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Research Paper

A Portable Surface Plasmon Resonance Biosensor for Rapid Detection of *Salmonella typhimurium*

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Abstract Here, the rapid detection of *Salmonella typhimurium* by a portable surface plasmon resonance (SPR) biosensor in which the beam from a diode laser is modulated by a rotating mirror is reported. Using this system, immunoassay based on lipopolysaccharides (LPS)-specific monoclonal anti-*Salmonella* antibody was performed. For the purpose of orientation-controlled immobilization of antibodies on the SPR chip surface, the cysteine-mediated immobilization method, which is based on interaction between a gold surface and a thiol group (-SH) of cysteine, was adopted. As a result, using the portable SPR-based immunoassay, we detected *S. typhimurium* in the range from 10^7 CFU/mL to 10^9 CFU/mL within 1 hour. The results indicate that the portable SPR system could be potentially applied for general laboratory detection as well as on-site monitoring of foodborne, clinical, and environmental agents of interest.

Keywords: Biosensor, surface plasmon resonance, portable SPR, *Salmonella typhimurium*, Foodborne pathogen

I. Introduction

Salmonella is a group of bacteria that can cause food poisoning [1]. Rapid detection of *Salmonella* is of great importance in the food industry and in the field of public health [2]. So far, many analytical methods based on enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of *Salmonella* [3,4]. Although ELISA is one of the most widely used techniques for detection of antigens, various enzymes, and reagents for colorimetric, fluorescent, or luminescent measurement are used in ELISA. Also, numerous PCR-based formats for *Salmonella* detection in food samples have been developed [5,6]. Recently, Woubit et al. have developed a 96-well PCR-microplate array adapted to read twelve food-threat agents and related pathogens including *Salmonella typhimurium* [7]. Nevertheless, PCR methods include laborious and time-consuming steps such as DNA isolation, DNA amplification, or DNA hybridization. Thus, development of a rapid, simple, sensitive, specific, and cost-effective technique for detection of *Salmonella* is necessary in the food industry.

As a label-free technique, the surface plasmon resonance (SPR) system does not require any tag or dye to recognize biomolecules such as nucleic acids, proteins, peptides, small molecules, and cells. SPR angle changes result from alterations in the refractive index upon binding of analytes to the gold surface. To date, a wide variety of SPR biosensors have been developed for various applications including foodborne pathogen detection, environmental monitoring, and medical diagnosis [8-10]. From a practical point of view, miniaturization is important in the development of a portable SPR-type biosensor. Traditional SPR devices are of large size and heavy weight. Thus, they are not portable or cost-effective for use in point-of-care (POC) applications. In this regard, it is noteworthy that current need for the rapid detection of biohazardous agents such as bacterial or viral pathogens that endanger public safety is driving increased demand for a portable SPR system.

A variety of immunoassays based on SPR-type sensors have been developed for detection of *Salmonella* [11-13]. The SPR system is capable of measuring biomolecular interactions in a rapid real-time manner, which is how immunobiochemical reactions occur. The SPR signal usually depends on the size of analytes to be detected directly after their binding to the antibody-modified gold surface. For this, an SPR biosensor could be a promising tool to monitor *Salmonella* infection, as bacterial cells are large enough to induce huge changes in SPR angle. Nevertheless, there have been very few studies on portable SPR systems for detection of *Salmonella*. In this study, a portable SPR-based

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immunoassay system using lipopolysaccharide (LPS)-specific monoclonal anti-*Salmonella* antibody was developed; its applicability is shown for general laboratory measurement as well as on-site monitoring of foodborne, clinical, and environmental materials.

II. Materials and Methods

1. Materials

Phosphate buffered saline (PBS, P4417), Bovine serum albumin (BSA, A7906), and thimerosal (T2299) were purchased from Sigma Aldrich Inc. (St. Louis, MO). Anti-Salmonella antibody directed against lipopolysaccharides (LPS) (ab8274) was purchased from Abcam Inc. (Cambridge, MA). The *S. typhimurium* cells (KCTC 2515) used in this study were obtained from the Korean Collection for Type Cultures (KCTC). The portable SPR instrument was purchased from MiCoBioMed CO., LTD. (Korea).

