N-Doped Graphene Oxide Decorated with PtCo Nanoparticles for Immobilization of Double-Stranded Deoxyribonucleic Acid and Investigation of Clenbuterol-Induced DNA Damage

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ABSTRACT: We demonstrate here a facile hydrothermal-assisted formation of PtCo alloy nanoparticles (NPs) and their simultaneous anchoring on the graphitic surface of N-doped graphene oxide (NGO). Doping induced nanopores in the carbon surface to facilitate the uniform and homogeneous anchoring of alloy nanoparticles. It was revealed that the formation of PtCo NPs on an NGO interface plodded excellent tendency toward double-stranded deoxyribonucleic acid (dsDNA). The dsDNA immobilization was enabled by the presence of several oxidation states of Pt and Co. The same property was further used to monitor the direct detection of dsDNA damage induced by clenbuterol via screen-printed carbon electrodes. Cyclic voltammetric and electrochemical impedance spectroscopic characterization traced well the dsDNA attachment on the modified electrode surface. Differential pulsed voltammetry was further used as a tool to monitor the characteristic guanine peak before and after incubating with clenbuterol used as a damage probe for the dsDNA. The findings can further be appurtenant in exploring dsDNA immobilization protocols and developing analytical methods for determination of various dsDNA damaging agents.

INTRODUCTION

Recently, numerous efforts have been devoted to the quest of finding newer therapeutic agents and probes to understand the DNA sequencing and specific genetic information. Efficient immobilization has been the matter of interest for several researchers. Direct adsorption, covalent binding, and probe conjugation are the widely used methodologies in these lines. Several organic and inorganic molecular species and hybrid materials have been used as an immobilizing support for this purpose. Among these, due to their water solubility, tunable molecular architecture, inertness, and stability under physiological conditions, the transition metals have been proven to be the ideal candidates for site-specific DNA binding. In this direction, cobalt metal (Co) being widely distributed in several biological systems have attracted much attention to study their interactions with DNA. Several covalent and noncovalent interactions of Co and double-stranded deoxyribonucleic acid (dsDNA) bases have been reported, indicating the great tendency of Co to interact with dsDNA. Another extensively studied metal for DNA interaction and binding is platinum (Pt). Pt has demonstrated an ample tendency, either in the form of mononuclear or bimodal complexes both via covalent and intercalation interactions.

Moreover, DNA–metal interactions due to the phosphatic oxygen and nitrogen atoms of DNA bases have widely been explored for sensitive and specific detection of several effectors. These belong to both environmental and biological backgrounds, which may damage the dsDNA in terms of distorting the nucleotide sequence or their hydrogen bonding. To study the quantitative damage of immobilized DNA under the influence of external co-factors, efficient dsDNA immobilization is necessary. Likewise, to limit the catalytic loading and increase the surface area of a substrate, the deposition of monolayers, submonolayers, and nanoparticles of bimetallic lineage over a variety of support materials has been widely explored. Engineering the electronegative environment of...
transition metals in alloy formation could tune up their physical and chemical properties according to the substrate requirements. However, very less efforts have been exerted to utilize such transition metal alloy nanoparticles as a DNA immobilization support. Besides the great affinity of dsDNA toward heavy metals, they can mitigate its structure as well, causing carcinogenesis. The elaborated quantification of dsDNA–metal complexation consequently could be a matter of great concerns to tailor as a useful analytical tool. Few efforts have been exerted for spectroscopic interactions of DNA with metallic and bimetallic complexes. Likewise, tailored designing of binuclear and polynuclear complexes with larger surface area, higher charge distribution, and desirable electronic structures renders efficient probes for dsDNA. Therefore, electrochemical-based studies of DNA immobilized on metal surfaces can potentially integrate several benefits including precise detection and higher sensitivity and specificity.

