared with screening-printed-electrodes (SPEs), the most accepted low-cost electrodes, the advantages of PGEs focus on that minimum instrumentation was required during the whole procedure which makes it feasible to fabricate

low-cost biosensors in ordinary laboratory. Moreover, the integrated PGE was found having higher capacity for DNA immobilization than those electrodes fabricated by standard sputtering based photolithography, which is essential and greatly helpful for the performance of biosensors. We believe that this method provides a practical and alternative technique for fabrication of gold electrode biosensor and it has great potential for wide-spread application, such as point of care testing (POCT), by further industrial standardization and optimization.

Acknowledgment

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Supporting information available

Supplementary data associated with this article can be found in the online version at

Reference

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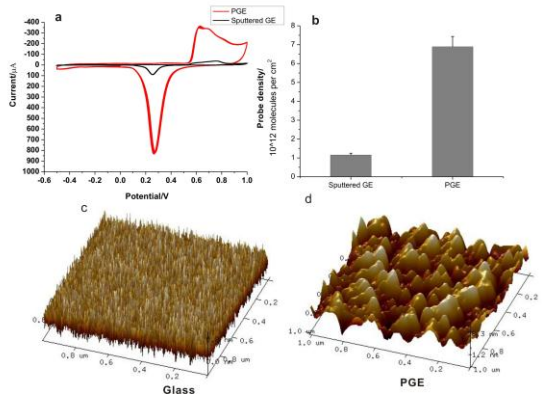
Graphical abstract




Highlights

1. PGE was found having extremely high capacity of DNA immobilization compared with gold electrodes fabricated by standard sputtering based photolithography

2. Unique surface feature containing nano-scale structure was observed on PGEs through morphology techniques.

3. A probable mechanism of carboxylic acid produced on polystyrene substrate after exposure to UV irradiation was proposed and discussed for the first time.