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Electrochemical detection of human papillomavirus DNA type 16 using a pyrrolidinyl peptide nucleic acid probe immobilized on screen-printed carbon electrodes

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ABSTRACT

An electrochemical biosensor based on an immobilized anthraquinone-labeled pyrrolidinyl peptide nucleic acid (acpcPNA) probe was successfully developed for the selective detection of human papillomavirus (HPV) type 16 DNA. A 14-mer acpcPNA capture probe was designed to recognize a specific 14 nucleotide region of HPV type 16 L1 gene. The redox-active label anthraquinone (AQ) was covalently attached to the N-terminus of the acpcPNA probe through an amide bond. The probe was immobilized onto a chitosan-modified disposable screen-printed carbon electrode via a C-terminal lysine residue using glutaraldehyde as a cross-linking agent. Hybridization with the target DNA was studied by measuring the electrochemical signal response of the AQ label using square-wave voltammetric analysis. The calibration curve exhibited a linear range between 0.02 and 12.0 μM with a limit of detection and limit of quantitation of 4 and 14 nM, respectively. This DNA sensing platform was successfully applied to detect the HPV type 16 DNA from a PCR amplified (240 bp fragment of the L1 gene) sample derived from the HPV type 16 positive human cancer cell line (SiHa), and failed to detect the HPV-negative c33a cell line. The sensor probe exhibited very high selectivity for the complementary 14 base oligonucleotide over the non-complementary oligonucleotides with sequences derived from HPV types 18, 31 and 33. The proposed sensor provides an inexpensive tool for the early stage detection of HPV type 16, which is an important biomarker for cervical cancer.

1. Introduction

Cervical cancer is one of the leading types of fatal cancer in women around the world. It mostly occurs in women within the age range of 30–50 years old, and the number of cervical cancer patients has increased continuously to a current level of around 500,000 people per year with a mortality of 200,000. Among these cases, 80% of all the patients are from developing countries that have limited public healthcare resources (Parkin et al., 2001).

Human papillomavirus (HPV) has been shown to be the major cause of cervical cancer (Bosch et al., 2002; Munoz et al., 1992; Walboomers et al., 1999). The virus is transmitted sexually and via skin and mucous membranes (Koutsky et al., 1988; Roman and Fife, 1989). HPV can be sub-classified into high-risk and low-risk groups, where only the high-risk HPV infections, such as HPV types 16 and 18, can cause cervical cancer (Davies et al., 2001; Gravitt et al., 1998; Jacobs et al., 1995; Van Den Brule et al., 2002).

Various techniques have been developed and applied over the last few decades for the diagnosis of HPV infections. Currently, the most widely used techniques for the screening and diagnosis of HPV infection are the Digene Hybrid Capture assay (HC2), Pap smear test and polymerase chain reaction (PCR) with generic primers (Gravitt and Jamshidi, 2005; Lorincz and Anthony, 2001). However, the first two aforementioned techniques have some disadvantages. In particular, these techniques exhibit a low sensitivity and specificity, require an expert to analyze the data, are time consuming, and require complicated and expensive instrumentation. Therefore, these techniques are unsuitable for countries with limited resources and personnel (Villa and Denny, 2006).

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More recently, new detection techniques, such as leaky surface acoustic wave and piezoelectric and fluorescence spectroscopy, have been applied for the detection of HPV, but these are still expensive and require complicated instrumentation (Fu et al., 2004; Ramanujam et al., 1994; Wang et al., 2009). In addition, the electrochemical detection of HPV-related sequences has also been developed (Civit et al., 2010, 2012; Sabzi et al., 2008). The high sensitivity, small sample volume requirement, low cost, simplicity and portability make electrochemical detection an excellent candidate for a point-of-care DNA diagnostic method.

The general principle of a DNA electrochemical biosensor involves the immobilization of a DNA or DNA-analog probe onto the electrode surface. Different types of electrodes such as gold, hanging mercury drop and various carbon-based electrodes can be used. Subsequent hybridization between the probe and the target DNA causes a change or shift in the electrochemical signal of the electroactive labels/indicators attached to the probes or to the hybrids formed on the electrodes (Abi and Ferapontova, 2012; Kang et al., 2012; Luo et al., 2008; Ozkan et al., 2002).