In these lines, to further tune up the functionality of metal nanoparticles and reduce the dosage of precious metals, higher conductivity and surface area are highly desirable. Owing to its large surface area and excellent thermal, optical, electrical, and mechanical properties, graphene has received enormous attention since its discovery. It has been used as a support material for many applications with the benefits of uniform dispersion and enhanced intrinsic properties of graphene. Moreover, in the process of metal anchoring on the graphitic surface, various functionalities and lattice defects of graphene oxide can help in uniform immobilization of metal nanoparticles.

The aim of this study is to synthesize more effective and comprehensive metal-based substrates for dsDNA immobilization. The bimetallic nanoparticles anchored on the graphene basal plan could develop newer bioaffinity protocols to electrochemically monitor the double-stranded DNA immobilization. Herein, a facile methodology for uniformly anchored PtCo nanoparticles onto N-doped graphene oxide has been reported. The doped nitrogen atoms play a decisive role in the uniform anchoring of Co atoms followed by the Pt attachment and overall morphology of the nanostructure.

Figure 1. Schematic representation of a synthetic route for NGO-PtCo nanohybrid followed by immobilization of dsDNA and clenbuterol-induced DNA damage studies.

Figure 2. (A) XRD pattern and (B) Raman spectra of NGO and NGO-PtCo hybrid materials, indicating the successful growth of PtCo nanoparticles on the NGO surface.
RESULTS AND DISCUSSION

The in situ synthesis of PtCo bimetallic nanoparticles onto the N-doped graphene oxide is illustrated in Figure 1. N doping resulted in the enhanced conjugation effect of basal $\pi$ electrons and lone pair electrons of nitrogen. Such an electron-rich environment of N-doped graphene oxide provides the ease of Co and Pt coordination and eventually provides more anchoring sites to the Pt and Co atoms on the basal plane.\(^\text{17}\)

X-ray diffraction (XRD), Raman spectroscopy, and Fourier transform infrared (FTIR) were used to investigate the molecular interactions and atomic-scale changes on the basal plane, as shown in Figure 2 and Figure S1. In the XRD patterns of NGO-PtCo and NGO, the appearance of a band at $26^\circ$ represented the (002) diffraction of graphene, whereas four peaks corresponding to (111), (200), (220), and (311) planes of Pt were also evidenced along with a Co representative band at $46^\circ$. Similarly, changes in the ID/IG ratio of the Raman spectra of both NGO (0.97) and NGO-PtCo (1.04) indicated the structural influence of PtCo nanoparticles onto the NGO. Consistent with the literature, anchoring of PtCo NPs

Figure 3. XPS core-level spectra C1s of (A) NGO and (B) NGO-PtCo. Core-level spectra of (C) Pt4f and (D) Co2p$_{3/2}$ of NGO-PtCo. Both fitted data and deconvoluted fitting components are presented here.

Figure 4. (a) SEM and (b) TEM, and (c, d) HRTEM of NGO-PtCo and EDS (energy-dispersive spectrometry) elemental mapping analysis for N, Pt, and Co elements of NGO-PtCo.
increased the ID and corroborated an increase in the IG band of graphene. In the FTIR spectra, the presence of the peaks at 1226, 1403, 1727, and 3405 cm\(^{-1}\) can be attributed to stretching vibrations of O–H, C–O, and O–H (deformation) and stretching vibration of C–OH, respectively. Moreover, the appearance of a skeletal vibrational peak of graphene at 1560 cm\(^{-1}\) under the influence of PtCo confirmed the NGO-PtCo synthesis, as shown in Figure S1.\(^{18}\)

