Electrochemical self-signal identification of circulating tumor DNA based on poly-xanthurenic acid assembled on black phosphorus nanosheets

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Abstract

A self-signal electrochemical identification interface was prepared for the determination of circulating tumor DNA (ctDNA) in peripheral blood based on poly-xanthurenic acid (PXTA) assembled on black phosphorus nanosheets (BPNSs) acquired through simple ultrasonication method. The electropolymerization efficiency was promoted due to the physical adsorption between the xanthurenic acid (XTA) monomers and BPNSs, which was beneficial to the enlargement of the signal response of PXTA. The assembled PXTA/BPNSs nanocomposite with attractive electrochemical activity was adopted as a platform for the recognition of DNA immobilization and hybridization. The probe ssDNA was covalently fixed onto the PXTA/BPNSs nanocomposite with plentiful carboxyl groups through the terminate free amines of DNA probes by use of the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydrosulfosuccinimide cross-linking reaction, accompanied with the decline of the self-signal response. When the hybridization between the probe ssDNA and the target DNA was accomplished, the self-signal response of the nanocomposite interface reproduced by virtue of the shaping of helix construction. The determination limit of the assembled DNA identification interface was 2.1×10^{-19} \text{mol/L}, and the complementary target DNA concentrations varied from 1.0×10^{-18} \text{mol/L} to 1.0×10^{-12} \text{mol/L}. The DNA identification platform displayed magnificent sensitivity, specificity and stability, and was efficaciously implemented to the measurement of ctDNA derived from colorectal cancer.

1. Introduction

Tumor is one of the growing global health care problems, with more than one million new diagnoses arose each year. It is a polygenic disease with genetic changes caused by disorders of related genes, with high morbidity and mortality [1]. At present, tissue biopsy is the most commonly used means of diagnosing cancer in clinical practice and is widely used to characterize tumor types. However, tissue biopsy is an invasive sampling method, and during the sampling process, it is likely to cause cancer misidentification due to the heterogeneity of the tissue itself or the failure to sample at the location of the lesion. In addition, real-time monitoring of tumors cannot be carried out due to the fact that biopsies themselves cannot be repeatedly sampled. Therefore, tissue biopsy technology still faces more challenges in terms of operating cost, diagnostic accuracy, and efficient treatment of cancer [2]. In order to overcome the above problems, some researchers have developed a new type of non-invasive test, called liquid biopsy. The assay is based on analysis of circulating tumor DNA (ctDNA), circulating tumor cells and other tumor-derived substances in plasma. They are derived from tumor tissue and exist in the blood, which can reflect information such as tumor development and drug resistance, and guide individualized precision treatment [3, 4]. Detection of ctDNA in liquid biopsy can reveal a variety of epigenetic alterations such as single nucleotide variation, copy number variation, methylation, and so on. It carries the information code of tumor tissue, is a good tumor detection biomarker, has high accuracy and safety in tumor detection, so it has attracted much attention and has been widely studied [5, 6].

In recent years, molecular biology techniques have developed rapidly, promoting the research and detection of ctDNA [7]. At present, the main types of methods for detecting ctDNA are PCR-based
methods, including real-time fluorescent quantitative PCR (qPCR), digital PCR (dPCR), amplification refractory mutation system PCR (ARMS-PCR). The other is based on next-generation sequencing (NGS) method. The shortcomings of PCR-based techniques and NGS method are that they require high cost and are time-consuming. In addition to this, several new technologies, especially nanotechnology-based biosensors, have great potential in detecting ctDNA, providing a simple and high-performance general strategy for low-cost clinical applications, mainly including electrochemical biosensors, fluorescent biosensors, surface enhanced Raman scattering biosensors, colorimetric/resonance light scattering biosensors, etc [8]. Particularly, electrochemical biosensing methods have received a lot of attention because of their high sensitivity, wide determination range, low cost, quick response, easy to miniaturize and carry. To improve the detection competence, multiple functional nanomaterials have been adopted for the building of electrochemical identification platforms, such as metal nanomaterials, nanostructured metal compounds, carbonaceous nanomaterials, transition metal sulfides nanosheets, and so forth [9–13].

