Rapid Detection of Foodborne Pathogens by Surface Plasmon Resonance Biosensors

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Manuscript received October 5, 2015; revised November 20, 2015.
doi: 10.17706/ijbbb.2015.5.6.329-335

Abstract: Here, we report rapid and sensitive detection of *Salmonella Enteritidis* with high specificity by using SPR (Surface Plasmon resonance) technique. Antibodies against *S. Enteritidis* were immobilized on to a gold sensor surface and different concentrations of serially diluted *S. Enteritidis* solutions were pumped in to the system. The changes in the response unit (RU) due to the binding of different concentrations of bacteria were measured. Regeneration of the sensor surface was carried out by using 0.05 % PBS + Tween 80 solution. The selectivity of the developed sensor was examined with *Listeria monocytogenes* which did not produce any significant response. Additionally, the ability of the developed sensor to detect *S. Enteritidis* in milk samples was tested. Based on these results, the developed SPR based biosensor may be used for rapid, label-free, sensitive and selective detection of bacteria in food materials.

Key words: Surface Plasmon Resonance (SPR), biosensors, *Salmonella Enteritidis*, *Listeria monocytogenes*, milk.

1. Introduction

Food safety is a public health challenge and there is a great interest on using devices allowing early, fast and label-free detection of bacteria to prevent outbreaks [1]. Precise and rapid quantification of low levels of food pathogens is necessary to guarantee microbial safety of the produce and to carry out research on the subject of persistence of the pathogens [1], [2]. Traditional techniques used to identify pathogens involve the time-consuming cultivation period [3]. Cultivated pathogens are identified by biochemical testing or more advanced molecular methods like PCR, which can amplify pathogen-specific nucleic acid targets [2]. These approaches are effective, but they target only a single pathogen per assay. The development of a multiplex detection method for simultaneous identification of several food pathogens is needed to improve the efficiency of detection [4]-[7]. Therefore, there is a current need for portable, real-time, multiplex pathogen detection technologies that can predict the safety of food.

Surface Plasmon Resonance (SPR) imaging is a sensitive, label-free method that can detect the binding of an analyte to a gold surface by the changes in refractive index that occur upon binding. SPR is an optical detection technique based on changes of the reflection and refraction of light and monitors the interactions occurring between biomolecules grafted on a biochip and target molecules within the sample (Fig. 1) [8],

[9].

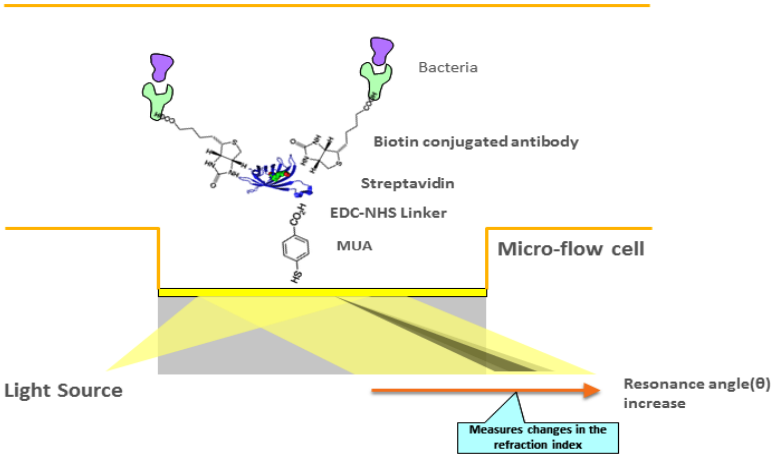

Fig. 1. Shematic presentation of proposed food pathogen detection technique with surface plasmon resonance.

The objective of this study was to develop a SPR based biosensor for the rapid, sensitive and selective detection of *S. Enteritidis*. The specificity of the sensor against other bacteria and detection limits, were determined. Bacteria determination was conducted also for milk sample directly.

