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Gold Nanoparticles/Polymer Nanocomposite for Highly Sensitive Drug-DNA Interaction

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The interaction of anticancer drug, mitomycin C (MC) and DNA immobilized on gold nanoparticles/poly(vinylferrocenium) (AuNPs/PVF^+) coated electrode was presented. This is the first attempt to prepare biocompatible nanoparticles/redox polymer composite in a one-step and easy electropolymerization procedure and then to use the coated electrode for MC-DNA interaction. The prepared electrode exhibits high sensitivity for the investigation of drug-DNA interaction.

Interaction of anticancer drugs with DNA is an important topic for understanding the nature of binding mechanism of these drugs to DNA and for studies in drug discovery/design and pharmaceutical development processes for remedy.[1] Different types of binding mechanisms exist: intercalation, groove binding, covalent binding/cross linking, DNA cleaving, and nucleoside-analog incorporation. These binding interactions result in structural change of both DNA and drug molecules to accommodate complex formation.[2] Therefore, investigation of interactions of anticancer agents with DNA has been employed by different techniques including DNA-footprinting, nuclear magnetic resonance (NMR), mass spectrometry (MS), spectrophotometric methods, vibrational spectroscopy (Raman and infrared), capillary electrophoresis, surface plasmon resonance (SPR) and electrochemistry. Among these techniques, electrochemical ones received considerable attention.[3]

Mitomycin C (MC) is a widely used chemotherapeutic agent which is generally effective for upper gastro-intestinal, anal cancers, and breast cancers. Previous researches indicate that MC had ability to bind to DNA covalently both mono and bifunctionality.[4] It binds to DNA by shielding the oxidizable groups of electroactive DNA bases such as guanine and adenine.[1b,4a,e] Electrochemical investigation of the interaction between mitomycin C and DNA was examined in previous works in order to develop new drug-related systems.[5]

The use of electroactive polymers as the transduction element has attracted great attention in the past.[6] However, to the best of our knowledge there is no data in the literature about the investigation of the interaction between MC and DNA immobilized onto nanoparticles/polymer nanocomposites. Polymer modification of the substrates offer great potential to help the identification of target compounds and the ability to control the properties of the obtained surfaces.[7] Furthermore, the use of nanomaterials/polymer nanocomposites in many life sciences has found great importance since nanomaterials improve the performance of the electroactive polymers by promoting electron transfer reactions and increasing the electroactive surface area of the sensing platform which has been already improved with the modification of electroactive polymers.[8] The combination of nanotechnology and polymer technology, thus offers wide applications for biological, medical, pharmaceutical and environmental purposes due to their good chemical, mechanical, electrical, optical and thermal properties. There have been promising attempts on nanoparticles/polymer composites based on electrochemical (bio)sensing, energy storage, forensic and nanoelectronics.[9] Particularly, with their perfect flexibility and good electrical, optical, and mechanical properties, they provide many advantageous compared to the conventional systems.

In this paper, we aim to develop a new sensing platform for sensitive DNA-drug interaction. This platform based on a nanoparticles/polymer host nanocomposite for DNA has been prepared via modification of gold nanoparticles (AuNPs) and poly(vinylferrocenium) (PVF^+) together in a single step electropolymerization. PVF^+ is a well-known redox polymer[6d,e] and it has been used as the indicator of different interactions in various studies due to its excellent electrochemistry.[9d,11] Since this is the first attempt to prepare biocompatible nanoparticles/redox polymer composite in a one-step and easy electropolymerization procedure, we aim to show the improved electrochemical characteristics of the polymer in the presence of AuNPs in the first part of the study. Thus, the performance of the electrode was compared with the PVF^+ coated electrode. Accordingly, the interaction between MC and DNA on the AuNPs/PVF^+ coated electrode was finally investigated. The electrochemical behavior of DNA immobilized AuNPs/PVF^+ coated electrode was examined in the absence/presence of MC with differential pulse voltammetry (DPV). The changes in the magnitude of electroactive DNA bases, guanine (G)and adenine (A) were used as the indicator of drug-DNA interaction. MC binds to DNA by shielding the oxidizable groups of electroactive DNA bases, as reported in previous works.[1b,4a] Thus, G and A peak currents diminished after the interaction of MC with DNA immobilized on the AuNPs/PVF^+ electrode in parallel to the literature. This sensing platform provides a stable, fast, cost-effective and sensitive DNA immobilization which improves the efficiency of drug-DNA interaction.