To determine the NGO-PtCo surface states and binding energies, X-ray photoelectron spectroscopy (XPS) was performed as shown in Figure 2 and Figure S2. Four distinct peaks at 284.50, 285.41, 286.54, and 289.21 eV were deconvoluted after carefully treating the raw data, which could be attributed to the C=\(\equiv\)C, C=\(\equiv\)N, C–OH, and O=\(\equiv\)C=\(\equiv\) bonds of the NGO-PtCo\(^{19}\) (Figure 3B). It is noteworthy that the similar peaks were obtained during the investigation of NGO XPS spectra (Figure 3A). However, the increase in binding energy of Pt4f core levels for the NGO-PtCo composite with respect to that of pure Pt, and the peak intensity and peak-to-peak ratio difference collectively indicate the presence of electronic species around the sp\(^2\) carbons.\(^{20}\) Further XPS investigations of NGO-PtCo graphed doublet peaks for Pt4f, which were further deconvoluted into several pairs of doublets, indicating the presence of Pt in multiple states. The intensive doublet at 74.41 and 72.10 eV represents the zerovalent metallic state, while the less intense set of peaks at 773.06 (Co metal), 783.04 (Co(II)), and 795.36 (Co(III)).\(^{19}\) Furthermore, detailed comparisons of different states of NGO and NGO-PtCo, for example, N1s and O1s, were also performed and shown in Figure S2 of the Supporting Information, confirming the PtCo alloy formation.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) also depicted the heterostructure of uniformly distributed PtCo NPs onto the graphene basal plane. As shown in Figure 4a, the SEM micrograph indicates the abundance of PtCo NPs uniformly adsorbed across the entire basal plane of graphene with a uniform particle diameter of about 10 nm. The elemental mapping of SEM images for N, Pt, and Co also indicates the uniformity of the elemental distribution throughout the graphitic substrate (Figure 4). Likewise, the TEM micrographs also supported these observations. A nearly transparent graphene film with highly dispersed uniform NPs throughout the graphene surface can be evidenced by TEM (Figure 4b) and HRTEM (Figure 4c,d).

Notably, it is evident in TEM and HRTEM micrographs of NGO-PtCo that negligible nanoparticles are scattered out of the graphene surface, which indicates the strong interaction between alloy nanoparticles and the graphene substrate. Likewise, the spacing measurements of lattice fringes with HRTEM rendered a d spacing value of 0.22 nm, a characteristic of face-centered Pt (111). For comparison, SEM and TEM investigation of NGO were also performed, which presented a clear picture of pristine nitrogen-doped graphene films, with the appearance of nanoparticles. These defects formed by N doping eventually facilitated PtCo NP formation and anchoring on the graphitic surface. Similarly, the TEM analysis of PtCo anchored graphene (PtCo-G) prepared under similar conditions showed uniform PtCo NPs with poor dispersity, as shown in Figure S3c. These observations furthermore supported our hypothesis of the influence of N doping onto the morphology and successful anchoring of alloy NPs.\(^{17,21}\)

Furthermore, dsDNA immobilization was monitored via changes in electron/charge transfer ability (CV spectra) and electron resistance (EIS curves) of NGO-PtCo in 1 mM solution of \([\text{Fe(CN)}_6]^{3-/4-}\), as shown in Figure 5. The NGO-PtCo-modified screen-printed electrode demonstrated the ease of electron transfer by showing the increased CV response as compared to the bare electrode. The redox current of ferro/ferricyanide (redox probes) was enormously increased, while the electron resistance value was decreased on modifying electrode with NGO-PtCo (Figure 5a). The DNA immobilization caused a decrease in the redox current and an increase in electron transfer resistance (Figure 5b), confirming the success of dsDNA adsorption onto the NGO-PtCo NPs. As a proof of concept to demonstrate the DNA immobilization, DNA was incubated on the bare screen-printed carbon electrode (SPCE), where there was a small peak at 1.2 V corresponding to the adenine of the adsorbed DNA (Figure S4). To validate the hypothesis of better dsDNA immobilization on NGO-PtCo-modified electrodes and identify the electrochemical signals of damaged DNA, we performed control experiments with dsDNA and CLB on bare electrodes, as shown in Figure S4. The results demonstrated clear evidence of the influence of NGO-PtCo on dsDNA immobilization. Moreover, a peak for damaged DNA (guanine peak at 0.55 V) was clearly differentiable from the oxidation peak (0.67 V) of amino groups of CLB. The quenching in the electron transfer could

