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Rapid detection of human papilloma virus using a novel leaky surface acoustic wave peptide nucleic acid biosensor

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ABSTRACT

A novel leaky surface acoustic wave (LSAW) bis-peptide nucleic acid (bis-PNA) biosensor with double two-port resonators has been constructed successfully for the quantitative detection of human papilloma virus (HPV). The bis-PNA probe can directly detect HPV genomic DNA without polymerase chain reaction (PCR) amplification, and it can bind to the target DNA sequences more effectively and specifically than a DNA probe. When the concentrations varied from 1 pg/L to 1000 μg/L, with 100 μg/L being the optimal, a typical linearity was found between the quantity of target and the phase shifts. The detection limit was 1.21 pg/L and the clinical specificity was 97.22% of that of real-time PCR. The bis-PNA probe was able to distinguish sequences that differ only in one base. Both the intraassay and interassay coefficients of variance (CVs) were <10%, and the biosensor can be regenerated for ten times without appreciable loss of activity. Therefore, this technical platform of LSAW biosensor can be applied to clinical samples for direct HPV detection.

1. Introduction

Sensor science and technology has progressed very rapidly during the last 20 years. Biosensor technology has many advantages and great potential for applications in many fields such as biochemistry, food industry, environmental protection and clinical analysis. It can be used to detect specific DNA sequences in real-time without labels such as radioisotopes, enzymes, and fluorophores (Lee et al., 2005; Lucarelli et al., 2008; Nakamura and Karube, 2003; Skladal et al., 2004; Tombelli et al., 2002). With the progress of the acoustic and microelectronic technique, a new leaky surface acoustic wave (LSAW) biosensor has been developed recently. LSAW is confined strongly on the surface of the device in the range of the acoustic wavelength, regardless of the thickness of the whole substrate. For this reason, the wave is potentially sensitive to any change in the surface, such as mass loading, viscosity and conductivity changes. Further, the development of photolithographic techniques for computer and telecommunication devices to optically transfer micro- and nanostructure patterns onto the substrate allowed fabrication of nanostructures implemented in modern biosensors (Berkenpas et al., 2006; Gronewold, 2007; Hirst et al., 2008; Moll et al., 2007; Tombelli et al., 2005). We have demonstrated the construction of a multi-channel 2×5 model of piezoelectric quartz microarray biosensor, which is based on the quartz crystal microbalances (QCMs) principles (Luo et al., 2006; Zhang et al., 2004, 2007). QCMs use a thickness shear mode vibration and are bulk wave sensors, where the waves propagate in the complete piezoelectric substrate. An increase in the detection sensitivity is directly proportional to the increase in frequency. That is, the higher the frequency is, the more fragile the substrate may be, as it becomes thinner. Thus, the detection sensitivity is limited.

At present, most biosensors are immobilized with DNA probes, causing two problems. One is the unspecific hybridization between many mismatch sequences and DNA probes. The other is that the detection sensitivity is not high enough, as the DNA probes are about 20 bases and usually cannot combine with long target sequences because of the lower combining force. To solve these problems, the target sequences underwent PCR amplification before detection with biosensors, while PCR is laborious and time consuming (Mannelli et al., 2003; Marrazza et al., 2001; Tombelli et al., 2000; Zhou et al., 2001). Therefore, it greatly limits wider applications of biosensor (Chen et al., 2005). However, the PNA probe provides an excellent way to solve the above problems. Peptide nucleic acid (PNA) is a nucleic acid analog in which the sugar phosphate backbone of natural nucleic acid is replaced by a synthetic peptide backbone usually formed from N-(2-amino-ethyl)-glycine

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units, resulting in an achiral and uncharged mimic. PNA can bind to homopurine targets in double-stranded DNA via strand displacement, instead of conventional triple helix formation as observed with natural oligonucleotides. The $(PNA)_2$/DNA triplexes are stable when the Watson–Crick base pairing PNA strand is in the antiparallel orientation relative to the DNA strand and the Hoogsteen strand is in the parallel orientation relative to the DNA strand. Bis-PNA exhibits high affinity and high sequence specificity in binding to double-stranded DNA, which has been confirmed in the theoretical and practical studies (Liu et al., 2005; Nielsen et al., 1991; Steichen et al., 2007; Wittung et al., 1994a,b). The bis-PNA monomers connected by a flexible linker, may also recognize duplex homopurine sequences of DNA by strand invasion, and form a stable PNA–DNA–PNA triplex with a looped-out DNA strand (Bentin et al., 2006; Egholm et al., 1993, 1995).

