Long-Range Surface Plasmon-Enhanced Fluorescence Spectroscopy Biosensor for Ultrasensitive Detection of E. coli O157:H7

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ABSTRACT:

A new biosensor platform for the detection of bacterial pathogens based on long-range surface plasmon-enhanced fluorescence spectroscopy (LRSP-FS) is presented. The resonant excitation of LRSP modes provides an enhanced intensity of the electromagnetic field, which is directly translated to an increased strength of fluorescence signal measured upon the capture of target analyte at the sensor surface. LRSPs originate from a coupling of surface plasmons across a thin metallic film embedded in dielectrics with similar refractive indices. With respect to regular surface plasmon-enhanced fluorescence spectroscopy, the excitation of LRSPs offers the advantage of a larger enhancement of the evanescent field intensity and a micrometer probing depth that is comparable to the size of target bacterial pathogens. The potential of the developed sensor platform is demonstrated in an experiment in which the detection of E. coli O157:H7 was carried out using sandwich immunoassays. The limit of detection below 10 cfu mL\(^{-1}\) and detection time of 40 min were achieved.

Safety concerns about food, drinking water, and disease control related to microbial pathogens have attracted increasing attention worldwide. Among others, Escherichia coli O157:H7 producing Shiga-like toxins is a widespread foodborne pathogen, and the infective dose is as low as 50 to 100 cells.\(^1\) According to the Centers for Disease Control (CDC), outbreaks of E. coli O157:H7 cause annually ten thousand illnesses, thousands of hospitalizations, and up to one hundred deaths in the USA. Conventional techniques used for the analysis of bacterial pathogens include culturing methods,\(^2\) polymerase chain reaction (PCR),\(^3\) and enzyme linked immunosorbent assays (ELISA).\(^4\) However, these techniques can be performed only in specialized laboratories and require trained personnel and laborious sample pretreatment. In addition, they are time-consuming as a standard confirmation process takes usually several hours or even days.\(^5\) Therefore, new tools that are suitable for routine detection and rapid identification of bacterial pathogens are urgently needed in order to reduce the risk to public health. Biosensors are analytical devices extensively researched for rapid detection of pathogens in food control.\(^6\) Among these, biosensors based on surface plasmon resonance (SPR) hold a prominent stage due to their advantage of real-time and direct detection method that can be implemented to portable devices.\(^7\) Unfortunately, insufficient limit of detection for the analysis of bacterial pathogens (typically larger than 10\(^3\) cfu mL\(^{-1}\)) was demonstrated by this technology up to date. In order to enhance the sensitivity of SPR biosensors for detection of bacterial pathogens, the amplification of the sensor response by nanoparticle assays,\(^8\) improved sample preparation,\(^9\) and more accurate SPR sensor schemes\(^10\) have been investigated.

In this paper, we report a new approach to enhance the sensitivity for the detection of bacterial pathogens in which SPR is combined with fluorescence spectroscopy. In this method, referred to as surface plasmon field-enhanced fluorescence spectroscopy (SPFS),\(^11\) fluorophore-labeled molecules captured on a metallic sensor surface are excited by the enhanced intensity of electromagnetic field of SPs. The binding of target molecules

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is detected via the strong intensity of fluorescence light emitted by fluorophore labels. We advanced SPFS through a sensor architecture that enables probing the sensor surface using long-range surface plasmons (LRSPs).\(^{12}\) These surface plasmon modes propagate along a thin metallic film embedded in dielectrics with similar refractive indices with lower losses, and thus, their resonant excitation provides higher field intensity enhancement compared to regular surface plasmons. In addition, they allow probing the whole volume of captured bacterial pathogens due to their evanescent field with up to several micrometers of penetration depth from the sensor surface.