As an emerging two-dimensional material, black phosphorus has a unique honeycomb structure, which can be prepared by the phase conversion process of white phosphorus or red phosphorus in a high temperature and high pressure environment, and is one of the allotropes of elemental phosphorus [14, 15]. The two-dimensional black phosphorus nanosheets (BPNSs) obtained after stripping of the bulk black phosphorus have layer-like structures similar to graphene, and the phosphorus atoms in the layer are connected by $\text{SP}^3$ hybrid covalent bonds, the difference is that graphene has only one bond, and there are two P-P bonds of different lengths in the black phosphorus sheet layer structure. The unique sheet structure of two-dimensional black phosphorus has high carrier mobility and adjustable band gap, which shows great application potential in many fields such as energy storage, catalysis, optoelectronic devices, biomedical and sensing [16–19]. In particular, due to its high specific surface area and fast electron transfer speed, two-dimensional black phosphorus has shown great application prospects in the fields of electrochemical sensing. However, due to the chemical instability of two-dimensional black phosphorus, its separate application in the field of electrochemical sensing has been less studied. Two-dimensional black phosphorus are commonly combined with other functional materials to effectively improve the chemical stability and obtain excellent electrochemical sensing performance [20–23]. For example, Kim et al. developed a sensitive and rapid bovine viral diarrhea virus detection system using few layer black phosphorus modified with gold nanoparticle [20]. Jia et al. employed Ni$_2$P nanocrystallines anchored on BPNSs for highly selective and sensitive non-enzymatic electrochemical detection of L-cysteine over glutathione [21].

Xanthurenic acid (XTA) is one of the metabolites of tryptophan, and when vitamin B$_6$ is deficient, the content of XTA in the urine increases. As a new type of conductive polymer, poly-xanthurenic acid (PXTA), has been utilized to identify the bioactive substances on account of its non-toxicity and splendid redox activity [24, 25]. In this work, we report for the first time that XTA was electropolymerized on the pre-prepared BPNSs surface to acquire a novel polymer nanocomposite, which provided a favourable platform for self-signal electrochemical identification of ctDNA in peripheral blood. The obtained
PXTA/BPNSs nanocomposite revealed wonderful internal electrochemical activities to denote the process of DNA immobilization and hybridization. As far as we know, such strategy has not been previously proposed for the measurement of ctDNA in liquid biopsy.

2. Experimental

2.1. Apparatus and materials

The electrochemical mensurations were executed by CHI 760E electrochemical analyzer (Shanghai CH Instrument Corporation, China) with a traditional three-electrode system: a carbon paste electrode (CPE, Ø = 3 mm) or modified CPE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. Scanning electron microscopy (SEM) was performed employing a ZEISS GeminiSEM 300 machine (Carl Zeiss, Germany).

The black phosphorus powder was purchased from Nanjing Xianfeng Nanomaterial Technology Company (China). XTA monomers were attained by Acros Organics Corporation (Belgium). Graphite powder (spectral pure, diameter about 30 µm) was obtained from Shanghai Colloid Chemical Company (China). 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were acquired from Shanghai Aladdin Biological Technology Corporation (China). The serum samples were gained by Linyi People's Hospital (China). All the chemicals and reagents used were of analytical grade.

The 18-base oligonucleotides probe DNA (ssDNA, 5’-NH$_2$AGC TGA TGG CGT AGG CAA-3’), its complementary target DNA (cDNA, 5’-TTG CCT ACG CCA TCA GCT-3’), one-base mismatched DNA (5’-TTG CAT ACG CCA TCA GCT-3’), three-base mismatched DNA (5’-TTG CAT ACG CTA TCA ACT-3’), and the noncomplementary DNA (ncDNA, 5’-TAC ATG TGC GAG CTC GAT-3’) were produced by Shanghai Sangon Biotechnology Company (China).

