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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.