AuNPs are one of the widely used nanoparticles in bioelectrochemistry because of their good biocompatibility, high electrical conductivity and high surface-to-volume ratio.[10] In the work, the surface of Pt electrode was modified with AuNPs/PVF^+ composite. PVF^+ can be coated onto the electrode surfaces by controlled constant potential electrolysis using its neutral form, poly(vinylferrocene) (PVF).[11] This polymer improves homogeneous

distribution, ease of modification and stability of AuNPs on the electrode surface during simple-one step electrooxidation process. The resulting composite layer has been shown useful for DNA immobilization and for the investigation of drug-DNA interaction.

In the first part of the study, the AuNPs/PVF^+ coated electrode was characterized by electrochemical methods including cyclic voltammetry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). Figure 1A illustrates the cyclic voltammograms (CVs) of AuNPs/PVF^+ coated electrode (a), PVF^+ coated electrode (b), and uncoated Pt electrode (c) in 50 mM phosphate buffer solution (pH 7.4). Uncoated Pt electrode doesn't show any electroactivity in the working potential range as expected (Figure 1A-c). However, cyclic voltammograms of the nanocomposite coated and polymer coated electrodes exhibit anodic/cathodic peaks due to ferrocenium/ferrocene centers of the polymer that are covalently bound to insoluble polymer skeleton. In the presence of polymer coated electrode, the anodic peak at about +0.41 V vs. Ag/AgCl belongs to the oxidation of ferrocene to ferrocenium ion and the cathodic peak at +0.21 V vs.Ag/AgCl exhibits the reduction of ferrocenium ion to ferrocene (Fig. 1A-b). With the addition of AuNPs, oxidation/reduction peak currents increased significantly indicating the fast electron transfer due to the nanoparticles (Fig. 1A-a).[6f] The anodic peak was at about +0.39 V vs. Ag/AgCl and the cathodic peak was at +0.23 V vs. Ag/AgCl. The electroactive surface coverages (Γ_{EA}) of the modified electrodes were measured using cyclic voltammetry at different scan rates and they were given in Table 1 (n=3).[12] The results indicated the increased electroactive surface area in the presence of AuNPs. In addition, the electrode surfaces were calculated at a scan rate of 100 mV s^{-1} and found as 0.1307 cm^2 and 0.0772 cm^2 for AuNPs/PVF^+/Pt and PVF^+/Pt, respectively.[12] Differential pulse voltammograms (DPVs) of AuNPs/PVF^+ coated electrode (a), PVF^+ coated electrode (b) in 50 mM phosphate buffer solution are shown as Figure 1B. The oxidation peak of the polymer was at +0.33 V in the presence of AuNPs, while it was at +0.34 V in the absence of AuNPs. DPV results supported our CV results by giving higher oxidation peak current with the AuNPs/PVF^+ coated electrode.

Electrochemical impedance spectroscopy was used to compare the electrochemical responses of AuNPs/PVF^+/Pt and PVF^+/Pt (Figure 1C-a,b, respectively) in 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4}$ redox probe. In order to differentiate the modifications on the electrodes, R_{ct} values were compared. The average R_{ct} values are 680.0 ± 2.4 Ω and 1050.0 ± 3.2 Ω for AuNPs/PVF^+/Pt and PVF^+/Pt electrodes, respectively (n=3). AuNPs/PVF^+ coated electrode had smaller R_{ct} value than the one obtained with PVF^+/Pt, since the electron transfer was facilitated in the presence of nanoparticles. Microscopic characterization of the AuNPs/PVF^+/Pt was performed with atomic force microscopy (AFM) (Fig. S1).

Table 1. Electroactive surface coverages for the coated surfaces at different scan rates.