The high specificity of peptide nucleic acid (PNA) (Egholm et al., 1992a, 1992b, 1993; Nielsen et al., 1991) made it especially suitable as probes for DNA diagnostics. The use of PNA probe in electrochemical DNA biosensors had been shown to give higher sensitivity and specificity, fast hybridization kinetics that is independent of ionic strength and require shorter probe length than DNA (Wang et al., 1996). Recently, a new conformationally restricted pyrrolidinyl PNA system with α/β-peptide backbone derived from D-proline/2-aminocyclopentanecarboxylic acid (acpcPNA) was developed (Vilaivan and Srisuwannaket, 2006; Vilaivan et al., 2011). AcpcPNA exhibits a stronger binding affinity and a higher specificity towards a complementary target DNA than DNA or Nielsen's PNA (Ananthanawat et al., 2010).. Because of these excellent properties, acpcPNA has been applied as a sensor probe to detect target DNA in combination with various detection techniques, such as fluorescence microscopy, matrix assisted laser desorption/ionization–time of flight mass spectrometry (MALDI–TOF-MS) and surface plasmon resonance (Ananthanawat et al., 2009; Ananthanawat et al., 2010; Boontha et al., 2008; Korkaew and Vilaivan, 2008). However, these platforms are still not ideal for routine diagnostic purposes.

In this work, an electrochemical biosensor for the detection of high-risk HPV type 16 DNA was developed using an acpcPNA probe complementary to a 14 base region unique to the L1 gene of HPV type 16 and anthraquinone (AQ) as the redox-active label. Analytical parameters, such as the sensitivity, specificity and reproducibility, were investigated. This developed method was applied in screening for HPV type 16 associated with cervical cancer.

2. Materials and methods

2.1. Chemicals and apparatus

Graphite powder (mesh size < 100) was purchased from Sigma Aldrich. Carbon ink and silver/silver chloride were purchased from Acheson, California, USA. The screen-printed block was made by Chaiyaboon Co. Ltd. (Bangkok, Thailand). Chitosan flakes (Mw= 15 kDa) with a degree of deacetylation of 90% were obtained from Seafresh Chitosan (Thailand). Glutaraldehyde (70% in water, Biochemica grade) was purchased from Fluka. Analytical grade 1-hydroxy-9,10-anthraquinone was obtained from Aldrich and used without further purification. Glacial acetic acid (analytical grade) for dissolving the chitosan flakes was received form Merck. Other analytical grade reagents, including NaCl, KH_2PO_4, Na_2HPO_4 and KCl, were purchased from Merck and used without further purification. Analytical grade diethylene glycol monobutyl ether and ethylene glycol monobutyl ether acetate, used as binder solution in the ink preparation step, were purchased from Merck. The cervical cancer cell-lines with (SiHa) or without (c33a) HPV 16 were obtained from the Human Genetics Research Group, Department of Botany, Faculty of Science, Chulalongkorn University. Synthetic oligodeoxynucleotides corresponding to partial sequences of L1 genes of HPV types 16, 18, 31 and 33 were purchased from Pacific Science (Bangkok, Thailand) to study the selectivity of the PNA probe. The forward and reverse primers used for the PCR of cell-line samples were obtained from Pacific Science (Bangkok, Thailand). The sequences of the DNA oligonucleotide and primers are as follows:

Forward primer: 5′-CACTATTTTGGAGGACTGGA-3′
Reverse primer: 5′-GCCTTAAATCCTGCTTGTAG-3′
HPV type 16: 5′-GCTGGAGGTGTATG-3′
HPV type 18: 5′-GGATGCTGCACCGG-3′
HPV type 31: 5′-CCAAAAGCCCAAGG-3′
HPV type 33: 5′-CACATCCACCCGCA-3′

The HPV type 16 DNA sequences derived from a partial sequence of the L1 gene was chosen as the target. Corresponding regions of the same gene in other types of HPV DNA were used to design the non-complementary DNA to study the specificity of detection. All electrochemical measurements were performed on a PGSTAT 30 potentiostat (Metrohm Siam Company Ltd.) and controlled with the General Purpose Electrochemical System (GPES) software. A screen-printed carbon electrode was used in this work. The electrode consisted of a three-electrode system. Silver/silver chloride ink was used as a pseudo-reference electrode. Chitosan-modified carbon ink (4 mm i.d.) was employed as the working and counter electrodes (see details of the preparation in Section 2.3). All measurements were conducted using a square-wave voltammetric method at room temperature (25 °C).