Figure 5. Electrochemical responses (A) cyclic voltammetry and (B) impedance spectroscopy of a screen-printed carbon interface modified with NGO-PtCo for DNA damage studies; (a) bare SPCE, (b) NGO-PtCo-modified SPCE, (c) dsDNA-immobilized modified electrode, and (d) CLB-treated dsDNA immobilized modified electrode. All the measurements were performed at 50 mV s\(^{-1}\) in 1 mM \([\text{Fe(CN)}_6]^{3-/4-}\).
be attributed to the bulky DNA molecules hindering the redox probe to be reached at the conducting interface of the modified SPCE. Moreover, the incubation of dsDNA-modified SPCE in CLB caused damage of dsDNA, leaving the space for probe approach for electronic interaction and resulting in an increase in the electron transfer and a decrease in resistance (Figure 5d). On more reason for the increase in the electron transfer could be due to the influence of protein adsorption. As proteins have characteristics of charge transfer, the immobilization cause an increase in the conductivity.

On similar lines, the differential pulsed voltammetry (DPV) was also used to monitor the dsDNA immobilization followed by damage caused by clenbuterol (CLB). An adenine characteristic peak at 1.2 was observed when dsDNA was incubated either on the bare electrode (Figure 6Ad) or the NGO-PtCo-modified electrode (Figure 6Ab). NGO-PtCo, being electroactive in nature, caused a broader peak appearance in the range of 1.1–1.3, which engulfed the characteristic peak of adenine in the case of curves b and c; however, the surface changes were clearly indicated by the change in the peak intensity in that area. On incubation with CLB, the intensity of adenine peak was decreased, and a new peak at 0.55 V emerged, denoted as a characteristic peak of the guanine base of DNA (Figure 6Ac). It is noteworthy that the guanine peak was absent before CLB incubation (Figure 6Ab), which could be attributed to the steric hindrance and bulkiness of the dsDNA proteins. A similar trend was observed in the case of cyclic voltammograms of bare and modified electrodes before and after incubation with clenbuterol (Figure 6B). The current response was increased on modification with NGO-PtCo, indicating the increased electron conductivity (Figure 6Bb). Moreover, the immobilization of dsDNA also corroborated the electron conductivity, which could be attributed to the ease of charge transfer in the case of protein presence. On incubation with CLB, the peak at 0.8 V was incredibly increased, indicating the availability of more proteins to the surface of the electrode (Figure 6Bc). This increase in the peak current could be attributed to the CLB-based dsDNA damage, resulting in more proteins available for adsorption on the electrode surface. CLB causes distortion of H bonding of complementary bases of dsDNA. Several graphene-based nanocomposites, including Au/GO/MoS2 and electroconverted Au NP/graphene films, have so far been used for the detection of a specific sequence of DNA, leading to the DNA damage. Moreover, several reports have demonstrated the use of graphene and metal nanocomposites for DNA hybridization studies. Biomolecule- and molecular beacon-probed graphene nanocomposites are yet another root for the detection of colorimetric-based DNA damage. Recently, multicomponent-based graphene nanocomposites (graphene/ionic liquid/Nafion/hemin/ cytosine Ag ions) have also been used in different types of DNA damage investigations. However, due to unique morphological features and characteristic affinities of Pt and Co toward DNA, the synthesized nanocomposite could provide better DNA adsorption as compared to the already reported adsorbents. The efficient immobilization of biomolecules due to unique morphological properties of uniformly distributed alloy nanoparticles is the advantage of the current ternary nano-hybrids toward DNA immobilization protocols.

**CONCLUSIONS**

We uniformly anchored here the uniformly distributed PtCo bimetallic nanoparticles onto the N-doped graphene oxide substrate and further accessed its affinity toward dsDNA attachment. The affinity of nucleotide bases toward the NGO-PtCo nanocomposite was further examined via electrochemical studies of a screen-printed interface modified with NGO-PtCo. Moreover, nanocomposite-assisted dsDNA immobilized electrodes were investigated for DNA damage studies under the influence of clenbuterol via electrochemical differential pulsed voltammetry. From our observations, it can be concluded that alloy NP-anchored graphene can represent a suitable and sensitive tool for the immobilization of dsDNA, and the same could efficiently be used in the detection of DNA damaging agents.

