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# Electrochemical paper-based peptide nucleic acid biosensor for detecting human papillomavirus

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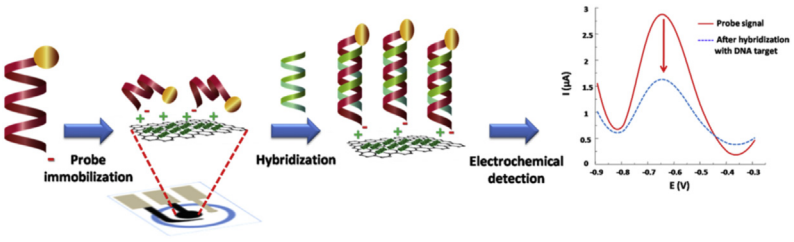
## HIGHLIGHTS

- A paper-based DNA biosensor using AQ-PNA probe and G-PANI modified electrode was first developed.
- This developed DNA biosensor was highly specific over the non-complementary DNA.
- This sensor was successfully applied to detect the HPV-DNA type 16 obtained from cancer cell lines.
- This sensor is inexpensive and disposable, which can be incinerated easily and safely after use.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A novel paper-based electrochemical biosensor was developed using an anthraquinone-labeled pyrrolidinyl peptide nucleic acid (acpcPNA) probe (AQ-PNA) and graphene-polyaniline (G-PANI) modified electrode to detect human papillomavirus (HPV). An inkjet printing technique was employed to prepare the paper-based G-PANI-modified working electrode. The AQ-PNA probe baring a negatively charged amino acid at the N-terminus was immobilized onto the electrode surface through electrostatic attraction. Electrochemical impedance spectroscopy (EIS) was used to verify the AQ-PNA immobilization. The paper-based electrochemical DNA biosensor was used to detect a synthetic 14-base oligonucleotide target with a sequence corresponding to human papillomavirus (HPV) type 16 DNA by measuring the electrochemical signal response of the AQ label using square-wave voltammetry before and after hybridization. It was determined that the current signal significantly decreased after the addition of target DNA. This phenomenon is explained by the rigidity of PNA-DNA duplexes, which obstructs the accessibility of electron transfer from the AQ label to the electrode surface. Under optimal conditions, the detection limit of HPV type 16 DNA was found to be 2.3 nM with a linear range of 10–200 nM. The performance of this biosensor on real DNA samples was tested with the detection of PCR-amplified DNA samples from the SiHa cell line. The new method employs an inexpensive and disposable device, which

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easily incinerated after use and is promising for the screening and monitoring of the amount of HPV-DNA type 16 to identify the primary stages of cervical cancer.



## 1. Introduction

The most important factors for diagnostic devices, especially for developing countries, are low cost, simplicity and speed of results for early screening and monitoring of disease biomarkers. To achieve this goal, paper-based analytical devices (PADs) have been widely used as an alternative device design for point-of-care (POC) applications [1–3]. Two detection modes that have been most frequently used with PADs include colorimetric and electrochemical detections. Since first reported by Dungchai et al. [4], PADs with electrochemical detection (ePADs) have increasingly attracted attention as they offer a combination of simplicity, low power requirements, low limits of detection, and ease of quantitation [5–8]. ePADs are therefore an ideal platform for developing sensitive, selective DNA biosensors for point-of-care applications.

In electrochemical DNA biosensors, many different electrode types have been used, including a gold [9–11], hanging mercury drop (HMDE) [12,13] and various carbon-based materials [14–17]. Carbon is considered a good electrode material due to its low cost, wide potential range, chemical inertness and low background current. Furthermore, carbon electrodes have a fast response time and can be easily fabricated in different configurations. These features make carbon suitable for use in ePAD DNA biosensors. However, the use of micro-scale electrodes as part of ePAD is a major obstacle due to limited sensitivity. To overcome this problem, graphene (G) has been used as a carbon-based nanomaterial and has achieved significant popularity due to the large specific surface area and unique electrochemical properties of G [18–20]. G-based electrodes exhibited superior performance compared to other carbon-based electrodes in terms of their electro-catalytic activity and electrical conductivity [21,22]. G has also been used in combination with various types of functional materials to fabricate high-performance electrodes. Among them, polyaniline (PANI) is a useful conducting polymer that has been widely used for electronic, optical and electrochemical applications such as enzyme-based biosensors and DNA assays due to its excellent environmental stability and unusual doping/dedoping chemistry [23–25]. Moreover, PANI improves the dispersion and reduces the agglomeration of the planar $sp^2$-carbon of G [23,26]. PANI also possesses free amino groups, which can act as a handle for the covalent immobilization of suitable detection probes via amide bonds [27,28]. Finally, doped PANI possesses the positive charge of the amino group, which can immobilize negatively-charged probes via electrostatic interactions. Thus, it is a challenge to evaluate alternative systems for immobilization of various bio-recognition elements via electrostatic interaction.