2.2. Synthesis and labeling of the PNA probe

The PNA probe used in this work was the conformationally restricted acpcPNA with a sequence of AQ–CATACACCTCCAGC–$LysNH_2$ (written in the N→C direction, AQ=anthraquinone), which was designed to be complementary to the HPV type 16 DNA. Lysinamide was included at the C-terminus as a PNA solubility enhancer and as a handle for immobilization of the PNA on the electrode via the amino group on the lysine side chain. The PNA was synthesized on a Tentagel resin equipped with a Rink amide linker by standard Fmoc solid-phase peptide synthesis, as previously described (Vilaivan and Srisuwannaket, 2006). The PNA probe was modified at the N-terminus with AQ by an acylation reaction. The commercially available 1-hydroxy-9,10-anthraquinone was first converted to 1-carboxymethoxyanthraquinone (Liu et al., 2011) by alkylation with *tert*-butyl bromoacetate, followed by subsequent deprotection by acidolysis (Figs. S1 and S2, supporting information (SI)). The N-terminal Fmoc group of the PNA on the solid support (0.5 μmol) was removed by a brief treatment with 2% 1,8-Diazabicycloundec-7-ene (DBU)+20% piperidine in dimethylformamide (DMF). The free amino group was then treated with 1-carboxymethoxyanthraquinone (4 equiv.), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxide hexafluorophosphate (HATU) (4 equiv.), and N,N-diisopropylethylamine (DIEA) (16 equiv.) in DMF overnight at room temperature (RT, 25 °C). The progress of the reaction was monitored by MALDI–TOF-MS analysis on a Microflex MALDI–TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). After completion of the reaction, the modified PNA on the solid support was treated with 1:1 (v/v) aqueous ammonia:dioxane in a sealed tube at 60 °C overnight to remove the nucleobase protecting groups. The AQ-labeled PNA probe (PNA-AQ) was then cleaved from the solid support with trifluoroacetic acid (TFA) and purified by reverse-phase HPLC (C18 column, 0.1% (v/v) TFA in H_2O–MeOH

gradient). The identity of the PNA-AQ was verified by MALDI–TOF MS analysis, and the purity confirmed to be >90% by reverse-phase HPLC.

2.3. Preparation of the chitosan-modified screen-printed carbon electrode (CHT-SPCE)

The SPCE used in this study consisted of a three-electrode system prepared as previously described (Khaled et al., 2008, 2010). The pattern of the electrode was designed using Adobe Illustrator. Except where stated otherwise (Section 3.3), the ink composition included a 4% w/v chitosan solution, graphite powder and carbon ink, which were mixed together at a ratio of 0.35:0.2:1, respectively, to give a final concentration of chitosan in the ink of 0.9% (w/v). The CHT-SPCEs were prepared in-house according to the design shown in Fig. 1. Silver/silver chloride ink was first printed onto a polyvinyl chloride (PVC) substrate as a base layer to be used as both the pseudo-reference electrode (RE) and the conductive pads. Next, the chitosan-modified carbon ink was printed onto the same PVC substrate as the second layer to form both the working electrode (WE) and counter electrode (CE). Finally, the insulator (nail polish) was screened as the last layer. The finished electrode was heated at 55 °C for 1 h to remove the solvent and dry the electrode.

2.4. Preparation of PNA and DNA solutions

The acpcPNA, PNA-AQ and DNA stock solutions were prepared in Milli-Q water and kept frozen prior to use. The concentration of each stock solution was determined spectrophotometrically from the calculated molar extinction coefficients at 260 nm (ε_{260}). The (more dilute) working PNA and DNA solutions were prepared in phosphate buffer pH 7.4 (PBS; 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 140 mM NaCl and 2 mM KCl).