In this study, we constructed a LSAW bis-PNA biosensor which allows direct detection of genomic DNA without PCR. To evaluate the accuracy of the system, we have chosen the HPV as the target pathogenic microorganism, which is a double-stranded DNA virus. Genital HPVs have been subdivided into low-risk and high-risk types. The latter includes HPV 16 and HPV 18, and is frequently associated with invasive cervical cancer (Dell'Atti et al., 2007; Matsukura and Sugase, 2008; Munoz et al., 2003). HPV 18 probe was immobilized on the gold electrode surface of the LSAW biosensor through thiol method and hybridized with complementary target sequence by an optimized procedure. The immobilized PNA probe with the complementary sequence, one base-pair mismatch sequence and two base-pairs mismatch sequence were investigated respectively. The main analytical parameters of the LSAW biosensor such as sensitivity, specificity, precision and reproducibility have been studied. Subsequently, the detection system was applied to evaluate 14 controls and 36 clinical samples which were confirmed to be HPV 18 positive by PCR.

2. Materials and methods

2.1. Reagents

Bis-PNA probe 5′-SH-$(CH_2)_6$-$(Lys)_3$-TTTTCTTCCT -$(egl)_3$-TCCTTCTTTT-Lys (egl denotes linker, SH denotes thiol group and Lys denotes lysine), was purchased from Chengdu CP Biochem Co., Ltd. (Chengdu, China). The probe was purified first with reversed-phase HPLC and characterized by MALDI-TOF spectrometry. The DNA probe labelled with thiol group at 5′-terminal (5′-SH-$(CH_2)_6$-TTTTCTTCCTCTGAGTCGCT-3′) was purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The target sequences were as follows:

- T1: 5′-AGCGACTCAGAGGAAGAAAA-3′ (complementary),
- T2: 5′-AGCGACTCAGAGGAGGAAAA-3′ (one base mismatch),
- T3: 5′-AGCGACTCAGAGGAGGTAAA-3′ (two bases mismatch).

pBR322-HPV 18 plasmid was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Plasmid extraction kit was purchased from BioDev-Tech. Co., Ltd. (Beijing, China).

2.2. Apparatus

The LSAW biosensor was obtained from the 26th Research Institute, Chinese Electronic Scientific and Technical Group Company (Chongqing, China). The data (the phase shift) were displayed on the main display screen and can be read directly by a computer connected to the network analyzer (R3767CG, ADVANTEST, Japan). The order was sent automatically to the network analyzer and the detection data were acquired from the LSAW biosensor and recorded on the computer through GPIB controlling card (Agilent Co., USA) simultaneously. Phase shift date collection, storage and analysis were processed by self-developed biosensor monitor system software (BSMS 1.0) (Fig. 1A).

2.3. Fabrication of LSAW biosensor device

The LSAW biosensor consisted of double two-port resonators including a detection channel and a reference channel and operated at 100 MHz. The device was of rectangular parallelepiped shape with dimensions of 20 mm × 10 mm and 800 μm thick, with 36° rotated, *y*-cut and *x*-propagation $LiTaO_3$ piezoelectric single crystals. Each channel included two-port resonators (Fig. 1B). The interdigital transducer (IDT) of LSAW biosensor was constructed by two single-phase unidirectional transducers (SPUDT) and a reflexed bar array on both sides of the LSAW biosensor. Each IDT was made by using metal evaporation, reactive ion etching (RIE) and ion sputtering techniques. IDT consisted of 100 electrodes, arranged in split-finger configuration to minimize overall transducer reflection. Each electrode was 5.1 μm in width and 17 μm in length, and the acoustic aperture was equal to 4.0 mm. The area between two IDTs was used for biological reaction; the upper surface of the reaction region was made from a gold-plated membrane in a vacuum environment by ion sputtering method, the piezoelectric porous layer between the gold film and $LiTaO_3$ crystals, and chromium is the preferred material (Fig. 1C).