2. Growth curve and preparation of antigen: *Salmonella typhimurium*

The growth curve of *S. typhimurium* was obtained using the standard plate count (SPC) method. *S. typhimurium* cells were grown in sterile liquid LB broth at 37°C during an incubation period of 24 hours. The culture medium was treated with thimerosal (1%, w/w) and incubated at room temperature for 36 hours to destroy *S. typhimurium* cells. The LB medium containing the killed *S. typhimurium* was centrifuged at 4,000 rpm for 10 minutes at room temperature. The precipitated *S. typhimurium* cells formed pellets on the bottom of the tube and were resuspended in fresh LB medium. The cellular pellets were washed three times with at least 3 ml of PBS (pH 7.4) buffer. Then, the final pellets were suspended again in the same buffer and stored at 4°C until used.

3. Preparation of Cys-tagged protein G

The Cys-tagged protein G construct was prepared as described previously [14]. Briefly, the plasmid, which contains the gene encoding the IgG-binding protein G and a Cys-tag, was transferred into *Escherichia coli* BL21 (DE3). The transformed cells were grown at 37°C until OD_{600} reached 0.6; isopropyl-β-d-thiogalactopyranoside (IPTG) was then added to a final concentration of 1 mM for protein induction. The transformants were grown at 25°C for an additional 16 hours. The recombinant protein was expressed in *E. coli* BL21 (DE3), and purified via immobilized metal affinity chromatography (IMAC). The purified protein was then stored in PBS (pH 7.4). The Bradford method with bovine serum albumin (BSA) was used to determine the protein concentration.

4. Configuration of a portable SPR system

The SPR sensor system using a rotating mirror uses laser diode rather than LED as a light source for emitting the incident light to the gold thin film. The rotating mirror having cylindrical shapes reflects the polarized incident light for radiating light with disk-shape. A cylinder lens help to focus the light rays which pass through a light shielding film, and the gold thin film absorbs the focused light to create surface plasmon resonance. A dielectric medium was provided under the gold thin film, and the light reflected from the metal thin film was captured by a CMOS image sensor.

5. Surface modification and SPR immunoassay

A pre-cleaned gold chip was rinsed with PBS (pH 7.4), which was used as a running buffer. The cysteine-tagged protein G at a concentration of 0.2 μg/mL was attached to the gold surface at room temperature by 100 μL injection of the solution through the SPR channel at a flow rate of 5 μL/min. Subsequently, anti-*Salmonella* antibody at a concentration of 0.1 μg/mL was immobilized in the cysteine-tagged protein G for *Salmonella* binding at the same flow rate. Then, the blocking buffer (PBS with 1% BSA), which will bind to all potential sites of nonspecific interaction, was applied on the antibody-modified surface to prevent non-specific binding. To evaluate the sensing performance of the portable SPR sensor in response to *Salmonella*, the bacterial sample solutions were introduced into the channel at concentrations of 10^7, 10^8, and 10^9 CFU/mL. The sample solutions were delivered into the channels using syringe pumps (Cole-Parmer, USA). All measurements were performed at room temperature.

III. Results and Discussion

1. Growth characteristics of *Salmonella typhimurium*

Figure 1 shows the *Salmonella* growth curve, obtained from the standard plate count (SPC) method, the most routinely used method for quantifying bacteria in foods. The growth curve of *S. typhimurium* exhibited three distinct phases including the lag phase (1-3 hours), the log or exponential phase (3-8 hours), and the stationary phase (8-10 hours), which are similarly shown in a typical growth curve for bacterial populations. The wild type *S. typhimurium* yielded a CFU count of approximately 14 $\log_{10}$CFU/mL at 8 h after incubation, and remained at that level.