	$\Gamma_{EA} * 10^{-8}$ (mol cm^{-2})	
Scan rate (mV s^{-1})	PVF^+/Pt	AuNPs/PVF^+/Pt
50	1.9±0.4	4.2 ±0.2
100	3.5 ±0.2	6.3±0.4
150	5.5 ±0.4	9.5 ±0.4

Since PVF^+ is a well-known redox indicator, due to its ferrocene/ferocenium centers, we also aimed to observe the changes in the oxidation peak currents of polymer as well as the changes in the oxidation peak currents of guanine and adenine. For this purpose, double-stranded DNA (dsDNA) immobilized AuNPs/PVF^+ coated electrode (dsDNA/AuNPs/PVF^+/Pt) and dsDNA immobilized PVF^+ coated electrode (dsDNA/PVF^+/Pt) were prepared via immobilization of dsDNA onto the positively charged modified electrodes. Figure 2A-a,b present the cyclic voltammetric behaviors of AuNPs/PVF^+/Pt and dsDNA/AuNPs/PVF^+/Pt in 50 mM phosphate buffer solution, respectively. Cyclic voltammetric behaviors of PVF^+/Pt and dsDNA/ PVF^+/Pt are shown in Figure 2B-a,b, respectively. CVs of dsDNA/AuNPs/PVF^+/Pt and dsDNA/PVF^+/Pt exhibited dramatic peak current diminutions, when compared to CVs of AuNPs/PVF^+/Pt and PVF^+/Pt since DNA exhibits less conductive character and decrease the electroactivity of the conductive nanocomposite/polymer by blocking the electroactive sites. Diminutions in the oxidation and reduction peak currents of the polymer were more significant with dsDNA/AuNPs/PVF^+/Pt (80.7% for the oxidation peak current) than dsDNA/PVF^+/Pt (66.3% for the oxidation peak current) since nanocomposite provided a larger surface area for dsDNA immobilization.

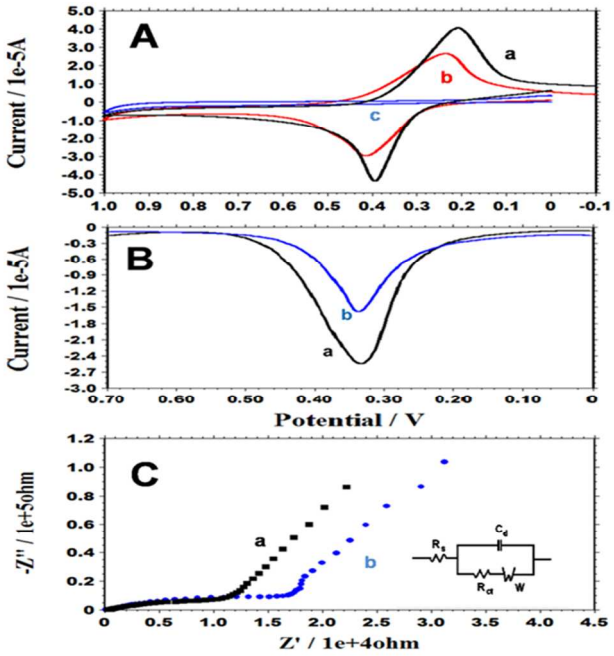

Figure 1. (A) CVs of AuNPs/PVF^+/Pt (a), PVF^+/Pt (b), and Pt (c) in 50 mM phosphate buffer (pH 7.4) containing 0.1 M $NaClO_4$, (B) DPVs of AuNPs/PVF^+/Pt (a), PVF^+/Pt (b) in 50 mM phosphate buffer (pH 7.4) containing 0.1 M $NaClO_4$,(C) Nyquist diagrams of AuNPs/PVF^+/Pt (a), and PVF^+/Pt (b) in 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4}$ redox probe (inset Figure 1C shows the equivalent circuit model used to fit the impedance data, Rs: solution resistance, Cd: space charge resistance at the electrode/solution interface, Rct: charge transfer resistance at the electrode/solution interface, and W: Warburg impedance due to the mass transfer to the electrode surface).