**EXPERIMENTAL SECTION**

**Materials.** *E. coli* O157:H7 standard was obtained from KPL (Gaithersburg, MD), and the non-pathogenic, but highly related, *E. coli* strain K12 was cultivated in our laboratory. The average diameters of heat-killed *E. coli* O157:H7 and K12 cells were determined by dynamic light scattering (DLS) with Zetasizer from Malvern Instruments (Worcestershire, UK) as 1177 and 869 nm, respectively. Capture polyclonal antibodies against *E. coli* O157:H7 (c-IgG; cat. No. ab75244) were purchased from Abcam (Cambridge, UK). Affinity-purified detection polyclonal antibodies (d-IgG) against *E. coli* O157:H7 (cat. No. 01-95-90) were acquired from KPL and labeled with Alexa-Fluor-647 dye (at a dye-to-protein molar ratio of about 4.5) using a labeling kit (cat. No. A-20186) from Invitrogen (Carlsbad, CA). The used fluorophores exhibited the excitation and emission wavelengths (cat. No. A-20186) from Invitrogen (Carlsbad, CA). The used fluorophores exhibited the excitation and emission wavelengths of λ<sub>ex</sub> = 633 nm and λ<sub>em</sub> = 670 nm, respectively. Samples for validating the method were prepared by spiking phosphate buffer saline containing 0.05% Tween 20 (PBST) with the *E. coli* O157:H7 (target analyte) or *E. coli* K12 (negative control) in the range 10<sup>3</sup>–10<sup>6</sup> cfu mL<sup>–1</sup>. Sandwich immunoassay was employed in which labeled d-IgG was bound to captured analyte from a PBST solution. The concentration of d-Ab of 1 nM was used for which no measurable nonspecific interaction with the surface was observed and the majority of accessible binding sites on the captured analyte were reacted with d-Ab (let us note, that *E. coli* O157:H7 carries multiple binding sites that are recognized by d-Ab). Poly(ethylene) glycol thiol (PEG-thiol) and carboxylic acid terminated thiol (COOH-thiol) were purchased from Sensopet (Bozem, MT). The protein coupling chemical reagents 1-ethyl-3-(3-dimethylpropyl)-carboimide (EDC) and N-hydroxysulfosuccinimide (NHS) were from Pierce (Rockford, IL).

**Sensor Instrument.** An angular spectroscopy of surface plasmon modes relying on the total attenuated reflection (ATR) method with Kretschmann configuration was employed in the long-range surface-plasmon-enhanced fluorescence spectroscopy (LRSP-FS) biosensor as described previously by our laboratory.\(^{12}\) This optical setup was used for the measurement of the intensity of reflected light (λ<sub>ex</sub> = 633 nm) and emitted fluorescence light (λ<sub>em</sub> = 670 nm) from a sensor surface as a function of time <i>t</i> and the angle of incidence θ of an excitation laser beam (λ<sub>ex</sub> = 633 nm); see Figure 1a. The sensor chip consisting of Cytop and gold layers deposited on a glass slide with the thickness of d<sub>θ</sub> = 887 and d<sub>m</sub> = 15.2 nm, respectively, was optically matched to a prism. A flow cell with a volume of 5 μL was pressed against the sensor chip in order to flow liquid samples with the flow rate 0.3 mL min<sup>–1</sup>. The gold surface was modified with mixed thiol self-assembly monolayer (SAM) of PEG-thiol and COOH-thiol. Through EDC/NHS coupling chemistry, c-IgG against *E. coli* O157:H7 was immobilized on the surface with a surface mass density of 2.4 ± 0.7 ng/mm<sup>2</sup> (details are presented in Supporting Information). Detection of the target analyte was performed using a sandwich immunoassay format. First, a sample (volume of 1 mL) was circulated through the sensor flow cell for 20 min. Then, the sensor surface was successively rinsed with PBST for 5 min, incubated with d-IgG for 10 min, and rinsed again with PBST buffer for 5 min. The analyte binding was observed after the incubation with d-IgG by measuring the angular fluorescence intensity spectrum R(θ) and from the time evolution of fluorescence intensity F(0) at an angle providing maximum signal strength.

