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High-throughput SPR sensor for food safety

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ABSTRACT

High-throughput surface plasmon resonance (SPR) biosensor for rapid and parallelized detection of nucleic acids identifying specific bacterial pathogens is reported. The biosensor consists of a high-performance SPR imaging sensor with polarization contrast and internal referencing (refractive index resolution 2×10^{-7} RIU) and an array of DNA probes microspotted on the surface of the SPR sensor. It is demonstrated that short sequences of nucleic acids (20–23 bases) characteristic for bacterial pathogens such as *Brucella abortus*, *Escherichia coli*, and *Staphylococcus aureus* can be detected at 100 pM levels. Detection of specific DNA or RNA sequences can be performed in less than 15 min by the reported SPR sensor.

1. Introduction

Biosensors represent a promising technology for detection of chemical and biological analytes. In recent years, biosensors were increasingly used in numerous important applications such as medical diagnostics, environmental monitoring, food safety, and security (Farre et al., 2007). Current biosensors utilize optical, mechanical, or electrochemical transducers. Electrochemical sensors employ enzymes or other electrochemically active compounds to generate electrical signal (Palchetti and Mascini, 2008). Biosensors with mechanical transducers include microcantilevers and bulk or surface acoustic wave sensing devices (Lange et al., 2008; Sobel and Ballantine, 2008). Optical biosensors based on surface plasmon resonance (SPR) provide high sensitivity without the use of molecular labels. In addition, they show promise for development of high-throughput systems for parallelized measurements (Homola, 2008). The number of publications on SPR biosensors for detection of analytes implicated in food quality and safety has been growing (Lazcka et al., 2007; Patel, 2006). SPR biosensor-based detection of bacterial pathogens has been demonstrated employing immunoreactions between bacteria and specific antibodies immobilized on the SPR sensor surface (Oh et al., 2002; Taylor et al., 2006). Alternatively, bacterial pathogens can be measured by detecting their specific nucleic acids, such as rRNA sequences which can be extracted from bacterial pathogens (Nelson et al., 2002). This approach provides high specificity, stability and speed compared to antibody-based detection of whole bacteria (Wang et al., 2007). Furthermore, it takes advantage of the well-developed DNA array technology that is compatible with high-throughput operation format desired in many important applications (Boozer et al., 2006; Nelson et al., 2002). The most common approach to parallelized SPR sensing is SPR imaging (Rich and Myszka, 2006; Schaferling and Nagl, 2006; Wassaf et al., 2006). The main drawback of the SPR imaging is its rather limited refractive index resolution and limit of detection, which are typically one or two orders of magnitude worse than those provided by the best SPR sensors based on spectroscopy of surface plasmons (Nelson et al., 2001; Phillips and Cheng, 2007). Recently, an alternative approach to SPR imaging employing polarization control has been developed. The introduction of polarization control in SPR imaging has significantly improved both sensitivity and operating range, bringing the performance of SPR imaging sensors close to that of the best high-resolution sensors based on spectroscopy of surface plasmons (Piliarik and Homola, 2008; Piliarik et al., 2007).

In this paper, we combine a novel SPR biosensor platform based on SPR imaging with polarization contrast and internal referencing and a DNA array to provide a platform for detection of fragments of nucleic acids identifying DNA or RNA sequences specific for selected foodborne pathogens.

2. Materials and methods

2.1. Surface plasmon resonance imaging sensor

In this study, we use a novel self-referencing SPR imaging sensor with polarization contrast developed at the Institute of Photonics

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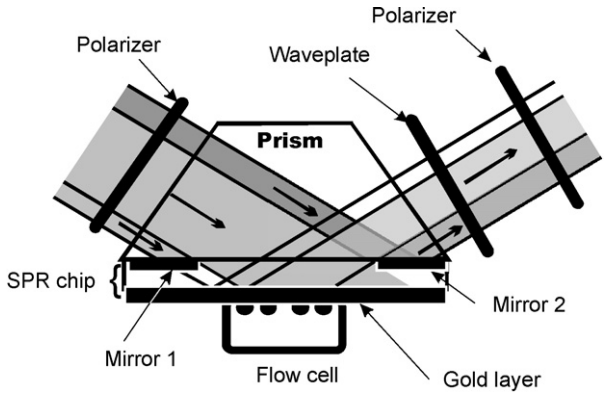

Fig. 1. Concept of SPR imaging with polarization contrast and internal referencing.

and Electronics (Piliarik and Homola, 2008). This sensor is based on the Kretschmann geometry of the attenuated total reflection method and prism coupling of light into a surface plasmon (Raether, 1988). In this configuration, the intensity and polarization of the reflected light depend on the coupling strength of the incident light beam to the surface plasmon. Therefore, a spatial distribution of refractive index changes can be correlated with a two-dimensional light intensity distribution as measured on a detector. A narrow-band light emitted by a superluminescent diode (central emission wavelength: 750 nm; full width at half maximum: 20 nm) is collimated and passed through a polarizer, a quarter-wave plate and made incident on the base of the coupling prism. Light reflected from the gold layer on the SPR chip interfaced with the prism is passed through another polarizer and imaged on a CCD camera. Telecentric imaging optics (Edmund optics GmbH, Germany) is used for the imaging to preserve array dimensions and avoid image distortion. Two gold mirrors are prepared on the prism surface and the SPR chip is attached directly to this surface via a refractive index matching fluid (Fig. 1). The light blocked and reflected by these mirrors provides dark and bright reference signals for real-time compensation for fluctuations in the dark current and intensity of incident light, respectively. Images acquired from the CCD camera are averaged (100 frames per record) and intensities from pixels within each measurement area are binned. The two polarizers and the wave-plate are set to extinguish the reflected light corresponding to a refractive index which is slightly lower than that of the used running buffer (Piliarik and Homola, 2008). Near this refractive index, a change in refractive index at the surface of the gold film results in a change in the polarization of reflected light and consequently in an increase in the intensity of transmitted light. An acrylic flow cell with gaskets made of 50-μm thick adhesive Mylar foil is pressed against the SPR chip to contain a liquid sample during the experiment. The gasket forms six flow chambers on the sensor surface, each containing a group of sensing channels. Each flow chamber is 0.9-mm wide. Length of the section of the flow chamber imaged on the CCD camera is 6.4 mm. A multichannel peristaltic pump is used to deliver six different samples simultaneously into the flow cell.