2. Experimental Section

2.1 Instrumentation

SPR-Mini device (Nanodev Scientific, Ankara, TURKEY) was used for SPR experiments. SPR-Mini with the dimensions of 25X10X15 cm is capable of monitoring RI changes with the sensitivity of 1.25×10^{-6} refractive index units (RIU). Autoclavable micro channel structure was designed to inhibit contamination. The flow of reagents into the flow cell (15 mm round gold chip) at a rate of 1.66 µl s^{-1} was controlled by using a multichannel cassette type peristaltic pump. The device has a built-in temperature control unit. Optics and sample holder were heated with a high precision PID controller enabling 0.1 ^{o}C precision. A 1.3 MP resolution CMOS camera was used for detection of resonance angle. A point source constant-current driven led with a wavelength of 850nm was used as a light source in the device.

2.2 Materials and Methods

2.2.1 Microorganisms

Salmonella Enteritidis and *Listeria monocytogenes* were obtained from KWIK STIK™**.** Cultures for the assays were grown on Tryptic Soy Broth (TSB-Merck) for 24 h at 37 C. One loop of sample from overnight culture was transferred to sterile TSB media, was grown to logarithmic phase. This culture was diluted to the level of 1×10^{7} cfu/ml measuring of the turbidity with a spectrophotometer. The culture medium was centrifuged (4000 *g* 15 min.) and collected. The pellet was washed with phosphate buffer saline (PBS) to remove all remaining TSB and centrifuged at same conditions. After the second centrifugation the microorganisms were resuspended with solution of PBS with % 0.05 Tween 80 and stored at 4°C until use.

2.2.2 Reagents

MUA (11 mercapto-1-undecanoic acid), EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), NHS (N-hydroxysuccinimide), streptavidin and phosphate buffer saline (PBS) were purchased from SIGMA ALDRICH. Biotin conjugated goat anti- *Salmonella* and *L. monocytogenes* polyclonal antibodies were obtained from KPL.

2.2.3 Preparation of gold surface

The production of Au coated SPR chips were performed by evaporation. Thin layer of Titanium was evaporated onto glass slides as an interfacial layer. A 47nm of 99.995 percent pure gold layer was evaporated onto titanium layer with a rotating sample holder. MUA monolayer coating on gold surfaces was prepared by immersing overnight gold substrates into 10 mM MUA solution in absolute ethanol.

2.2.4 Immobilization of antibodies

Primarily, the chip surface was washed with deionized water flow 15 minutes. A base line was obtained by injecting PBS onto the clean gold surface. Two mL of 1:1 EDC (100mM) +NHS (50mM) solution and Streptavidin (20 µg) were injected sequentially to the flow cell and their adsorption were monitored until surface saturation was observed. Excess and weakly adsorbed streptavidin was washed off by injecting PBS for 30 minutes. Then, biotin conjugated antibodies were injected to the sensor chip. PBS was used to remove excess antibodies. Reversibly bound antibodies were cleaned from the surface by injecting 0.05 % PBS Tween 80 solution.

2.2.5 Detection of bacteria

The 1 mL of solutions containing bacteria at different concentrations were injected in to the flow cell. The unbound antigens were pre-washed by passing PBS through the flow cell. Binding of bacteria cells to the sensor surface was marked by the corresponding resonance angle changes. After each analysis the sensor was regenerated by injecting 0.05 % PBS Tween 80. The sensitivity of the developed sensor for *Salmonella* detection was determined by following the same assay procedure for *L. monocytogenes*. The resonance angle changes obtained for each bacterium were compared. In order to measure the performance of the produced sensor chip, pasteurized whole milk is contaminated with different concentrations of food pathogens and injected on to functionalized gold surface.