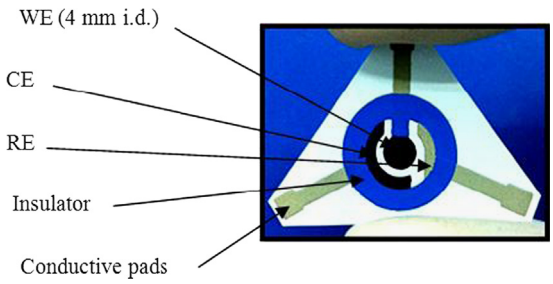

Fig. 1. The design of the three-electrode CHT-SPCE system.

2.5. Immobilization and hybridization of the PNA probe

The PNA-AQ probe was covalently immobilized onto the CHT-SPCE surface as previously described (Yi et al., 2003). First, 10 μL of a 5% (w/v) aqueous solution of glutaraldehyde (as cross-linking agent) was dropped onto the electrode surface and incubated at 40 °C for 3 h, and the electrode was then washed three times with PBS prior to dropping 5 μL of the PNA-AQ probe solution (15 μM) onto the electrode surface and then left in a small box to prevent the evaporation of the solution, at RT overnight. The excess and non-specifically adsorbed PNA-AQ probe on the electrode surface was removed by washing twice with PBS. The hybridization experiment was performed by dropping 10 μL of the target DNA solution (oligonucleotide (15 μM) or denatured PCR product) onto the electrode surface. After 15 min, the electrode was rinsed with PBS to remove the non-specifically adsorbed DNA. PBS (30 μL) was dropped onto the electrode surface again, and the electrochemical measurement was performed. The immobilization and hybridization procedures are summarized in Fig. 2.

2.6. DNA amplification

A 240 bp fragment from the HPV L1 gene region was PCR amplified from the SiHa (HPV type 16 positive) cell line, with the HPV negative C33a cell line (negative control), using the HPVL1-F (5′-CACTATTTTGGAGGACTGGA-3′) and HPVL1-R (5′-GCCTTAAATCCTGCTTGTAG-3′) primers as detailed in Section 1.9 of the SI (http://www.ncbi.nlm.nih.gov/nuccore/JQ004098.1, 2013). The reaction contained 0.4 μM of each of the primers, 0.2 mM deoxynucleotide triphosphate mixture, 1 × buffer (KCl, Tris)+1.5 mM $MgCl_2$, 0.5 U of Taq polymerase and 100 ng/μL of the cell line DNA sample. The amplification was performed as 95 °C for 10 min followed by 35 cycles at 95 °C for 10 s, 52 °C for 30 s and 72 °C for 30 s, and finally 7 min at 72 °C. The obtained PCR products were analyzed by size resolution in comparison with known molecular weight markers using 2% (w/v) agarose gel–TBE electrophoresis, and uv-transillumination visualization.

2.7. Sample preparation and electrochemical measurement

Prior to detection of cell line samples (SiHa as positive control, C33a as negative control), the DNA duplexes were first denatured in NaOH as previously described (http://fruitfly4.aecom.yu.edu/labmanual/41.html, 2013). Briefly, 10 μL of the PCR product was pipetted into a microcentrifuge tube together with 10 μL of 1.0 M