2.2. Preparation of the PXTA/BPNSs/CPE

The BPNSs were produced by an ultrasonic exfoliation means. Detailedly, 10 mg of bulk black phosphorus powder was added into 10 mL of water solution, and then ultrasonicated for 10 h, and ultimately a suspension was gained. The gained suspension was sedimentated by use of centrifugation for 30 min at 12000 rpm to get rid of the surplus black phosphorus, and the supernatant was removed during the sedimentation. After sedimentated through two cycles, the sediment was dried and re-dispersed into the water solution, and the homogenous BPNSs suspension was acquired with the concentration of 1.0 mg/mL.

The CPE was prepared as reported formerly [26]. The surface of CPE was pre-processed through polishing with a weighing paper. 10 µL of the suspension was overlayed onto the pre-processed surface of CPE, and after natural dryness, the BPNSs/CPE was fabricated. The fabricated BPNSs/CPE was immersed into 0.3 mol/L phosphate-buffered saline (PBS, pH 5.5) comprising 500 µmol/L XTA monomers with the
potential cycling between 1.2 V and −0.6 V for 20 cycles with the scanning rate of 100 mV/s, and the PXTA/BPNSs/CPE was accomplished. For contrast, the PXTA/CPE was also achieved by means of the alike steps.

2.3. DNA immobilization and hybridization

The probe ssDNA was attached onto the PXTA/BPNSs/CPE through covalent combination between the amine groups of ssDNA probes and the carboxyl groups of PXTA. The PXTA/BPNSs/CPE was immerged into 0.3 mol/L PBS (pH 7.0) embracing 5.0 mmol/L EDC and 5.0 mmol/L NHS for 1 h for the activizing of the carboxyl groups of PXTA. Afterwards, 10 µL of PBS (pH 7.0) containing 1.0×10⁻¹² mol/L ssDNA probes was coated onto the surface of the above activized electrode for 2 h, cleansed with PBS (pH 7.0) to eliminate the unfixed probe ssDNA, and this probe-caught electrode was designated as ssDNA/PXTA/BPNSs/CPE.

For hybridization, 10 µL of solution (PBS, pH 7.0) including cDNA (1.0×10⁻¹² mol/L) was spread onto the ssDNA/PXTA/BPNSs/CPE for 90 min, and then the electrode was washed with PBS to remove the unhybridized cDNA, subsequently by dint of drastically purged with ultrapure water to eradicate the unhybridized cDNA. Besides, the hybridization between the probe-caught electrode and one-base mismatched DNA (1.0×10⁻¹² mol/L), three-base mismatched DNA (1.0×10⁻¹² mol/L), or ncDNA sequence (1.0×10⁻¹² mol/L) was fulfilled through the analogous procedures.

2.4. Electrochemical determinations

The cyclic voltammetry (CV) determinations were carried out in 0.3 mol/L PBS (pH 7.0), and the potential scanning ranges were between 0.2 V and −0.6 V with a scanning rate of 100 mV/s. The electrochemical impedance spectroscopy (EIS) measurements were realized in 0.3 mol/L PBS (pH 7.0) with the voltage frequencies in the range from 10⁵ Hz to 1 Hz and the AC voltage amplitude of 5 mV.

Except exclusively illustrated, all the determinations were implemented at room temperature. The reported consequence was the evenness of three independent mensurations.

3. Results and discussions

3.1. SEM characterization of the PXTA/BPNSs composite

Figure 1. near here.