2.4. Extraction of HPV genomic DNA

The purified full-length plasmid DNA of HPV 18 was used as a standard for the development method. Then the Hind III was used as a marker. Molecular grade pure water instead of DNA extract was used as negative control.

2.5. Immobilization of the bis-PNA and DNA probe

The probes of bis-PNA or DNA were immobilized on the surface of the crystal, which was prepared as follows. The gold membrane of the biosensor was soaked and rinsed firstly for 10 min in cleaning solution (30% H_2O_2:98% H_2SO_4 = 1:3) and then rinsed repeatedly with ultrapure water and finally dried with pure nitrogen gas. The bis-PNA and DNA probes (2.0 μmol/L) were immobilized on the gold electrode surface through the thiol method.

2.6. Responses of the LSAW biosensor in different conditions

After bis-PNA probe was immobilized on the detection channel of LSAW biosensor, 25 μL of 20 mmol/L PBS buffer with pH 6.6 was added respectively to the detection and the reference channel. The phase (P_0) was monitored until a steady baseline was obtained at 55 °C. Then 25 μL at a final concentration of 100 μg/L target genomic DNA was added respectively to the detection and reference channel to hybridize with bis-PNA probe at 55 °C. PBS buffer was added to the reference channel as negative control. The final concentration of 100 μg/L epidemic encephalitis type B solution was added to the sensor for independence control. These experiments were detected with phase. When reaction ended, the other steady-state phase was taken as P_1. The phase shifts were calculated by equation $\Delta P = P_1 - P_0$. The equilibrium time for hybridization was also recorded.

Duplicate tests of the HPV DNA were performed by detecting low, medium and high concentrations containing 10 μg/L, 100 μg/L and 1000 μg/L. For each sample, six times repeated tests in one day have been performed for intraassay. These tests were repeated in 6 consecutive days (mean of three duplicates per day) in the same manner for interassay.