**EXPERIMENTAL SECTION**

**Synthesis of PtCo Anchored N-Doped Graphene Oxide (NGO-PtCo).** Prior to N doping, graphene oxide (GO) was synthesized via the Hummers’ method with slight modifications. Typically, for N doping, 4 mL of GO aqueous suspension (5 mg/mL) was dispersed in 30 mL of water followed by the addition of 6 g of urea (200 mg/mL as a N precursor) and stirred for 3 h. Then, the solution was transferred to a Teflon-lined stainless steel autoclave (100 mL), and hydrothermal treatment of the reaction mixture was performed at 180 °C for 5 h. The resultant mixture was then centrifuged and washed with water and ethanol followed by vacuum drying at 55 °C to get a black powder termed as NGO. To further synthesize PtCo-grafted NGO, 100 mg of as prepared NGO was dispersed in 400 mL of ethanol followed by the addition of PtCl4 (100 mg), CoCl2·6H2O (40 mg), and sodium acetate (700 mg) under vigorous stirring. As a reducing agent, NaBH4 was quickly introduced to the reaction mixture, and the resultant mixture was stirred for 4 h at room temperature. The mixture was further centrifuged and washed with ethanol and water, and the resulting black precipitate was dispersed in 30 mL of water and stirred for 4 h. The solution was then centrifuged and washed with water and ethanol followed by vacuum drying at 55 °C to get NGO-PtCo. To further examine the electrochemical activity of NGO-PtCo, cyclic voltammetry (CV) was performed on a three-electrode system using a bare SPCE as a working electrode, NGO-PtCo-modified SPCE as an electrode, and an Ag/AgCl as a reference electrode. The electrolyte solution was 1 M HClO4 containing 0.1 M KCl. The electrochemical system was operated on a CHI 660B electrochemical workstation (Shanghai Chenhua Instruments).

**DPV Measurements.** Differential pulse voltammetry (DPV) measurements were performed using a CHI 660B electrochemical workstation with a three-electrode system using bare SPCE, NGO-PtCo-modified SPCE, NGO-PtCo performed after modification, and NGO-PtCo after modification as a working electrode, and Ag/AgCl as a reference electrode. The electrolyte solution was 1 M HClO4 containing 0.1 M KCl. The electrochemical system was operated on a CHI 660B electrochemical workstation (Shanghai Chenhua Instruments).
temperature. Finally, the product was collected and washed via centrifugation in plenty of deionized water and ethanol.

dsDNA Immobilization and Damage Studies. To evaluate the bimetallic nanoparticles as a reinforcement for dsDNA adsorption onto the graphene substrate surface, an adequate amount of dsDNA (dissolved in 50 mM NaCl solution) was incubated for several hours onto the working interface of the carbon-printed electrode. Prior to further treatment of dsDNA-modified electrodes, they were water washed and immersed in a working buffer (0.1 M acetate buffer, pH 4.4) to remove unbonded dsDNA and NaCl crystals. Thirty microliters of clenbuterol (CLB; 2 × 10⁻⁵ M) was then incubated on the modified electrode for a time of 120 s followed by rinsing with distilled water. The obvious changes in cyclic voltammetry (CV)-based and electrochemical responses on dsDNA immobilization evidenced the easy and successful immobilization of dsDNA onto the NGO-PtCo surface. All the cyclic voltammetric studies were performed at scan rate of 50 mV s⁻¹ in 1 mM [Fe(CN)₆]³⁻/⁴⁻. The modified electrodes were eventually scanned for differential pulsed voltammetry (DPV) in 100 μL of acetate buffer (pH 4.4). The appearance of an intrinsic guanine peak in DPV measurements, which were absent before CLB treatment, indicated the damage of dsDNA and hence the successful immobilization of dsDNA onto the NGO-PtCo. The same was also observed with cyclic voltammetry and electrochemical impedance spectroscopy studies.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02184.

Details of materials used, FTIR, XPS, and DPV curves of control experiments along with SEM images (PDF)

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Notes
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