SEM was implemented to characterize the morphology of the PXTA/BPNSs composite. The representative SEM graphics of the BPNSs and PXTA/BPNSs composite were illustrated in Fig. 1. Manifestly, the BPNSs (Fig. 1A) presented a laminated structure with sleek superficies, and the nanosheets were overlapping with each other and cumulated on the electrode surface. When the PXTA was assembled onto the BPNSs, the gained PXTA/BPNSs composite (Fig. 1B) appeared a rough surface
and possessed a number of protrusions, and which might be ascribable to that the vegetating of the PXTA on the surface of the BPNSs shaped harsh membrane.

3.2. Electrochemical responses of the PXTA/BPNSs/CPE

Figure 2. near here.

A couple of quasi-reversible redox peaks (peak a and b) appeared in Fig. 2A illustrated that PXTA had been successfully formed on the electrode surface. The definite oxidation peak in Fig. 2A (peak c) suggested the oxidation and electropolymerization of XTA. It can be seen that the peak currents of PXTA/BPNSs/CPE (blue line in Fig. 2A) were markedly larger than that of PXTA/CPE (red line in Fig. 2A), denoting that the BPNSs were propitious to the electropolymerization of XTA monomers. The BPNSs with large surface area could be integrated with the XTA monomers by right of physisorption, and hence furnished abundant nucleation positions for the polymerization of the monomers.

As illustrated in Fig. 2B, in order to further research the function of the BPNSs on the polymerization of the monomers, the signal responses of diverse modified electrodes were paralleled by observing the self-signals of PXTA in 0.3 mol/L PBS (pH 7.0). As displayed at the bare CPE (curve a), there were no noticeable anodic or cathodic signals. However, the curve b of the PXTA/CPE exhibited a couple of redox responses, which demonstrated the shaping of the polymer membrane on the electrode surface. In contrast with the signal responses of the PXTA/CPE (curve b), much larger peak values were observed at the PXTA/BPNSs/CPE (curve c), confirming that the existence of the BPNSs could upgrade the self-signal responses of PXTA, as a result of that the BPNSs with large surface area which might provide a splendiferous electron transmission channel between the PXTA molecules and the electrode.

The quantity of the BPNSs could have momentous impact on the capability of the PXTA/BPNSs membrane. The self-signal responses of the PXTA/BPNSs membrane enlarged progressively, accompanied with the increment of the quantity of the BPNSs, and the maximum value was gained at 10 µL of 1.0 mg/mL BPNSs. The low concentration of the BPNSs will bring about too sparse BPNSs membrane, so the few conjunction sites of the sparse BPNSs membrane with the XTA monomers conduce to the frail physisorption between the BPNSs and XTA monomers. Nevertheless, when the concentration of the BPNSs surpassed 1.0 mg/mL, the signal responses depressed, which might be due to that much dense BPNSs membrane would cumulate together and hence impress the conductibility of the obtained nanocomposite membrane. Thereby, 10 µL of 1.0 mg/mL BPNSs was employed in experiments.

The signal responses of the PXTA/BPNSs membrane could also be impacted by the electropolymerization factors, particularly the polymerization cycles. The maximum signals of the PXTA/BPNSs membrane arrived at the 20th cycle, and then diminished with further polymerization (Figure S1). Accordingly, a 20-cycle polymerization was adopted in experiments.

3.3. Self-signal identification of DNA immobilization and hybridization
The self-signal variations of the PXTA/BPNSs/CPE in 0.3 mol/L PBS (pH 7.0) before and after probe immobilization and hybridization were explored by CV technique, and the results were displayed in Fig. 3. The PXTA/BPNSs/CPE presented favourable electrochemical self-signal responses in a neutral circumstance (curve a), just the same as announced above. After the fixation of the probe ssDNA onto the surface of the PXTA/BPNSs nanocomposite by covalent conjunction, the self-signal responses of the ssDNA/PXTA/BPNSs/CPE declined strikingly (curve b) in contrast to that at the PXTA/BPNSs/CPE (curve a). The flexibleness shape attributes of the probe ssDNA could transform the conformation and kept down the effectual electron transport \[26\]. Consequently, the self-signals of the ssDNA/PXTA/BPNSs layer depressed (“signal-off”). Contrarily, when the hybridization of the probe ssDNA with the target DNA was achieved, the self-signal responses emerged an increment (curve c, signal-on) in comparison with the curve b, and which might be on account of the following clarications. In contrast with the probe ssDNA with flexibleness configuration, the formative dsDNA exhibited unelastic and structured skeleton, which resulted in that the electron transportation along the double-helix arose much speedier than that along the single-chain framework \[27, 28\]. The above results testified that the self-signal alterations of the PXTA layer could be applied for direct identification of DNA immobilization and hybridization by means of CV technology without the augment of any extraneous indication agents.

