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Comparison of sensing strategies in SPR biosensor for rapid and sensitive enumeration of bacteria

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ABSTRACT

Rapid and sensitive detections of microorganisms are very important for biodefence, food safety, medical diagnosis and pharmaceutics. The present study aims to find out the most proper bioactive surface preparation method to develop rapid, sensitive and selective bacteria biosensor, based on surface plasmon resonance (SPR) spectroscopy. *Escherichia coli* (*E. coli*) was used as a model bacterium and four sensing strategies in SPR were tested. Three of these strategies are antibody immobilization methods that are non-specific adsorption, specific adsorption via the avidin–biotin interaction, and immobilization of antibodies via self-assembled monolayer formation. The fourth strategy is a novel method for bacteria enumeration based on the combination of the SPR spectroscopy and immunomagnetic separation with using gold-coated magnetic nanoparticles. According to results, the most efficient SPR method is the one based on gold-coated magnetic nanoparticles. This method allows to specifically separate *E. coli* from the environment and to quantify rapidly without any labeling procedure. The developed method has a linear range between 30 and 3.0×10^4 cfu/ml, and a detection limit of 3 cfu/ml. The selectivity of the method was examined with *Enterobacter aerogenes* and *Enterobacter dissolvens*, which did not produce any significant response. The usefulness of the method to detect *E. coli* in real water samples was also investigated, and the results were compared with the results from plate-counting method. There was no significant difference between the methods ($p > 0.05$).

1. Introduction

Detection and identification of harmful bacteria and their associated toxins to prevent illness are critical to the food industry, homeland security, and in clinical diagnosis and therapeutics (Sapsford and Shriver-Lake, 2008). Due to this reason, rapid identification and quantification of bacteria has great importance. Identification of bacteria is usually performed by culture-based and colony-counting methods (Dudak and Boyaci, 2009). In spite of their sensitivity and reliability (Lazcka et al., 2007), these methods are time-consuming and labor-intensive (Masco et al., 2007). In addition to these, a complex series of tests is generally required before any identification. The results of such tests are often difficult to interpret (Wilkins et al., 1999). The existing studies have shown that the new approaches have advantages according to culture-based and colony-counting methods in terms of rapidity and simplicity. However, a great majority of these sensitive techniques requires expensive chemicals and also time consumption, such as secondary antibody usage and/or labeling procedures. Despite the deficiency of the field, the disadvantages of several methods can be overcome by the promising technique the surface plasmon resonance (SPR) spectroscopy.

Surface plasmon resonance is a charge–density oscillation that may exist at the interface of a metal and a dielectric (Homola et al., 1999). The SPR signal relies on refractive index changes in the evanescent field at the metal surface (Schuck and Zhao, 2010). In recent years, SPR spectroscopy has received a great deal of attention because of offering label-free and rapid detection of various antigens. However, due to their large size and morphology, detection of live bacteria results in a number of limitations, including limited penetration of bacteria to the electromagnetic field, the low refractive index difference between the bacterium cytoplasm and the aqueous environments in which detection is usually performed, the availability and accessibility of corpuscular antigens on the bacterium surface binding to biorecognition elements (Taylor et al., 2008). Various treatment methods of bacteria are employed to overcome these challenges (Ladd et al., 2006), including heat killing (Taylor et al., 2006), soaking in 70% ethanol (Koubova et al., 2001; Taylor et al., 2005), and the combination of treatments such as heat killing and detergent