**RESULTS AND DISCUSSION**

The coupling of a laser beam to LRSPs supported by the thin gold film is manifested as a narrow dip in the reflectivity spectrum R(θ); see Figure 1b. Through an analysis of the reflectivity spectrum, we calculated the penetration depth of LRSPs of λ<sub>p</sub> = 1124 nm (defined as the distance from the surface at which the amplitude of the field intensity |E| drops by 1/e) which is comparable to the average size of the target analytes. As seen in Figure 1b, the capture of target *E. coli* O157:H7 analyte followed by the binding of fluorophore-labeled d-IgG was manifested as a strong peak in angular fluorescence spectrum F(θ) centered at the angle θ at which LRSPs are resonantly excited. The peak intensity of F(θ) increased with the concentration of *E. coli* cells. Let us note that the capture of bacteria induced no significant shift of the LRSP dip in the angular reflectivity spectrum R(θ) for

![Figure 1](image-url)

Figure 1. (a) Optical setup of a LRSP-FS biosensor, surface architecture, and detection assay. (b) The angular reflectivity (left axis) and fluorescence (right axis) spectra measured after the analysis of *E. coli* O157:H7 at the concentrations between 10<sup>3</sup> and 10<sup>6</sup> cfu/mL (curves clearly indicated in the graph).
the analyte concentrations below $10^5$ cfu mL$^{-1}$ (see inset in Figure 1b), indicating a superior sensitivity of SPFS with respect to SPR-based detection of binding-induced refractive index changes.

In order to determine the key performance characteristics of the LRSP-FS biosensor, the peak fluorescence signal was measured as a function of time $F(t)$ upon the analysis of samples containing target (E. coli O157:H7) and control (E. coli K12) analytes. The detection experiment was performed in cycles, in which the sensor surface was subsequently incubated with the analyte followed by the binding of labeled d-IgG. Figure 2 shows the time evolution of the sensor signal $F(t)$ measured for samples with analyte concentrations between $10^6$ and $10^8$ cfu mL$^{-1}$. The abrupt increase of the fluorescence signal $ΔF \sim 5 \times 10^5$ cps after the injection of d-IgG was associated with the excitation of fluorophore molecules present in a bulk solution. Afterward, the fluorescence signal gradually increased in time due to the affinity binding of d-IgG to the E. coli O157:H7 captured on the sensor surface. After rinsing with PBST, a fast decrease in the fluorescence signal was observed due to the removal of d-IgG from the bulk solution followed by a gradual decrease of the signal $F$ that is caused by the dissociation of the complex of c-IgG, E. coli O157:H7, and d-IgG. The data in Figure 2 reveal that, for E. coli O157:H7, a strong fluorescence signal was emitted and its intensity increased with increasing analyte concentration. In the control experiment, no response due to the binding of the analyte E. coli K12 was observed as the fluorescence signal $F$ dropped back to the original baseline after the incubation in d-IgG and rinsing with PBST.

The sensor response $ΔF$ was defined as a difference between the fluorescence signal $F$ measured before and after the incubation with d-IgG; see Figure 2. The calibration curve for the analysis of E. coli O157:H7 that is presented in Figure 3 was averaged from a triplicate measurement, and the error bars show the obtained standard deviation (SD) that is attributed to chip to chip variability and inaccuracy in incubation times. From this curve, the limit of detection (LOD) below 10 cfu mL$^{-1}$ was determined for which the linear fit of calibration curve reaches 3-fold SD of the baseline fluorescence signal $3σ(F) = 314$ cps.

This limit of detection is in the range required by the Advisory Committee for Food and Dairy Products (ACFDP) in the UK, and the analysis time of 40 min is highly superior compared to that needed for the conventional analysis.

**CONCLUSIONS**

In conclusion, a new biosensor platform for ultrasensitive detection of bacterial pathogens was developed on the basis of long-range surface plasmon-enhanced fluorescence spectroscopy (LRSP-FS). The performance characteristics of this method were evaluated for E. coli O157:H7, and a limit of detection (LOD) below 10 cfu mL$^{-1}$ and a detection time of 40 min per test were achieved. The high specificity was demonstrated by the lack of response for the strongly related but nonpathogenic E. coli strain K12. With respect to the conventional SPR biosensor technology, a substantially lower limit of detection (about 3 to 4 orders of magnitude lower) was achieved, which lies in the range required by regulatory bodies for the analysis of samples relevant to food control.

**ASSOCIATED CONTENT**

Supporting Information. Experimental details, including the characterization of the sensor surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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