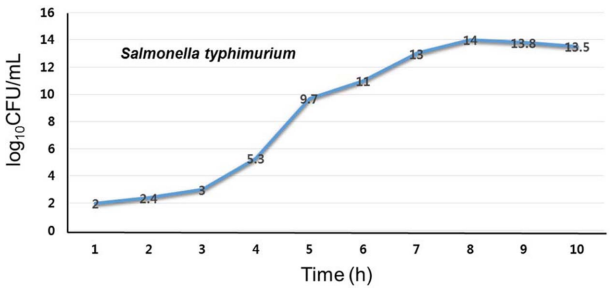

Figure 1. Graphic representation of typical growth curve of *S. typhimurium* in culture medium.

2. Characteristics of rotating mirror-based SPR instrument

Surface plasmons (SPs) are composed of oscillating waves of free electrons that travel along the interface between a metal thin film and a dielectric. When incident light enters a thin layer region via a dielectric medium (i.e., a prism), the SPs are excited and cause a plasmon resonance wave if the incident angle exceeds the critical angle of the prism. Surface plasmon resonance (SPR) is characterized by the resonant oscillation of free electrons. SPR angle (or resonance angle) is highly sensitive to variations of the refractive index. Thus, the value of the resonance angle depends on changes in the refractive index of the metal surface. SPR detection modules are based on this theoretical background for detection of a wide range of target analytes. The prism-based Kretschmann configuration, in which a laser is used as a light source because it can deliver a fast-pulse and high-intensity beam to a target, is adopted in most SPR applications to measure the SPR angle. In this mode, reflected light may cause flaring or ghosting, resulting in an interference problem, which can deteriorate the image quality [8,15]. In order to establish an SPR detection system for more elaborate measurement of analytes, an electrical measurement unit with extreme stability and precision is required. For this reason, the use of lasers as light sources is not suitable for the development of compact and portable SPR biosensor systems. To solve this issue, a light-emitting diode (LED) could offer a hopeful alternative to laser-based systems, because LEDs produce no interference problems. SPR sensors normally use an angular reflection spectrum based on a monochromatic light source. However, LED light is not monochromatic, while a laser emits nearly monochromatic light, occurring at a single wavelength. The LED spectral widths range from 20 nm to 170 nm; this range induces a broad angular spectrum in an SPR sensor, lowering the SPR sensitivity. In this case, an additional monochromatic filter should be applied to offer a narrow range of wavelengths. Oscillation mirror-based instruments, in which a mirror adjusts the incident angle at which surface plasmons are excited, also allow the detection of analytes of interest. However, for the mirror format, the oscillation mirror should be exactly synchronized with the optical excitation of the photodiode to read the intensity of the reflected light. For this reason, oscillation mirror-mediated SPR systems may not be suitable for portable applications. To solve the abovementioned problems, a rotating mirror instead of a prism as a dielectric medium was integrated into the SPR system to develop a hand-held SPR device (Figure 2).

The rotating mirror adjusts the angle of incidence, optimizing it for the SPR. Using this portable SPR to modulate the laser beam with a rotating mirror of cylindrical shape, changes in resonance angle were determined by measuring the reflected intensity of light upon binding of *Salmonella* to the immunologically modified SPR surface.

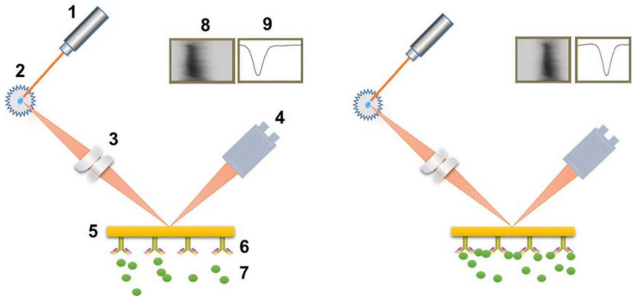

Figure 2. Configuration of a rotating mirror-based SPR system.1. Laser diode; 2. Rotating mirror; 3. Cylinder lenses; 4, CMOS image sensor; 5. Gold film; 6. Probe antibody; 7. Analytes; 8. Reflected SPR image; 9. Linear profile. Adapted from [15].