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lysing (Taylor et al., 2005), heat killing and ultrasonication (Taylor et al., 2006). However, these treatment methods have insufficient sensitivity to detect the low concentrations of bacteria. On the other hand, different surface modifications and assay types, including avidin–biotin-characterized SPR assays, sandwich assays with primary and secondary antibodies, self-assembled monolayers (SAM) based SPR assays were also developed for the detection of various bacteria. *E. coli* detection was realized by avidin–biotin-characterized SPR based immunoassay with a detection limit of 9×10^1 cfu/ml (Dudak and Boyacı, 2007). Subramanian et al. (2006) used the polyethylene glycol terminated alkanethiol mixed self-assembled monolayers for *E. coli* O157:H7 detection by direct assay, Protein G and a sandwich-type assay. The detection limits of the assays were 10^6 cfu/ml, 10^4 cfu/ml, and 10^3 cfu/ml respectively. A SAM monolayer of 16-mercaptoundecanoic acid was used by Abdelghani et al. (2010) and *E. coli* detection was performed by direct assay. The detection limit of the assay was 10^4 cfu/ml. In another study; a mixed SAMs of two thiols of different chain lengths was used to reduce steric hindrance for detection of *Acidovorax avenae* subsp. *citrulli*. The obtained detection limits were 10^6 cfu/ml and 5×10^5 cfu/ml in direct detection and sandwich assays, respectively (Puttharugsa et al., 2011). As mentioned by Wei et al. (2007), more research is needed to understand if the variability in the sensitivity reported by different methods is due to the methodology itself or the different sample and target preparation protocols used in the studies.

In this study, three different antibody immobilization methods which are non-specific adsorption, specific adsorption via the avidin–biotin interaction, immobilization of antibodies on the SPR surface via self-assembled monolayer (SAM) formation and the method which combines the SPR spectroscopy and immunomagnetic separation with gold-coated magnetic nanoparticles were tested and compared for bacteria enumeration. In line with this purpose, *E. coli* was used as a model bacterium. The analytical performance of the methods with respect to linear range, detection limit, and response time was studied, and the results are presented in this paper. After determining the most sensitive method, the selectivity of this method was tested using other coliform bacteria and also the ability of the method in real water samples was investigated.

2. Materials and methods

2.1. Materials

N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), absolute ethanol, sodium chloride, potassium chloride, MES monohydrate, fluorescamine, sodium hydroxide, concentrated hydrochloric acid, tween 20, triton X-100, KH_2PO_4, Na_2HPO_4, acetone and 11-mercaptoundecanoic acid (11-MUA) were purchased from Sigma-Aldrich (Taufkirchen, Germany). N-hydroxysulfosuccinimide sodium salt (NHS) and avidin were purchased from Pierce Biotechnology (Bonn, Germany). Biotin conjugated rabbit anti-*E. coli* polyclonal antibodies were obtained from Fitzgerald Industries International, Inc. (Acton, MA, USA).

2.2. Solutions and buffers

Phosphate buffered saline (PBS, 0.1 M) was prepared using KH_2PO_4, Na_2HPO_4, NaCl, and KCl, and adjusting the pH with NaOH. PBST was prepared by mixing PBS with 0.05% Tween 20 (v/v). The molar concentration of the MES buffer used in this study was 0.05 M and the pH of the MES buffer was adjusted to 6.5 by adding NaOH. The solution used for cleaning the SPR surface was 0.1 M NaOH containing 1% Triton X-100. All solutions were prepared with deionized water (18 MΩ cm).

2.3. Microorganisms

E. coli, *E. aerogenes*, and *E. dissolvens* were obtained from Refik Saydam National Type Culture Collections, Ankara, Turkey. The stock cultures for assay were grown on Tryptic Soy Broth (TSB; Merck KgaA, Germany) at 37 °C for 24 h. The cultures were centrifuged at 8000 rpm for 10 min. The cell pellets were then washed twice with PBS and bacteria samples were prepared by diluting the cultures in PBS. The number of colony forming units for each sample was detected by plating 100 μl solution on Sorbitol MacConkey Agar (SMAC; Merck KgaA, Germany) and incubated at 37 °C for 24 h. Owing to the sorbitol positive character of generic *E. coli*, pink colonies formed on the plates were then counted for *E. coli* enumeration.

2.4. Instrumentation

The Spreeta™ sensors (Texas Instruments, Dallas, TX, USA) were used for SPR experiments. The gold surface of the sensor was removed with aqua regia solution according to Faid et al. (2006), and the gold-coated slides (Aluminosilicate Glass Slides Coated with 500 A Au, Platypus Technologies, Madison, WI, USA) was coupled to the sensor surface by using immersion oil due to usage of gold coated glass slides instead of the gold surface of the SPR sensor. Indeed, it could be possible to coat the multiple surfaces with SAM monolayer at the same time. The components of the SPR system used in our experiments are a 3-channel flow cell, 12-bit DSP electronic control box, Goldman syringe pumps (Biasis Ltd. Sti., Ankara, Turkey) and 4-way switching valves (Upchurch Scientific, Oak Harbor, WA, USA).