The DNA hybridization time was researched. The CV responses of the dsDNA/PXTA/BPNSs/CPE augmented with extending the hybridization time and arrived at a steady value at 90 min. When further lengthening the hybridization time, the CV signals had no notable intensication (Figure S2). Ultimately, the hybridization time of 90 min was exploited in experiments.

3.4. Specificity of the constructed recognition platform

To research the specificity of the constructed recognition platform, distinct DNA species derived from the KRAS gene were schemed to imitate the principal interferences, comprising one-base mismatched DNA, three-base mismatched DNA and ncDNA. As exhibited in Fig. 4, the variations of the Bode plots in 0.3 mol/L PBS (pH 7.0) took place after the hybridization of the ssDNA probes. The curve a represented the Bode plot of the PXTA/BPNSs/CPE. As shown in the curve f, when the probe ssDNA was connected onto the PXTA/BPNSs nanocomposite, the impedance value had a prominent increment, illustrating the satisfying loading of the probe ssDNA. The impedance response of the dsDNA/PXTA/BPNSs/CPE (curve b) brought down transparently after the hybridization of the probe ssDNA with the cDNA, which might be owing to that dsDNA revealed higher conduction competence than ssDNA. When the ssDNA/PXTA/BPNSs/CPE hybridized with the ncDNA (curve e), the impedance value altered little in comparison with the probe electrode (curve f), illuminating that nearly no hybridization came true. The impedance responses could be distinguished from the curve b when hybridized with the one-base mismatched DNA (curve c) and three-base mismatched DNA (curve d), which could be ascribed to that the base mismatch induced the incomplete hybridization. Correspondingly, the DNA biorecognition
platform based on the PXTA/BPNSs nanocomposite maintained splendid specificity to distinguish distinct DNA species.

### 3.5. Sensitivity of the developed recognition interface

Figure 5. near here.