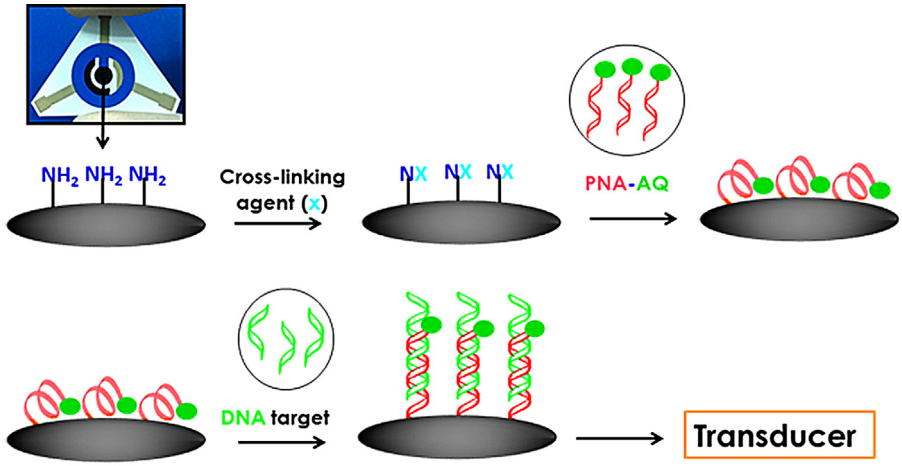

Fig. 2. Schematic illustration of the immobilization of the PNA-AQ probe onto the CHT-SPCE and subsequent hybridization with the target DNA.

NaOH and incubated at RT for 5 min. The reaction was then neutralized by the addition of 15 μL of 3.0 M NaOAc buffer (pH 5.0), and the denatured DNA was diluted with PBS to 100 μL. For electrochemical measurements, the denatured DNA (10 μL) was dropped onto the modified electrode and allowed to sit for 15 min for hybridization. The electrode was then rinsed twice with PBS. Before the electrochemical detection, PBS (30 μL) was dropped onto the electrode surface, followed by the subsequent electrochemical measurement. The probe signal after hybridization was observed and compared with the signal in the absence of the sample.

For evaluation of the specificity, the electrochemical measurement was performed as above using the complementary oligodeoxynucleotide (HPV type 16: 5′-GCTGGAGGTGTATG-3′) and the non-complementary oligodeoxynucleotides corresponding to partial sequences of the L1 genes of HPV types 18 (5′-GGATGCTG-CACCGG-3′), 31 (5′-CCAAAAGCCCAAGG-3′) and 33 (5′-CACATCCA-CCCGCA-3′).

In all cases the SWV was performed with 25 Hz frequency, 50 mV amplitude and 30 mV step potential.

3. Results and discussion

3.1. Characterization of the CHT-SPCE

Prior to PNA immobilization, the finished CHT-SPCE was characterized using 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in a 0.5 M KCl solution. The result shows a reversible peak of $[Fe(CN)_6]^{3-/4-}$ for the electrode characterization step, which indicated that the electrode was ready for use (Fig. S3, SI).

3.2. Immobilization and hybridization of the PNA-AQ probe with the DNA target

To make the PNA probe electrochemically detectable, it was first modified with the redox-active AQ via amide bond formation between a carboxy-containing AQ label and the free amino at the terminus of the PNA probe. The successful attachment of the AQ label to the PNA probe was confirmed by MALDI–TOF MS. The unlabeled PNA probe showed a mass peak at m/z=4644.6 (calcd m/z for $M \cdot H^+$=4645.1) and after labeling the m/z increased to 4909.7 (calcd m/z for $M \cdot H^+$=4910.7) (Fig. S4, SI). The mass increment of 265 Da coincides with the mass of the AQ label, thus indicating the successful labeling of the PNA probe. The electrochemical behavior of the non-immobilized PNA-AQ probe was investigated using square-wave voltammetry (SWV), where the redox peak of AQ appears at approximately −0.9 V (Fig. S5, SI). This peak was then used to confirm the successful immobilization of the PNA-AQ probe onto the electrode surface and to observe the electrochemical response after hybridization with the target DNA.