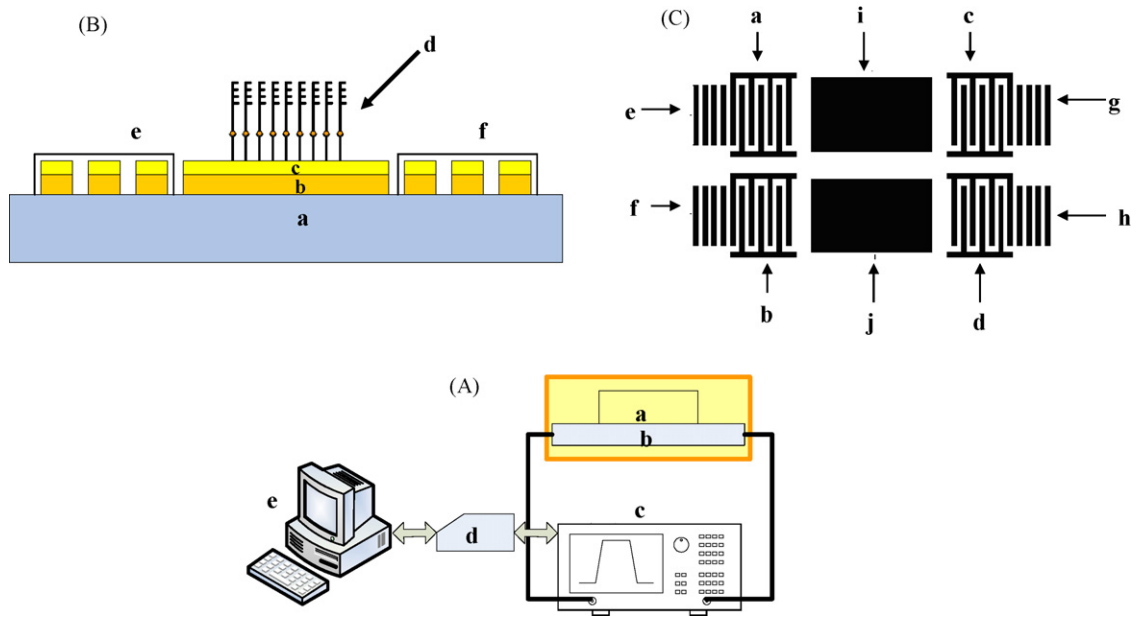

Fig. 1. (A) The detecting platform of LASW biosensor. (a) Hybrid cell, (b) LSAW biosensor, (c) network analyzer, (d) GPIB card, and (e) personal computer. (B) Schematic view of the leaky surface acoustic waves (LSAW) biosensor. (a) The lithium tantalate ($LiTaO_3$) piezoelectric crystals, (b) chromium (Cr) can enhance the adsorption capacity between $LiTaO_3$ crystal and the gold membrane, (c) reaction area made of a gold-plated membrane, (d) biology sensitivity layer, (e) input resonator, and (f) output resonator. (C) The double two-port resonators. (a) Input IDT of detection channel, (b) input IDT of reference channel, (c) output IDT of detection channel, (d) output IDT of reference channel, (e) input reflexed bar array of detection channel, (f) input reflexed bar array of reference channel, (g) output reflexed bar array of detection channel, (h) output reflexed bar array of reference channel, (i) reaction area of detection channel, and (j) reaction area of reference channel.

The same concentration probes were immobilized and hybridized with complementary targets. Experiments were repeated ten times. After each reaction, 1 M HCl, 1 M NaOH and piranha solution were used respectively to wash the reaction area and to explore the regeneration of the sensor.

2.7. Hybridization reaction

2.7.1. Effect of target sequence concentration

The procedures were similar to those mentioned above. Bis-PNA probe (2.0 μmol/L) was first immobilized on the surface of LSAW biosensor. Then 25 μL target HPV genomic DNA with different concentrations (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1.0, 10, 10^2, 10^3 μg/L) were added respectively to the detection and reference channel to hybridize bis-PNA probe at 55 °C. The phase shift (ΔP) and equilibrium time of the reaction were recorded and analyzed. Linear regression was applied between phase shift and the target concentration. Hence, the linear range was determined.

2.7.2. Hybridization with synthetic oligonucleotides targets

Bis-PNA and DNA probes (2.0 μmol/L) were immobilized on the biosensor at room temperature. After PBS buffer was equilibrated at 55 °C, 2.0 μmol/L target sequences T1, T2 and T3 for final concentration were added to the detection and reference channels respectively. The shift of phase (ΔP) and the equilibrium time for hybridization reaction were recorded.

2.7.3. Hybridization of clinical samples

The study was approved by the local ethics committee, and participants signed informed consent. Clinical samples (subjects' secretions) from patients undergoing routinely HPV screening and from healthy volunteers at Southwest Hospital (Chongqing, China) were removed from the spatula and collected in a Falcon tube containing sterilized saline water. The DNA extraction was performed by using DaAn Gene DNA kit (DaAn Gene Co., Ltd. Guangzhou, China) according to the manufacturer's protocol.

Different concentrations of HPV genomic DNA were obtained from 36 patients and 14 negative controls. HPV has been detected previously in the samples using the reference method of PCR (LightCycler® II, Roche). The detection of HPV 18 in the clinical samples was performed by the LSAW biosensor. Bis-PNA probe (2.0 μmol/L) was immobilized firstly on the detection channel as described above. Then 25 μL of the solution of HPV 18 DNA extracted from each subject's secretions was added respectively to the detection channel and reference channel to hybridize with the bis-PNA probe at 55 °C. The phase shift and the hybridization time were also recorded.

2.8. Statistical analysis

All experiments were performed at least three times. Data were analyzed with SPSS 13.0 statistical software. Phase shift data were presented as the mean values ± standard deviation. One-way ANOVA was used to determine the statistical significance of the differences among target concentration groups. Paired-samples *t*-test was used to compare the difference between PNA and DNA probes. McNemar test was used to compare the difference between LSAW biosensor and PCR. Statistical significance was set to $p < 0.05$. Kappa test was used to compare the consistency between LSAW biosensor and PCR. Statistical consistency was set at $p < 0.01$.

3. Results

3.1. Stability and precision

The dual channel circuit developed in our team was of strongly vibromotive ability in liquid phase and its stability of phase could also meet the need of our experiment ($\Delta P \leq 0.2°$). The stability and consistency of dual channels sensor were very good either in liquid or gas phase.