3. Results and Discussion

The changes in resonance angle during binding of EDC+NHS, streptavidin and immobilization of biotin conjugated polyclonal antibodies against Salmonella to the sensor surface were investigated. The changes in the resonance angle induced by the binding of bacteria at various concentrations are shown in Fig. 2 (The red line is the average of signal). Resonance angle was increased during the binding of streptavidin. The immobilization of the anti-Salmonella through avidin/biotin chemistry led to a remarkable change in resonance angle. Signal increased with increasing bacterial binding following the injection of Salmonella solutions to the flow cell. As shown in Fig. 3, we found that the detection limit for Salmonella is 1×10^2 cfu/mL which is one of the best results obtained with SPR compared to results given in the literature. A good correlation was obtained between bacteria concentration and the change in resonance angle.

The selectivity of the SPR biosensor was tested using dilution series of the *L. monocytogenes*. After performing *Salmonella* binding experiments, cross-contamination experiments of were conducted. We have injected *L. monocytogenes* on to Salmonella antibody functionalized gold surface to investigate the sensitivity of the developed sensor by using the same assay procedure. The binding of *L. monocytogenes* to the Salmonella antibody was shown in Fig. 4. According to the results, a small amount of *L. monocytogenes* was bonded to *the S. Enteritidis* antibody coated surface. It was observed that the response induced by the non-specific binding (*L. monocytogenes*) was much lower than that of the response induced by *S.Enteritidistidis*. Additionally, resonance angle changes obtained from *L. monocytogenes* at different dilution levels were smaller than that of the detection limit of target pathogen, which shows the high specificity of the biosensor.

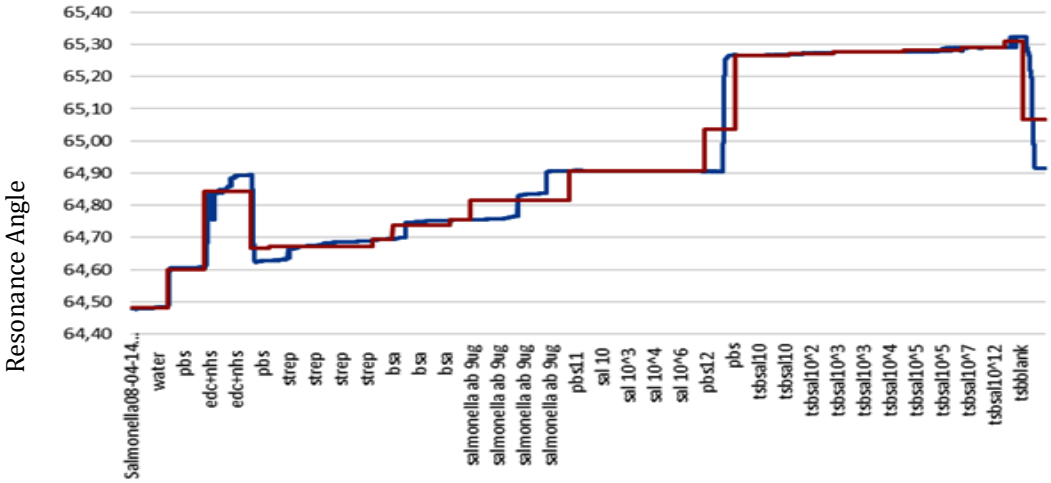

Fig. 2. Changes in resonance angle during the functionalization of the Au surface and *S. Enteritidis* flow. Blue line is the original signal. For better demonstration red line is calculated as the average of resonance angle for each chemical flow.

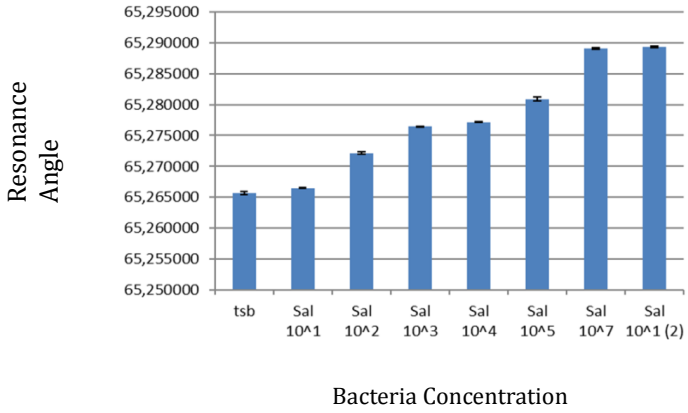

Fig. 3. Changes in resonance angle with *S. Enteritidis* concentration.