Fluorescence observations were performed using a Leica DMI4000 B microscope equipped with a 100× oil immersion lens (HCX APO U-V-I, 1.30 NA), a Leica CTR4000 CCD camera and a Leica EL6000 compact light source (Leica, Wetzlar, Germany). The images were taken using the Filter Cube A (Leica, Wetzlar, Germany) with an excitation filter of BP340–380 nm, a dichromatic mirror of 400 nm, and a suppression filter of LP 425 nm.

2.5. Performing the sensing strategies

2.5.1. Non-specific adsorption of E. coli antibodies onto the SPR surface

A schematic of the method is shown in Fig. 1A. First, a baseline was established by injecting PBS to the sensor surface and the flow rate of the SPR system was set to be 20 μl/min. 700 μl of antibody solution (50 μg/ml) in PBS was injected to the flow cells, and its adsorption onto the gold surface was monitored. The unbound antibody was washed off using PBS. *E. coli* solutions were then injected into the flow cells for 25 min. The unbound antigen was washed with PBS passed through the flow cell. Binding of *E. coli* cells to the sensor surface was marked by the corresponding response unit (RU) changes.

2.5.2. Specific adsorption of E. coli antibodies onto the SPR surface via the avidin–biotin interaction

Fig. 1B illustrates the general scheme of the method. In detail, flow rate of the SPR system was set to be 20 μl/min. PBS buffer was first flowed through the sensor until a steady PBS baseline was established. The gold surface was modified with 700 μl of avidin solution (50 μg/ml) followed by PBS wash. A 700 μl solution of biotin conjugated *E. coli* antibodies (50 μg/ml) was then selectively immobilized onto the sensor surface via avidin–biotin interaction and PBS wash was performed to remove unbound antibody molecules. 500 μl