Real-time monitoring of HPV reaction was performed by using the LSAW biosensor system and the method of detecting phase. During the process of hybridization, the phase can reflect the qual-

Table 1
Precision and measured concentrations obtained in LSAW biosensor.

Target concentration	Intraassay ($n=6$)			Interassay ($n=6$)[a]		
(μg/L)	Mean (°)	S.D. (°)	CV (%)	Mean (°)	S.D. (°)	CV (%)
10.00	2.17	0.13	5.99	2.38	0.17	7.14
100.00	2.84	0.18	6.34	2.96	0.21	7.09
1000.00	2.07	0.14	6.76	2.25	0.19	8.44

[a] Interassay was conducted on 6 sequential days, with 3 runs/day and with 3 replicate tubes per day at every target DNA concentration.

ity load. The phase shift of the detection channel was significantly obvious but the phase shift of the reference channel was not. The ΔP between two channels was the real change induced by the biological reaction, and it could eliminate effectively the influence of the environment. So in this hybridization reaction, phase shift was 2.8°. However, phase shift was not found in the independent control and negative control. This is shown in supplementary information S1.

The intraassay and interassay CV for the novel LSAW biosensor were 5.99% and 7.14% for 10.00 μg/L, 6.34% and 7.09% for 100.00 μg/L, 6.76% and 8.44% for 1000.00 μg/L respectively (Table 1). The analytical precision was also calculated with 3 replicates in one analytical run on a single control. The intraassay CV showed very low SD (mean CV = 6.36%) and the interassay CV had a slightly higher SD (CV = 7.56%), however, they were both acceptable for clinical detection (CV < 10%).

3.2. Calibration curves and linearity

The calibration curve was obtained by incubating variable concentrations of target DNA with bis-PNA probe (Fig. 2). The phase shift was enhanced firstly and then tended gently to hybridization between the probe and the target DNA when concentration of target DNA varied from 1 pg/L to 1000 μg/L, and 100 μg/L was the optimal. No evident change tendency was observed in equilibrium

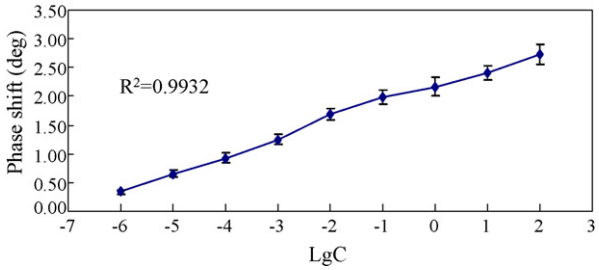

Fig. 2. Standard curve for quantitative detection of HPV DNA with LSAW biosensor. C: Target sequence concentration (μg/L), LgC: target concentration denary logarithm. The plots show the phase shift (°) on the *y*-axis as a function of increasing HPV concentrations on the *x*-axis. Each data point represents an average of least three times measurements ± standard deviation.

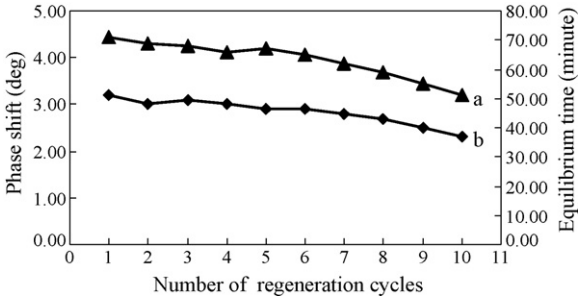

Fig. 3. Relationship of responses activity changes of LSAW biosensor and number of regeneration cycles. (a) Equilibrium time and (b) phase shift.

time for hybridization. The statistical linear regression equation was $\Delta P = 0.3010\mathrm{lgC} + 2.1753$ among the range of target concentration from 1 pg/L to 100 μg/L. The correlation coefficient was 0.9932 according to the statistical analysis. Moreover, no phase shift was detected by the biosensor when the PBS buffer was negative control.