DPVs of dsDNA/AuNPs/PVF^+/Pt and dsDNA/PVF^+/Pt were recorded in order to examine the magnitudes of guanine and adenine oxidation signals (Fig. 3A-a,b, respectively). Enhanced oxidation peak currents (2-fold for G and 4-fold for A) were observed with dsDNA/AuNPs/PVF^+/Pt compared to dsDNA/PVF^+/Pt after 30 min 250 ppm dsDNA immobilization onto the electrodes. Thus, further studies were performed with dsDNA/AuNPs/PVF^+/Pt in order to provide better sensitivity. In addition, the oxidation peak of the

polymer was monitored in the same voltametric scan with guanine and adenine oxidation peaks and enhanced polymer oxidation peak current (2-fold) was obtained in the presence of AuNPs with a lower oxidation peak potential.

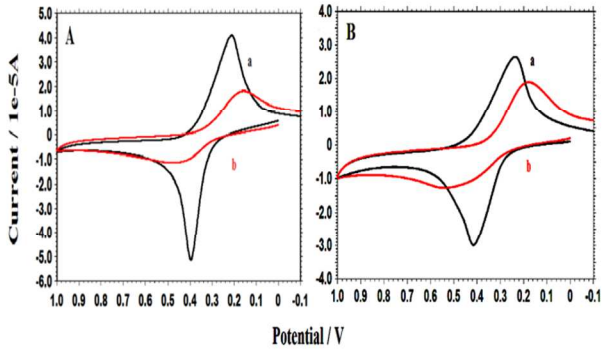

Figure 2. CVs of (A) AuNPs/PVF$^+$/Pt (a), dsDNA/AuNPs/PVF$^+$/Pt (b), (B) PVF$^+$/Pt (a), dsDNA/PVF$^+$/Pt (b) in 50 mM phosphate buffer (pH 7.4) containing 0.1 M $NaClO_4$.

dsDNA/AuNPs/PVF$^+$/Pt was then interacted with 100 ppm MC immersing the electrode into the MC solution for 15 min and the interaction of MC with dsDNA onto AuNPs/PVF$^+$/Pt was examined. Fig. 3B-a and b represent the DPVs of dsDNA/AuNPs/PVF$^+$/Pt and dsDNA/AuNPs/PVF$^+$/Pt after MC interaction in 50 mM acetate buffer solution (pH 4.8). According to the figure, when MC was interacted with dsDNA, guanine and adenine oxidation signals diminished dramatically (%38.8 and %78.2, respectively). This behavior was similar to the reported studies presenting the shielding effect of MC on oxidizible groups of guanine and adenine.[1b,4a,e,6e]

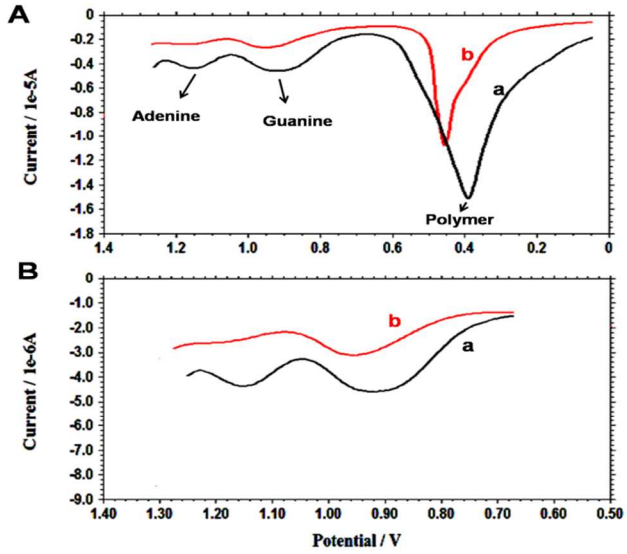

Figure 3. DPVs of (A) AuNPs/PVF$^+$/Pt (a), PVF$^+$/Pt(b), (B) AuNPs/PVF$^+$/Pt (a), AuNPs/PVF$^+$/Pt after 15 min of MC interaction (b) in 50 mM acetate buffer (pH 4.8).