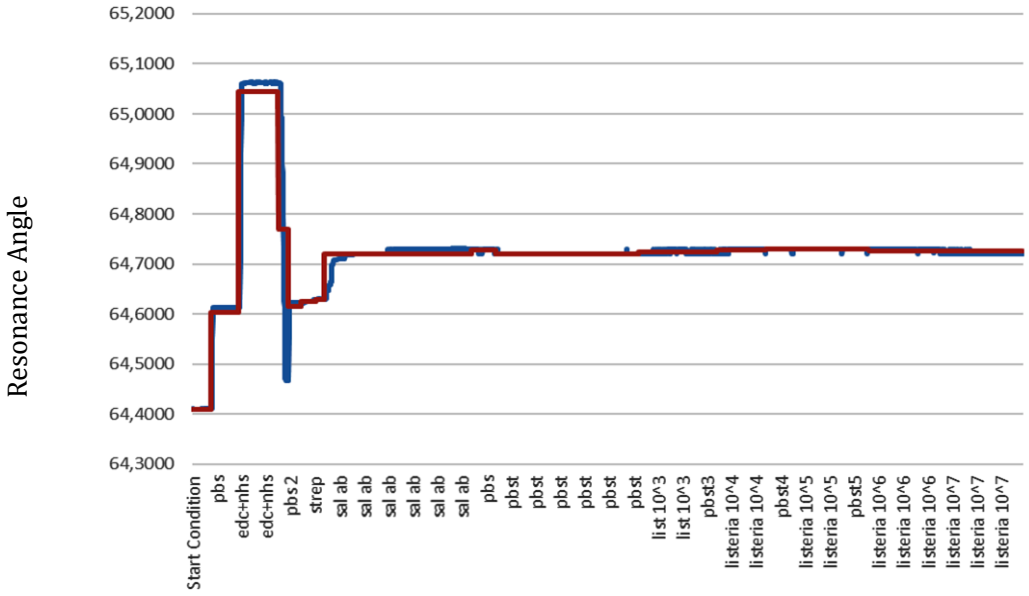

Fig. 4. Changes in resonance angle during functionalization and *L. monocytogenes* flow. Blue line is the original signal. For better demonstration red line is calculated as the average of resonance angle for each chemical flow.

Chip surfaces were imaged after bacteria attachment by using SEM. According to Fig. 5-A, *S. Enteritidis* are attached to the SPR chip surface in higher amount and more uniform than the *L. monocytogenes* (Fig. 5-B).