3.3. Specificity of the bis-PNA probe

The bis-PNA probe showed much higher specificity on hybridization with the DNA target sequence than the DNA probe (Table 2). The phase shift was about 0.71° when bis-PNA was hybridized with T2, which had one mismatch, while it shifted about 1.56° for the DNA probe. No significant response was detected for bis-PNA probe with the two bases mismatch sequence. However, the phase shift was 0.91° for the DNA probe with the two bases mismatch sequence. The results showed that the bis-PNA probe was able to distinguish the complementary strand from the one base mismatch strand. At the same time, the PNA probe gave a short hybridization equilibrium time.

3.4. Reproducibility

The experiment was repeated ten times with one biosensor and one sample, and there were observed no significant changes of phase and equilibrium time. After five regenerating cycles, the binding activity of the biosensor has retained 90.6% of the original, and after ten regenerating cycles, the binding activity has retained 71.9% of the original. Value of CV remained (CV = 9.71%), i.e., less than 10%, which indicates a good reproducibility of the LSAW biosensor (Fig. 3).

3.5. Quantitative detection of clinical sample and comparison

Among 36 clinical cases being diagnosed for HPV 18 infection by PCR, 35 samples were detected correctly by the LSAW biosensor. The positive rate was 97.22% (Table 3). The DNA concentrations of HPV 18 in the 36 clinical cases varied from 1.40×10^2 to 9.11×10^7 copies

Table 2
Results of match or mismatch target sequences hybridized to bis-PNA and DNA probes.

Immobilized probe	Target	ΔP for target reaction (°) ($\bar{x} \pm$ S.D.)	CV (%)	Equilibrium time for target reaction (min) ($\bar{x} \pm$ S.D.)
PNA	T1	$2.87 \pm 0.22^{*}$	7.67	$60.45 \pm 9.32^{**}$
	T2	$0.71 \pm 0.10^{*}$	14.08	$48.98 \pm 7.34^{**}$
	T3	ND[*]	ND[*]	ND[*]
DNA	T1	2.03 ± 0.24	11.82	65.58 ± 10.24
	T2	1.56 ± 0.14	8.97	59.67 ± 8.47
	T3	0.91 ± 0.13	14.29	51.32 ± 8.19

ΔP: Phase shift. ND: Not determined. bis-PNA probe group vs. DNA probe group.
[*] $p < 0.01$.
[**] $p < 0.05$.

Table 3
Comparison results of PCR and LSAW biosensor for the detection of HPV genomic DNA.

Quantitative PCR method	LSAW biosensor method		Total
	Positive	Negative	
Positive	35	1	36
Negative	0	14	14
Total	35	15	50

The amount of HPV genomic DNA in each clinical sample was compared with a conventional PCR.

per milliliter clinical sample (i.e., 1.21 pg/L to 0.78 μg/L). The HPV 18 was not detected in the 14 controls with either of the two methods. The range of phase shift was from 1.1° to 4.5° and the time consumed was from 38 min to 75 min. The noise of the system was about 0.24° in our preliminary test. The detection limit appeared when the signal was three times stronger than the noise. The phase shift was 1.1° when the DNA concentration was 1.21 pg/L (four times bigger than the noise), so it was chosen as the lowest detection limit. According to the Kappa test, $p < 0.01$, Kappa > 0.75, there exists the significant consistency between the two methods.

4. Discussion

Cervical carcinoma is one of the most common neoplasias among women worldwide. Infection of HPVs is involved in the carcinogenesis of this cancer (Braun et al., 2004). Early detection for HPVs is crucial for early treatment and improved the outcome of cervical carcinoma. Therefore, fast detection method is needed urgently for the detection of HPV. To our knowledge, this is the first study to detect HPV genomic DNA by using LSAW biosensor, providing a rapid, sensitive, specific, and convenient technique for the detection of dsDNA in the clinical laboratory.

In QCM sensor, the energy is distributed throughout the substrate, which reduces the energy and sensitivity on the sensing surface. However, in LSAW biosensor, the energy propagation path is highly localized inside the guiding layer (Gronewold, 2007). The resonator of LSAW biosensor is composed from a pair of IDTs and reflexed bar array. IDTs can generate and detect an acoustic wave propagating on the same surface of a piezoelectric substrate. Reflexed bar array can increase the signal noise ratio and the phase shift of the signal detection. In the previous experiment, we found the resonator sensor improved the sensitivity of biosensor obviously and reduced equilibrium time of the hybridization reaction compared with the delay linear style sensor. Besides its high sensitivity, LSAW biosensor also has superiority such as small size, robustness, and low fabrication cost (Chiu and Gwo, 2008; Lange et al., 2008). Hence, the biosensor has a wide variety of biomedical applications.