The competency of quantitative recognition of the developed recognition interface was explored by employing the ssDNA/PXTA/BPNSs/CPE to hybridize with the complementary target DNA of variant concentrations sprung from the KRAS gene, and the achieved Bode plots were revealed in Fig. 5A. The difference value (namely $\Delta \log Z$) between the log $Z$ of the probe-caught electrode and that after hybridized with the target DNA was hired as the measurement indication. The outcomes suggested that the $\Delta \log Z$ value was linear with the logarithm of the KRAS gene target sequence concentrations changing from $1.0 \times 10^{-18}$ mol/L to $1.0 \times 10^{-12}$ mol/L (Fig. 5B), and a detection limit of $2.1 \times 10^{-19}$ mol/L was appraised by virtue of $3\sigma$ (where $\sigma$ was the relative standard deviation (RSD) of the blank solution, $n = 11$) with the regression equation $\Delta \log Z = 0.12 \log C + 2.2643$ ($R^2 = 0.9918$). In contrast with some other antecedently projected indicator-free electrochemical gene recognition systems (Table 1), the proposed recognition tactics employing the PXTA/BPNSs nanocomposite in this work opened out the high-blooded idiosyncrasy with low determination limit and extensive linear range.
<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Signal source</th>
<th>Detection method</th>
<th>Detection range (mol/L)</th>
<th>Detection limit (mol/L)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/CuS-acetylene black/GCE</td>
<td>[Fe(CN)]$_6^{3-/-4-}$</td>
<td>DPV</td>
<td>1.0×10$^{-13}$ to 1.0×10$^{-9}$</td>
<td>2.0×10$^{-14}$</td>
<td>29</td>
</tr>
<tr>
<td>AuNpsPANI/GCE</td>
<td>[Fe(CN)]$_6^{3-/-4-}$</td>
<td>EIS</td>
<td>1.0×10$^{-15}$ to 1.0×10$^{-8}$</td>
<td>6.94×10$^{-17}$</td>
<td>30</td>
</tr>
<tr>
<td>graphene-Nafion/GCE</td>
<td>[Fe(CN)]$_6^{3-/-4-}$</td>
<td>EIS</td>
<td>1.0×10$^{-13}$ to 1.0×10$^{-10}$</td>
<td>2.3×10$^{-14}$</td>
<td>31</td>
</tr>
<tr>
<td>CNT aerogel electrode</td>
<td>[Fe(CN)]$_6^{3-/-4-}$</td>
<td>EIS</td>
<td>1.0×10$^{-13}$ to 1.0×10$^{-10}$</td>
<td>1.1×10$^{-12}$</td>
<td>32</td>
</tr>
<tr>
<td>sulfonated polyaniline-graphene oxide/CPE</td>
<td>electrode material</td>
<td>EIS</td>
<td>1.0×10$^{-13}$ to 1.0×10$^{-7}$</td>
<td>3.2×10$^{-14}$</td>
<td>33</td>
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<tr>
<td>RFSP/WS$_2$/GCE</td>
<td>electrode material</td>
<td>DPV</td>
<td>1.0×10$^{-16}$ to 1.0×10$^{-8}$</td>
<td>2.1×10$^{-17}$</td>
<td>34</td>
</tr>
<tr>
<td>FMNs/MoS$_2$/GCE</td>
<td>electrode material</td>
<td>DPV</td>
<td>1.0×10$^{-16}$ to 1.0×10$^{-8}$</td>
<td>1.2×10$^{-17}$</td>
<td>13</td>
</tr>
<tr>
<td>PXTA/BPNSs/CPE</td>
<td>electrode material</td>
<td>EIS</td>
<td>1.0×10$^{-18}$ to 1.0×10$^{-12}$</td>
<td>2.1×10$^{-19}$</td>
<td>this work</td>
</tr>
</tbody>
</table>

### 3.6. Stabilization and repetitiveness of the recognition system

The long-standing stabilization of the schemed DNA recognition system was checked. Six identically-equipped ssDNA/PXTA/BPNSs/CPEs was conserved in PBS (pH 7.0) at 4°C for one month, and the EIS responses still remained alike magnitudes in contrast with the original probe electrode (Figure S3), manifesting the superior stabilization of the gene recognition system. The repetitiveness of this DNA biorecognition platform was estimated by mansurating 1.0×10$^{-12}$ mol/L target DNA by way of six uniformly-manufactured probe-caught electrodes (Figure S4), and a RSD of 5.62% for the response alterations was assessed, which elucidated that the constructed biorecognition system possessed an exceptional repetitiveness for gene analyses.

### 3.7. Examination of the serum samples

The projected self-signal recognition platform was exploited for the identification of the ctDNA related to the KRAS gene derived from colorectal cancer in genuine serum samples. The recovery experimentions
were executed by mixing four categories of target DNA of established concentration into the blood specimens of healthy postulants by means of the standard addition manner, and examined five times for every specimen. The recovery ratios were in the scope of 95.0-102.0% with the RSDs of 4.76–6.08% \((n = 5)\), as presented in Table 2. The aforesaid results sufficiently testified that the schemed bioidentification system had amazing accurateness, which could be performed to tell apart the authentic ctDNA specimens.