A number of methods for the immobilization of PNA/DNA probes onto electrode surfaces have been reported. These methods include physical immobilization, such as electrostatic adsorption, or chemical immobilization via covalent attachment of the probe onto the electrode surface (Abi and Ferapontova, 2012; Kang et al., 2012; Luo et al., 2008; Ozkan et al., 2002; Xu et al., 2001). Chemical immobilization is more attractive because it provides a more stable linkage in a controllable orientation and so results in a potentially reusable electrode (Lai et al., 2006). Amide bond formation, mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), is frequently employed to immobilize PNA/DNA probes onto carboxyl or amino-functionalized electrodes. However, this immobilization protocol is not ideal due to the decomposition of EDC and EDC-activated species in aqueous solution (Lei et al., 2002; Gilles et al., 1990). Accordingly, in this work the covalent attachment of the PNA-AQ probe onto the electrode was attempted using the biocompatible and environmentally friendly chitosan. The electrode was first modified with chitosan by simple mixing of the dissolved chitosan into the carbon ink. Next, the PNA-AQ probe was immobilized onto the CHT-SPCE by cross-linking of the amino groups on the lysine residue and on the chitosan with glutaraldehyde. The immobilization of the PNA-AQ sensor probe on the CHT-SPCE was confirmed by monitoring the redox peak at around −0.9 V. After immobilization of the probe, the modified electrode was then hybridized with the target DNA (5′-GCTGGAGGTGTATG-3′). In the presence of an equimolar quantity of the complementary target DNA (corresponding to HPV type 16), the electrochemical response was decreased three-fold (Fig. 3A). The decreased electrochemical response is explained by the higher rigidity of the PNA-DNA duplexes compared to the unhybridized PNA probe, which affected the accessibility and electron transfer between the redox-active AQ label and the electrode surface (Fig. 3B) (Abi and Ferapontova, 2012; Farjami et al., 2011). The signal change of the AQ label could be more clearly observed than that of methylene blue as the redox indicator under identical conditions (see Fig. S6, SI).

3.3. Influence of the amount of chitosan on the electrochemical signal response of the sensor probe

Chitosan was used as the amino group donor on the electrode for functionalization with the PNA-AQ probe. As expected, increasing the amount of chitosan from 0.22% to 0.90% (w/v) in the carbon ink increased the obtained electrochemical signal (Fig. S7, SI). Although a chitosan concentration of 0.90% (w/v) was not likely to represent the highest probe signal (the probe electrochemical signal vs. chitosan concentration had not reached a plateau) this concentration was still selected as the optimal amount of chitosan for preparing the electrodes because higher chitosan concentrations formed a gel that was poorly dispersed in the carbon ink.

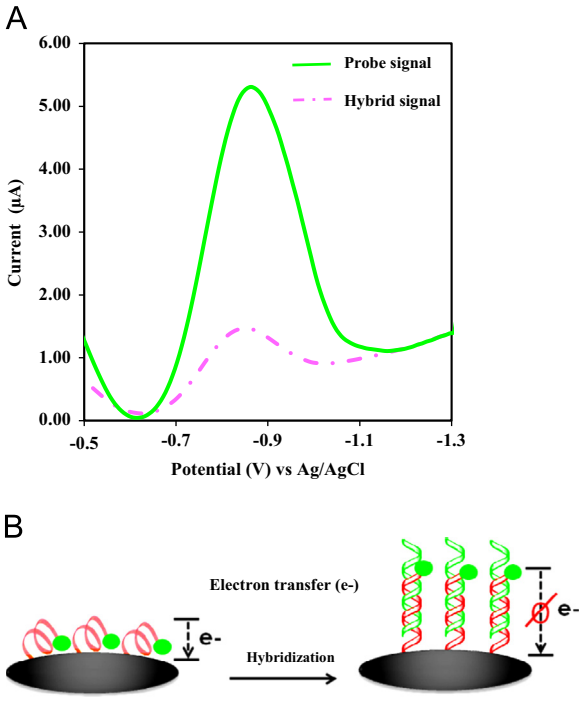

Fig. 3. (A) Representative electrochemical signal responses of the PNA-AQ probe (immobilized on the chitosan-SPCE) obtained from SWV before and after hybridization with an equimolar concentration of the target DNA. (B) Schematic illustration of the electron transfer space before and after hybridization of the PNA probe.

3.4. Influence of the amount and concentration of PNA-AQ probes

Next, the concentration of PNA-AQ probe in the immobilization step was optimized by varying the concentration of the PNA-AQ probe from 5 to 25 μM. No PNA-AQ probe signal was detected when less than 15 μM PNA-AQ was used, but clear signals were observed at PNA-AQ probe concentrations of 15 μM or greater. However, at concentrations higher than 15 μM PNA-AQ, the addition of the HPV type 16 oligomer DNA could not suppress the probe signal. Therefore, a concentration of 15 μM PNA-AQ was selected because it was the minimum concentration that provided clear and reproducible signals, both before and after hybridization with the target DNA (Fig. S8, SI).