2.2. Refractive index calibration

In the refractometric experiment, a series of liquid samples with known refractive indices were sequentially flowed through the flow-cell. The samples were water solutions with different amounts of NaCl and their refractive indices were determined using a commercial refractometer (DSR-lambda, Schmidt Haensch GmbH, Germany). The samples were sequentially pumped through the flow-cell and incubated with the sensor surface until a stable baseline was established. One hundred and twenty measuring areas were defined in the image of the sensor surface and intensity was averaged across each area to yield output signal for each sensing channel. The output signal was normalized using the bright and dark signals reflected and blocked by the reference mirrors and the sensor output was calculated for each sensing channel in real time.

2.3. Selection of sequences

Five short DNA sequences were selected as a model system to represent specific fragments corresponding to RNA sequences from 16S ribosomal RNA of three different bacterial pathogens (*Brucella abortus*, *Escherichia coli*, and *Staphylococcus aureus*). The selected sequences are known to specifically identify these pathogens. 5′-(CGC TCC AGC CTA ACT GAA)-3′ (Herman and Deridder, 1992) and 5′-(TCC AGC CTA ACT GAA CCA TA)-3′ (Romero et al., 1995) are specific for *B. abortus*, 5′-(GTC CCC CTC TTT GGT CTT GC)-3′ (Nelson et al., 2002) and 5′-(TAT TAA CTT TAC TCC CTT CC)-3′ (Huijsdens et al., 2002) are specific for *E. coli*, and 5′-(CCT AAA AGG TTA CTC CAC CGG CT)-3′ (Greisen et al., 1994) is specific for *S. aureus*. The DNA targets were used instead of ribosomal RNA fragments mainly because of better stability of the DNA.

2.4. Reagents

Oligo(ethylene glycol) alkanethiol (OEG) (HS-C11-EG2-OH) was purchased from ProChimia Surfaces (Poland). Bovine serum albumin (BSA), tris buffer, phosphate buffered saline (PBS) (0.01 M phosphate, 0.137 M sodium chloride, 0.0027 M potassium chloride, pH 7.4), sodium chloride (NaCl) and magnesium chloride ($MgCl_2$) were purchased from Sigma–Aldrich (St. Louis, MO). Thiolated DNA sequences Bab970SH (5′-/5ThioMC6-D/CGC TCC AGC CTA ACT GAA-3′), Bab960SH (5′-/5ThioMC6-D/TCC AGC CTA ACT GAA CCA TA-3′), E441SH (5′-/5ThioMC6-D/TAT TAA CTT TAC TCC CTT CC-3′), E180SH (5′-/5ThioMC6-D/GTC CCC CTC TTT GGT CTT GC-3′) and Sau1463SH (5′-/5ThioMC6-D/CCT AAA AGG TTA CTC CAC CGG CT-3′) were purchased from Integrated DNA Technologies (Coralville, IA). Bab970SH and Bab960SH are specific to *B. abortus*, E441SH and E180SH are specific to *E. coli*, and Sau1463SH is specific to *S. aureus*. Complementary DNA sequences Bab970c (5′-TTC AGT TAG GCT GGA GCG-3′), Bab960c (5′-TAT GGT TCA GTT AGG CTG GA-3′), E441c (5′-GGA AGG GAG TAA AGT TAA TA-3′), E180c (5′-GCA AGA CCA AAG AGG GGG AC-3′), and Sau1463c (5′-AGC CGG TGG AGT AAC CTT TTA GG-3′) were purchased from Masaryk University (Brno, Czech Republic).

2.5. Preparation of SPR DNA chips

SPR sensor chips, made of BK7 glass (Schott, Inc., USA), were cleaned in a UV-ozone cleaner and coated with Ti adhesion layer (thickness ~1) and SPR active Au layer (thickness: 49.5 nm) by electron beam evaporation in vacuum. Coated chips were rinsed with absolute ethanol, deionized water and blown dry with nitrogen. Chips were then cleaned in a UV-ozone cleaner for 20 min, followed by rinsing with absolute ethanol and deionized water and dried with nitrogen stream. An array of sensing channels was prepared on the sensor surface by means of microspotting solution of 10-μM thiolated DNA probes in PBS. Commercial microarrayer OmniGrid (Genomic Solutions, USA) was used for the microspotting of sensing channels in such a way that the area corresponding to each flow chamber contained a series of channels with different DNA probes (three spots per sequence). Stealth pins (946MP9, TeleChem International, Inc., USA) with the tip dimensions approximately 300 μm × 300 μm and with an uptake channel for large arrays printing were utilized to create sensing channels of dimen-