The reflected rays in the portable SPR system are likely to contain light of one wavelength and to travel in a straight line, thereby minimizing the interference phenomenon and maximizing the uniformity of the light intensity distribution. As a readout device, a CMOS image sensor was exploited to read the light reflected off the metal layer (gold thin film); the SPR signal was transferred to a laptop computer via USB port. The flow cell consists of two channels with dimensions of 5.5 mm (L)×1.0 mm (W)×0.2 mm (D) on a single SPR chip.

3. Orientation-controlled immobilization of antibody using cysteine-tagged protein G

For the purpose of orientation-controlled immobilization of antibodies on the SPR sensor surface, the cysteine-mediated immobilization method, which is based on the interaction between a gold surface and the thiol group (-SH) of cysteine, was adopted. Particularly, protein G fused with Cys-tag was used as an antibody-capturing agent because protein G has the ability to bind to fragment crystallizable regions (Fc regions) of heavy chains of antibodies. The protein G-mediated antibody immobilization strategy is a single step method with no need for antibody pre-modifications such as pre-activation or coupling. Therefore, immobilized antibodies mediated by protein G do not lose their binding strength or specificity. Studies have demonstrated that an SPR immunosensor based on the orientation-controlled immobilization of antibodies mediated by Cys-tagged protein G offered considerably enhanced sensitivity compared with conventional covalent immobilization methods, which are prone to random orientation of probe antibodies. Lee et al. reported that a protein G-mediated orientation-controlled immobilization technique for antibodies resulted in an improvement of the analytical performance of a surface plasmon resonance-based immunoassay by decreasing the possibility of steric effects in antibodies binding to their antigens [14]. The recombinant protein G fused with Cys-tag was expressed in *Escherichia coli* culture; the expressed protein was purified using IMAC (Immobilized Metal Affinity Chromatography).

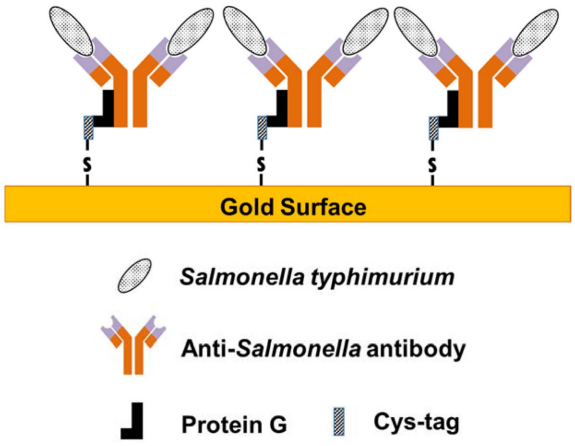

Figure 3. Cys-tagged protein G-mediated well-oriented immobilization of probe antibody. Not drawn to scale.

The purified cysteine-tagged protein G was further applied to the immobilization of monoclonal antibody against *S. typhimurium* for SPR measurements (Figure 3).

4. Detection of *S. typhimurium* using the portable SPR

The rotating mirror-based portable SPR device was developed for rapid and specific detection of *Salmonella*. *Salmonella* contamination is quite common in all foods, causing health problems and economic loss. It takes approximately 3-4 days for conventional cultural methods to confirm the identity of presumptive positive colonies. Currently, rapid detection methods for *Salmonella* are important for the food industry. In this study, using the portable SPR instrument, *S. typhimurium* was detected in a real time manner within 1 hour. The gold surface was modified with the Cys-tagged protein G for well-oriented immobilization of antibodies, as described in the "Materials and Methods" section. The probe antibody immobilized on the gold surface was then reacted with *Salmonella* cells, and the association of target cells with the antibody-functionalized surface was measured by the portable SPR.