3.5. Calibration curve

After formation of the CHT-SPCE-PNA-AQ using the optimized chitosan and PNA-AQ probe concentrations (Sections 3.3 and 3.4), a calibration plot of the difference in the current signals (ΔI) obtained from the SWV analysis and various test DNA (the different 14 nucleotide oligomers) concentrations relative to that without any test DNA was constructed. As expected, a lower AQ signal was observed when the electrode was exposed to higher target DNA concentrations (Fig. 4A), and the calibration curve exhibited a linear range between 0.02 and 12.0 μM with an R^2 value of 0.996 (Fig. 4B). From this data, the derived limit of detection (LOD) (at S/N=3) and limit of quantitation (LOQ) (at S/N=10) were calculated to be 4 nM and 14 nM, respectively.

3.6. Selectivity of the detection

The selectivity of the detection, which largely reflects the performance of the sensor probe, was examined by comparing the signals obtained from the CHT-SPCE-PNA-AQ in the presence of the 14-nucleotide oligomer target corresponding to HPV type 16 DNA and non-complementary 14-nucleotide oligomers (HPV types 18, 31 and 33). The probe signal was only decreased significantly in the presence of the complementary DNA, with negligible signal changes with the other three non-complementary DNA sequences that were tested (Fig. 5). Therefore, it can be concluded that the immobilized PNA-AQ probe selectively binds to the HPV type 16 DNA target sequences. It is important to note that the discrimination was already perfect under the non-stringent conditions used and therefore no further attempts (such as blocking or changing the hybridization conditions) were made in order to improve the specificity further.

Based on previously reported specificity of PNA for even a single nucleotide change (Wang et al., 1996), the high selectivity is not unexpected given the large level of sequence dissimilarity (10–13/14 nucleotides, 71–93%) between the target (HPV type 16) and these three non-target oligonucleotide sequences.

3.7. Detection of HPV type 16 DNA fragments in a PCR-amplified HPV infected cell line

To evaluate the performance of the CHT-SPCE–PNA-AQ in the analysis of real (PCR-amplified) DNA samples, two DNA samples were prepared by PCR amplification (~240 bp fragment of the HPV L1 region) of the extracted DNA from the SiHa cell line (HPV type 16 positive), with the PCR reaction product from the HPV negative C33a cell line used as a negative control. The results were consistent with expectations in that the probe signal decreased after exposure to the PCR reaction products from the HPV type 16-positive cell line in a concentration-dependent manner, but no signal change was detected with the PCR reaction from the HPV negative cell line (Fig. 6 and Fig. S9, SI). Thus, this developed electrochemical sensor appears to have good selectivity and has the potential to be successfully applied to detect HPV type 16 DNA in PCR samples.

4. Conclusions

A novel electrochemical biosensor for the detection of high-risk HPV type 16, based on an AQ-linked acpcPNA sensor probe conjugated to a CHT-SPCE was successfully developed and applied to detect the HPV type 16 specific 240 bp PCR amplicon from the HPV L1 region. The electrochemical signal was clearly decreased only in the presence of the correct target DNA. The calibration curve exhibited a linear range between 0.02 and 12.0 μM, with high coefficients of 0.996, and a LOD and LOQ of 4 and 14 nM, respectively. Furthermore, the sensor probe showed very high selectivity against non-complementary 14-base oligonucleotide, including HPV types 18, 31 and 33 DNA. The advantages of this