The effects of experimental parameters, such as MC concentration and MC interaction time with dsDNA were examined to find out optimum analytical conditions. In order to investigate the effect of MC concentration on the response of this biosensing system, dsDNA/AuNPs/PVF$^+$ modified Pt electrodes were interacted with MC, which had different concentrations. Fig. 4A presents the effect of different concentrations of MC on the response of the nanocomposite modified electrode based on the guanine oxidation signal (R^2=0.9945). The changes in the oxidation signal of adenine were also presented in Fig. 4B (R^2=0.9923). The results show that there are gradual diminutions in the oxidation signals of guanine and adenine. The responses remained almost constant after a concentration level of 125 ppm of MC. The modified electrode showed a very good reproducibility. The relative standard deviation was calculated as 2.25% for 25 ppm MC for adenine oxidation signal (n=3). The diminutions in the oxidation peak current of adenine after the interaction of dsDNA/AuNPs/PVF$^+$/Pt with 25, 50, 75, 100 and 125 ppm MC were 47.9%, 59.1%, 71.1%, 78.2%, and 87.8%, respectively based on the oxidation peak current obtained with dsDNA/AuNPs/PVF$^+$/Pt before the interaction.

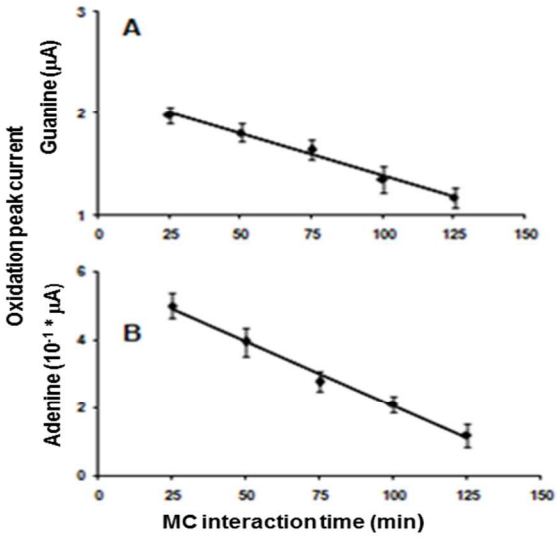

Figure 4. The changes in the oxidation peak currents of (A) guanine, (B) adenine after interaction of dsDNA/AuNPs/PVF$^+$/Pt with different concentrations of MC (n=3).

The effect of MC interaction time with dsDNA on the coated electrode was examined. Fig. 5 shows the response of the dsDNA/AuNPs/PVF$^+$/Pt based on the changes in the oxidation signal of guanine after various MC interaction times (R^2=0.9890). The changes in the oxidation signal of adenine were presented as Fig. ~~S1~~S2. The oxidation signals of guanine and adenine remained almost constant after an interaction time of 25 min. The relative standard deviation was calculated as 2.75% for 5 min of MC interaction for guanine oxidation signal (n=3).The interaction between MC-dsDNA was even detectable in the short interaction time.

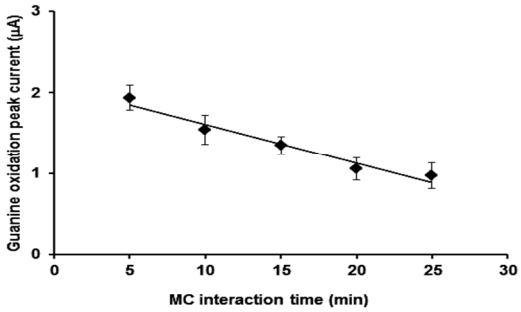

Figure 5. The changes in the oxidation peak currents of guanine after interaction of dsDNA/AuNPs/PVF$^+$/Pt with 100 ppm MC at different interaction times (n=3).

The interaction of anticancer drugs with DNA is an important topic for understanding the nature of many cancer types and for the investigation of developing new drug systems in chemotherapy. As a conclusion, the detection platform that was developed in this study provided rapid, cost-effective, reliable and sensitive results for MC-dsDNA interaction having comparability to the reported ones.[4a,4e,6e] The nanocomposite coated electrode can be useful for future applications including detection of specific DNA sequences, investigation of different anticancer drugs-DNA interactions and development of controlled drug release systems.

Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental data and Figure S1. See DOI: 10.1039/b000000x/

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Table of Contents

We demonstrate a gold nanaparticles/polyvinylferrocenium (AuNPs/PVF^{+}) coated platinum (Pt) electrode for highly sensitive DNA-anticancer drug interaction.