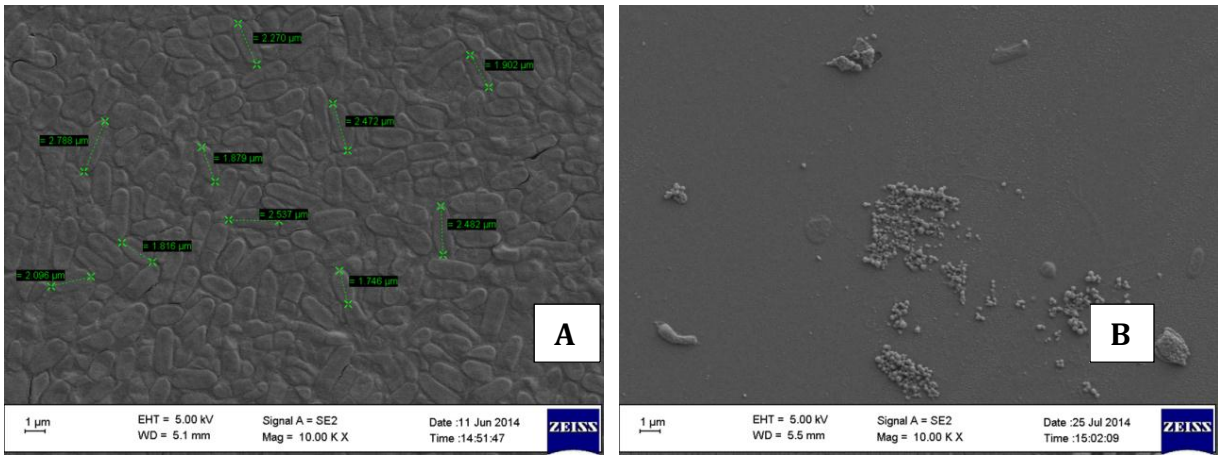

Fig 5. SEM images of Sal-ab functionalized SPR chip after *S. Enteritidis* (A) and *L. monocytogenes* (B) flow.

The ability of developed sensor to detect *S. Enteritidis* in food samples was investigated. UHT pasteurized whole milk samples inoculated with *S. Enteritidis* to test proposed biosensor platform for commercial applications. A detectable signal increase was observed onto functionalized chip with increasing contamination level (Fig. 6).

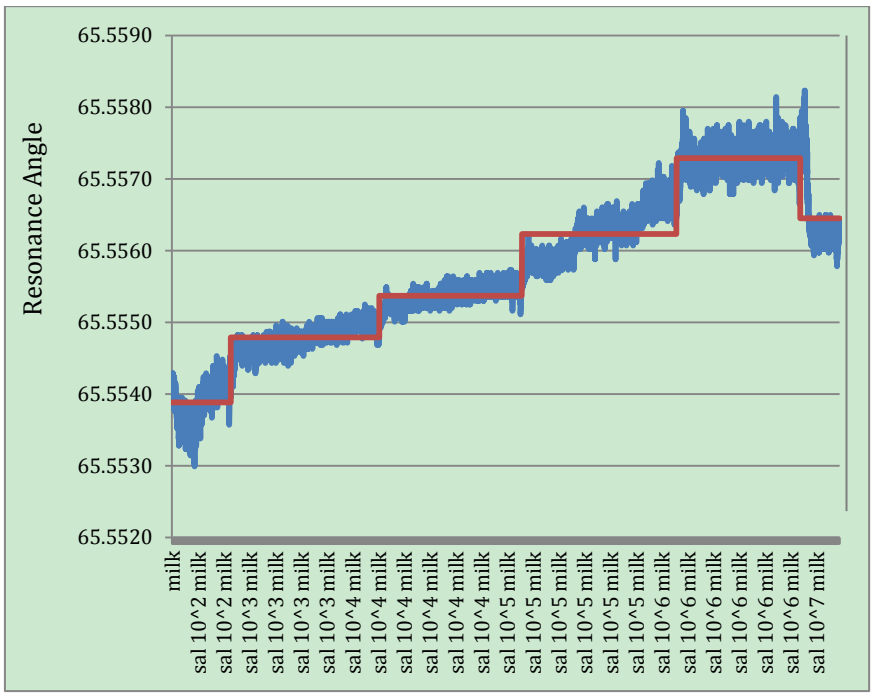

Fig. 6. Resonance angle change vs pathogen contaminated whole milk with different concentrations. Blue line is the original signal. For better demonstration red line is calculated average of resonance angle for each concentration.

4. Conclusion

L. monocytogenes and *Salmonella* spp. are foodborne pathogens which are responsible for the high

proportion of the hospitalizations in the world. There is a current need for portable, real-time, multiplex pathogen detection technologies that can predict the safety of food. In this study, surface plasmon resonance (SPR) imaging was successfully used for sensitive, label-free detection and quantification of food pathogens in less than 5 hours. The demonstrated system can potentially be generalized to other pathogens for the selected food materials.

Acknowledgment

This work was supported by the Scientific Research Foundation, Mersin University, Turkey (Project nr 2015-TP3-1165) and Nanodev Scientific Ltd. (Bilkent Cyberpark/Ankara-Turkey). The authors gratefully acknowledge all this support given for this research.

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