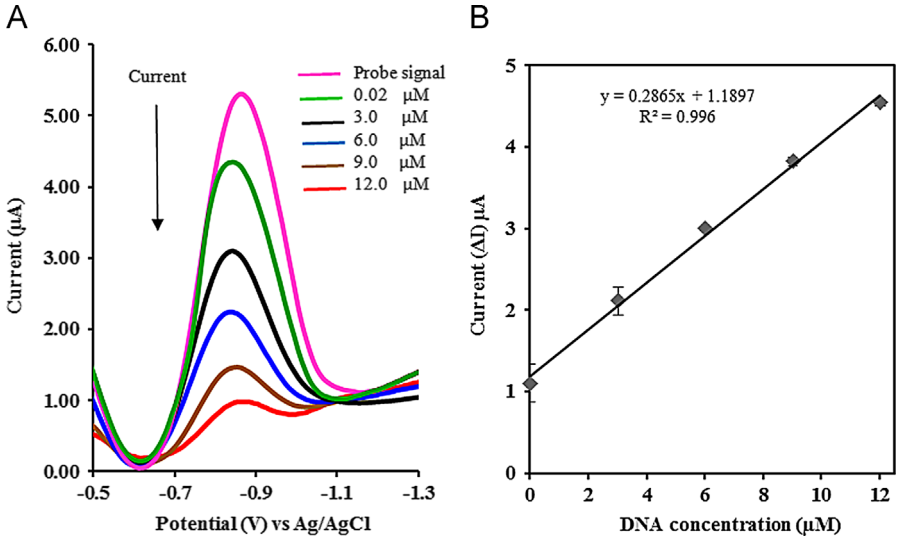

Fig. 4. (A) Representative SWV of the CHT-SPCE–PNA-AQ after addition of the target DNA (0.02–12.0 μM). (B) Calibration plots between the change in the probe electrochemical current (ΔI) and the target DNA concentration at optimized parameters.

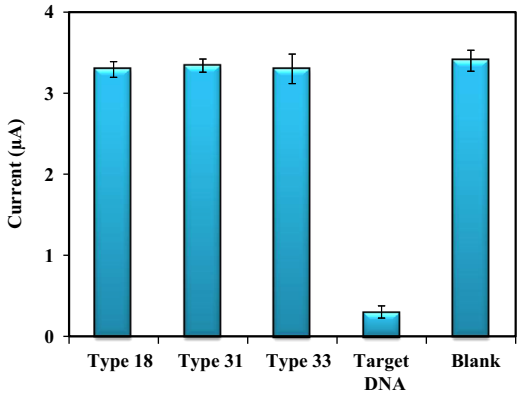

Fig. 5. Effect of various 14-base oligonucleotides (sequence from different HPV types) at 15 μM on the electrochemical signal response obtained from the CHT-SPCE–PNA-AQ.

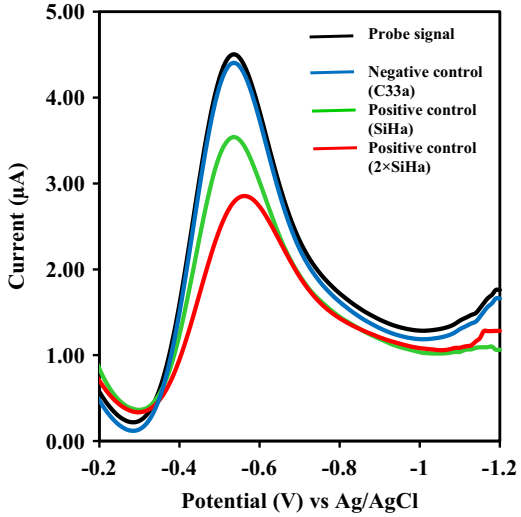

Fig. 6. Comparison of SWVs of the electrochemical signals of CHT-SPCE–PNA-AQ probe in the absence of DNA, in the presence of PCR reaction (240 bp amplicon) from a HPV negative (C33a), and HPV type 16 positive (SiHa) cell-lines.

platform include the ease of electrode preparation and probe immobilization. The electrode can be prepared inexpensively and requires only small sample volume. The specificity is excellent under non-stringent hybridization conditions. The instrument set up is also simple, therefore the present technique should be suitable as a novel tool for HPV screening in the developing countries. Disadvantages of this technique include the necessity of immobilizing the PNA probe, the inability to reuse and the rather high detection limit by the standard of DNA detection. Nevertheless, the detection limit is sufficiently good to allow detection of HPV type 16 DNA from PCR samples. Improvements of other aspects are underway.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.11.023.

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