Time saving is a significant feature of the LSAW biosensor. The total time spent on sample preparation, detection cell assembly and real-time detection was within 3 h, less than in the existing methods. The clinical samples can be detected directly without PCR amplification, and detection cell can be assembled in 1 min before real-time reaction procedure, which can be monitored via the phase changing trends on the screen, resulting in the shortened total analysis time.

Precision can reflect the stability of a detection method. In our study, CV of the interassay was slightly higher than that of the intraassay. However, they were both lower than 10%. Our study and others' experiments indicate that the stability of the LSAW biosensor system is satisfactory (Conneely et al., 2007; Mytych et al., 2009).

PNA probe has high specificity and it can be used to distinguish matched and mismatched base pairs correctly and easily. If there is one mismatched base pair in about 15 base pairs in a PNA–DNA hybrid, the Tm would decrease by 8–20 °C. They cannot be hybridized entirely with two bases mismatch. In this experiment PNA probe with different target sequence hybridization had a significant difference in phase shift. However, when the PNA probe hybridized with the two mismatch bases of the target sequence, the phase did not change. This implies the PNA hybridized with nucleic acid sequences with high sequence specificity. However, when the DNA probe hybridized respectively with three kinds of target sequences with different complementary degrees, the phase shift was not significantly different, indicating a low specificity of DNA probe to mismatch base. Therefore, the PNA probe can improve the detection specificity of the sensor.

According to the trend line, saturation is encountered with more than 100 μg/L HPV genomic DNA because of the complete combination of all immobilized bis-PNA. In this case, if the concentration of the target sequence increased, the phase shift cannot become greater, which suggests clearly that the LSAW biosensor can provide quantitative determination of HPV in the range from 1 pg/L to 100 μg/L. The linear range is within the most ordinary variant range of the HPV concentrations in clinical samples as detected by quantitative PCR.

The regeneration of the biosensor is an important factor for the development of practical biosensors. To reuse the biosensor, it is necessary to use some reagents to destruct the key connection between probes and targets, which can make the sensor surface cleaned completely. Therefore, we carried out experimental use of reproducibility of biosensor. The results have shown that the LSAW biosensor has good reproducibility. 1 M HCl, 1 M NaOH and cleaning solution could wash off everything covered on the reaction area including probe, target and hybridized nucleic acid molecules. The reaction area of the LSAW biosensor and IDTs was covered by gold on the surface of $LiTaO_3$ crystal. Thus, it cannot be injured by the cleaning process.

HPV genomic DNA from clinical samples can be detected directly with bis-PNA probe. HPVs are subdivided into low-risk and high-risk types (HPV 16 and 18). In this experiment, the universal primers of HPV 16 and HPV 18 were used in the PCR, but our bis-PNA was highly specific for HPV 18. The sample not detected successfully may be HPV 16. In our previous experiment, Hepatitis B virus (HBV) genomic DNA from clinical samples can be detected directly with QCM, with the lowest detection limit 8.6 pg/L (Yao et al., 2008). Therefore, sensitivity is about seven times as high as the QCM sensor. The LSAW biosensor has significant importance for clinical application. Detection limit of LSAW biosensor was 1.21 pg/L to 0.78 μg/L, which was calculated by quantitative PCR. The range of HPV DNA concentrations was located in the detecting linear range of the LSAW biosensor exactly.

5. Conclusions

In this study, we developed a novel LSAW biosensor capable to rapidly detect HPV with high selectivity and sensitivity. This detector combines the sensitivity of LSAW with the specificity of bis-PNA recognition and provides a highly versatile platform for the detection of other viral bimolecular. The LSAW biosensor has many advantages with the well-controlled surface structure, such as its reproducibility of the assay, the easier regeneration of the biosensor and prevention of nonspecific adsorption. The LSAW biosensor is of good stability, precision, reproducibility, automatization and real-time detection, which can meet the needs of clinical laboratory diagnosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.04.034.

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