For most electrochemical DNA biosensors, a probe that is designed to detect a specific sequence of target DNA is first immobilized on the electrode. Then, the electrochemical signal of an electroactive species, which was either covalently attached to the probe or added later as an indicator, is recorded and compared before and after the hybridization with the complementary DNA target [12,29]. The probe is a key parameter that determines the detection selectivity. While most DNA biosensors employ short oligodeoxynucleotide probes, several alternative probes have been used with great success. Peptide nucleic acid (PNA) [30,31], a synthetic DNA mimic with a peptide-like backbone of repeating N-(2-aminoethyl)-glycine units replacing the sugar-phosphate in natural DNA or RNA, has attracted increasing interest as the probe for electrochemical DNA biosensors [12,32–34] due to its sequence-specific binding to DNA or RNA, resistance to nuclease and protease enzymes, and strong binding to the target DNA. Recently, Vilaivan's group [35–37] proposed a new conformationally constrained pyrrolidinyl PNA system (known as acpcPNA) that possesses an α,β-peptide backbone derived from D-proline/2-aminocyclopentanecarboxylic acid. This new acpcPNA demonstrates a stronger binding affinity and higher specificity towards complementary DNA compared to DNA and Nielsen's PNA, and there are several applications of acpcPNA as a probe for DNA biosensors [38–40].

Human papillomavirus or HPV is the common virus that can be passed through any type of sexual contact. There are some high-risk types of HPV including type 16 and 18, which can cause abnormal changes to the cells of the cervix. These changes can lead to cervical cancer which, is one of the most important health problems for woman. The mortality occurring from cervical cancer has continuously increased especially in developing countries that have limited medical facilities [41].

In our previous work [38], the electrochemical sensor based-on acpcPNA probe for HPV detection was reported. Although the good limit of detection (LOD) was achieved, the use of PVC-based sensor was costly and the procedure relies on covalent method for probe immobilization was complicated and time-consuming. Cost is the most critical challenge for disposable POC electrochemical sensors. In this work, using PAD has potential to address all of challenges of disposable sensor in term of low-cost and simple fabrication. Electrostatic immobilization is also a key step of this work. This method eliminated the complicate steps of covalent process and significantly reduced time. Moreover, inkjet printing used for electrode modification step is advantageous due to it provided electrode reproducibility and high pattern resolution [42]. Inkjet-printing is scalable of mass production, while small amount of modifier is wasted in the modification process.

Here, we aim to develop a novel paper-based electrochemical DNA biosensor using an AQ-labeled acpcPNA probe in combination with a G-PANI modified electrode. In this new DNA biosensor, the acpcPNA probe labeled with anthroquinone (AQ) was first incorporated onto a G-PANI-modified ePAD using electrostatic immobilization. In the absence of complementary DNA, a strong signal was observed for the AQ. Hybridization with the complementary DNA target resulted in a decrease of the electrochemical signal that linearly correlated with the concentration of the target. The application of the biosensor to the sensitive detection of HPV DNA type 16 is demonstrated. The proposed method is applicable to the screening and monitoring of HPV-DNA type 16 in the primary stage of cervical cancer, a disease that leads to the death of women around the world, particularly in developing countries that have limited resources for public healthcare.

## 2. Materials and methods

### 2.1. Chemicals and materials

Graphene (G) was purchased from A.C.S (Medford, USA).