The PBS buffer solution (pH 7.4) was introduced to the flow cell of the SPR device; then, 10^7, 10^8, and

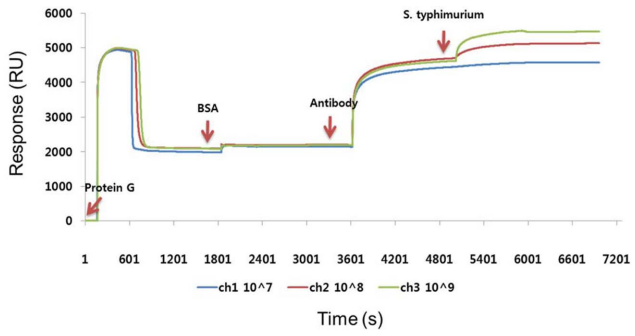

Figure 4. Rapid detection of *Salmonella* using the portable SPR biosensor. The SPR sensor surface was modified with anti-*Salmonella* monoclonal antibody. To kill the pathogenic cells, PBS solution spiked with *S. typhimurium* cells was treated with thimerosal (1%, w/w) and then applied to the anti-*Salmonella* antibody modified surfaces.

Table 1. Summary of SPR measurement.

Channel	Ru shift		*S. Typhimurium* Conc	ΔRU
	Cys-Protein G (0.2 mg/mL)	Antibody (0.1 mg/mL)		
Channel 1	2,000	2,430	10^7CFU/mL	+128.3
Channel 2	2,000	2,500	10^8CFU/mL	+431.9
Channel 3	2,000	2,500	10^9CFU/mL	+845.3

10^9 CFU/mL of *S. typhimurium* was flowed through the chip surface for 10 min at a flow rate of 5 μL/min for SPR measurement; finally, the flow channel was flushed with PBS again to remove loosely bound or unbound *Salmonella* cells. The PBS solution was passed through the gold surface until a steady baseline was achieved. The interaction between the antibody-functionalized surface and the target cells was investigated in comparison with changes in the SPR angle. An increase in the SPR signal was observed when sample treated with the PBS solution was spiked with *S. typhimurium.*

Upon return of PBS flow, the SPR signal remained constant. To evaluate the sensing performance of the portable SPR sensor in response to *Salmonella*, various concentrations of *S. typhimurium* cells suspended in PBS were examined. As shown in Figure 4 and Table 1, the difference of SPR angle shifts of 10^7 CFU/mL, 10^8 CFU/mL, and 10^9 CFU/mL *S. typhimurium* were +128.3 RU, +431.9 RU, and +845.3 RU, respectively. The SPR measurement was performed within 1 hour using the portable SPR device in real time. Barlen et al. reported that *S. Typhimurium* and *S. enteritidis* could be detected using an SPR-based sensor at 2.5×10^5 CFU/mL and 2.5×10^8 CFU/mL, respectively. Compared to this study, the sensing performance of the current system was at an unsatisfactory level. Thus, further work will be needed to achieve the desired sensitivity. The results indicate that the use of the portable SPR could be important for studies that examine food poisoning in POC settings. Portable systems typically weigh less than 23 kg (or 50 lb). The BIAcore 3000 immunoassay system, one of the most widely used commercial SPR platforms, weighs 50 kg (or 110 lb), so the instruments are not appropriate for POC applications. However, the hand-held SPR device is compact and lightweight enough to be carried by hand, and uses lower sample volume, thereby making it more suitable for POC applications.

IV. Conclusions

Here, a portable SPR-based immunosensor for detection of one important food pathogen, in which a rotating mirror modulates a laser beam as an incident light source, was described. Especially, an SPR instrument having a small and lightweight module as a portable monitoring and diagnostic device for point-of-care (POC) application was

used for rapid monitoring of *Salmonella.* In this study, we evaluated the performance of SPR immunosensing for rapid detection of spiked *S. typhimurium* in PBS within 1 hour. Although the attained limit of detection of the hand-held SPR used in the current study was not low enough (approximately 10^7 CFU/mL), the results at least showed the possibility that the portable SPR system can be possibly applied in food safety monitoring or disease diagnosis using bacterial pathogen-specific antibodies, if appropriate biomarkers are available. Thus, future optimization remains to improve the sensitivity.

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