<table>
<thead>
<tr>
<th>Serum samples</th>
<th>Target DNA added (M)</th>
<th>Target DNA detected (M)</th>
<th>Recovery (%)</th>
<th>RSD (%, (n = 5))</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(1.0\times10^{-18})</td>
<td>(0.95\times10^{-18})</td>
<td>95.0</td>
<td>4.76</td>
</tr>
<tr>
<td>3</td>
<td>(1.0\times10^{-15})</td>
<td>(0.97\times10^{-15})</td>
<td>97.0</td>
<td>5.32</td>
</tr>
<tr>
<td>4</td>
<td>(1.0\times10^{-12})</td>
<td>(1.02\times10^{-12})</td>
<td>102.0</td>
<td>6.08</td>
</tr>
</tbody>
</table>

### 4. Conclusions

In short, a groundbreaking electrochemical biosensing platform to determine the ctDNA related to the KRAS gene with high sensitivity and specificity was designed. The performance of the BPNSs was notably upgraded through PXTA assembly. Besides, using the BPNSs as a substrate effectually anchored PXTA, attaining PXTA/BPNSs nanocomposite that can synergetically intensify the signal response of the sensing interface. Significantly, the self-signal mutations of the PXTA layer evoked by DNA immobilization and hybridization could be susceptibly identified by way of CV and EIS techniques. Under optimum surroundings, the detection limit was as low as \(2.1\times10^{-19}\) mol/L. The specificity, stabilization, and repetitiveness of the biosensor were explored and presented acceptable outcomes, making it a potential implement for recognizing the ctDNA in correlative clinical diagnosis.

### Declarations

**Author contributions**

**Xinyu Yan:** Investigation, Methodology, Data curation, Editing. **Wei Zhang:** Conceptualization, Methodology, Investigation, Funding acquisition, Writing and Editing. **Jimin Yang:** Investigation, Data curation, Formal analysis, Funding acquisition.

**Declaration of Interest Statement**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Statement of Studies in Humans**

The authors declare that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association. The informed consent has been obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

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Figures
**Figure 1**

SEM graphics of the BPNSs (A) and PXTA/BPNSs (B).
Figure 2

(A) Electropolymerization process of XTA at diverse electrodes: PXTA/CPE (red line) and PXTA/BPNSs/CPE (blue line). (B) The CV curves of the bare CPE (a), PXTA/CPE (b) and PXTA/BPNSs/CPE (c) recorded in 0.3 mol/L PBS (pH 7.0).
Figure 3

The CV curves of the PXTA/BPNSs/CPE (a), ssDNA/PXTA/BPNSs/CPE (b) and dsDNA/PXTA/BPNSs/CPE (c) recorded in 0.3 mol/L PBS (pH 7.0).
Figure 4

Bode plots recorded at the PXTA/BPNSs/CPE (a), ssDNA/PXTA/BPNSs/CPE (f),
dsDNA/PXTA/BPNSs/CPE (hybridized with cDNA, b), hybridized with ncDNA (e), one-base mismatched
DNA (c), and three-base mismatched DNA (d) in 0.3 mol/L PBS (pH 7.0).
Figure 5

(A) Bode plots recorded at the ssDNA/PXTA/BPNSs/CPE (a) and after hybridized with the KRAS gene target sequences of variant concentrations: (b) $1.0 \times 10^{-18}$, (c) $1.0 \times 10^{-17}$, (d) $1.0 \times 10^{-16}$, (e) $1.0 \times 10^{-15}$, (f) $1.0 \times 10^{-14}$, (g) $1.0 \times 10^{-13}$ and (h) $1.0 \times 10^{-12}$ mol/L in 0.3 mol/L PBS (pH 7.0). (B) The calibration plot of $\Delta \log Z$ versus the logarithm of the KRAS gene target sequence concentrations.

Supplementary Files

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