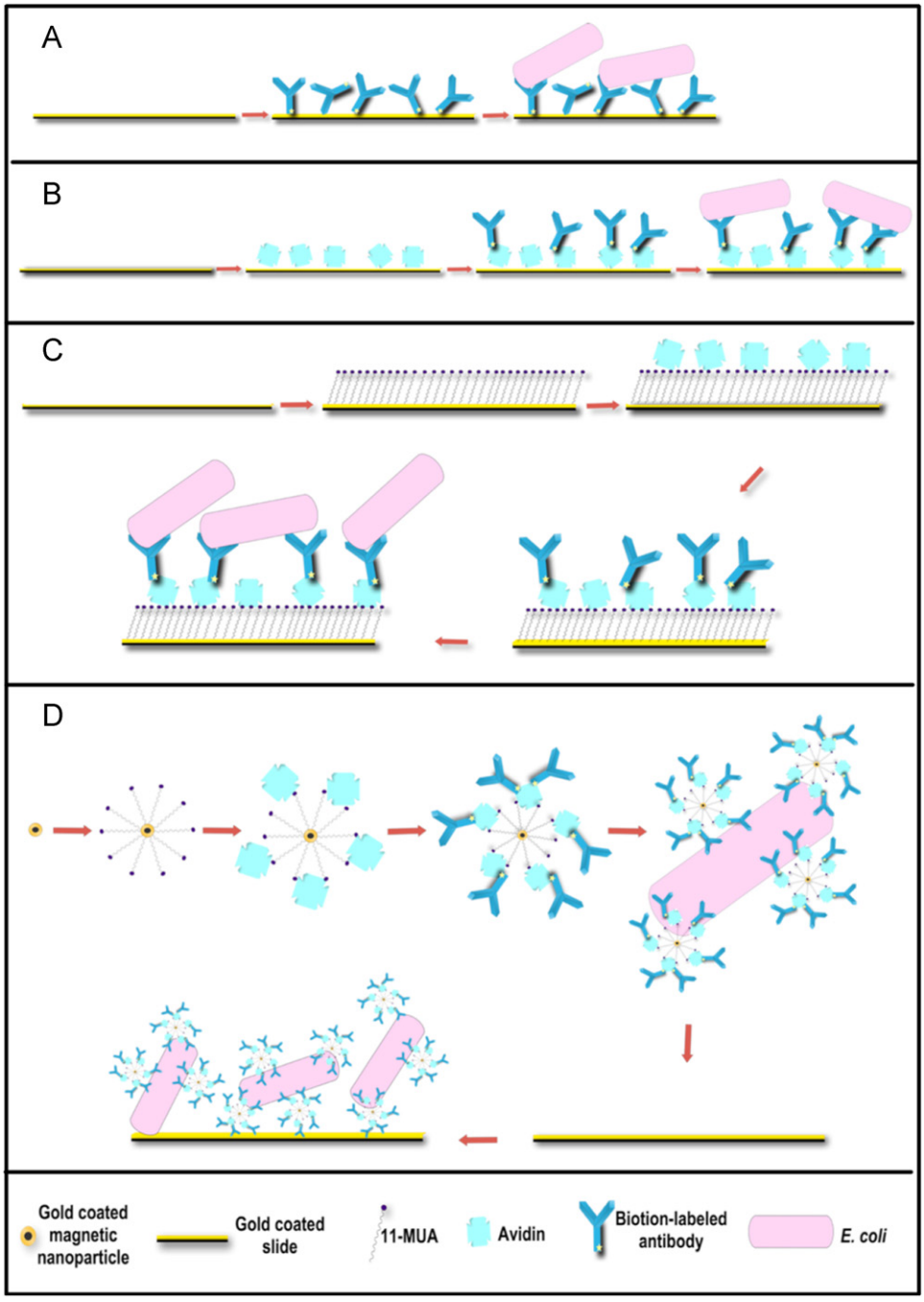

Fig. 1. Schematic illustration of sensing strategies in SPR biosensor (A) non-specific adsorption of antibody onto the SPR surface and *E. coli* detection, (B) oriented immobilization of antibody onto the SPR surface using (via) avidin–biotin interaction and *E. coli* detection, (C) covalent immobilization of antibody onto the SPR surface and *E. coli* detection, (D) magnetic gold nanoparticle based assay protocol comprised of the steps, immunomagnetic separation of *E. coli* and SPR measurements for *E. coli* enumeration.

of the *E. coli* solutions (3.5×10^1–3.5×10^5 cfu/ml) were injected to the flow cell in order to detect *E. coli*.

2.5.3. *Immobilization of E. coli antibodies onto the SPR surface via self-assembled monolayer formation*

Gold-coated slide was cleaned with piranha solution (70% conc. H_2SO_4:30% H_2O_2) and rinsed with deionized water prior to SAM formation. SAM of 11-MUA (50 mM in absolute ethanol for 15 h) was formed on the gold-coated slide surface at room temperature. Then, the slide was rinsed with ethanol and subsequently, rinsed with deionized water and dried with nitrogen. The modified slide finally was placed to the flow cell of SPR system. Flow rate of the system was set to be 20 μl/min and MES buffer was injected to the sensor surface. For the activation of SAM formed sensor surface, 0.2 M EDC/0.05 M NHS in MES buffer was injected to the flow cell for 30 min and the baseline was established by injecting MES buffer. After the activation, avidin (50 μg/ml) in MES buffer was injected to the flow cell for 30 min. The unbound avidin was removed from the system by injecting MES buffer. Biotin-labeled antibody solution (50 μg/ml) in MES buffer was injected to the sensor surface for 30 min. For the removal of unbound antibody, the system was rinsed by injecting MES buffer and then baseline was established by continuous flow of PBS. The SPR responses of the sample solutions

were obtained with 500 μl injection of *E. coli* dilutions (5.2×10^1–5.2×10^5 cfu/ml) prepared with PBS buffer. In Fig. 1C, the general scheme of the method is outlined.

2.5.4. Method based on the combination of immunomagnetic separation and SPR spectroscopy

This method composes of the three key steps, including synthesis of gold-coated magnetic nanoparticles, immunomagnetic separation and SPR measurements. In addition, the information about the performance of IMS technique was obtained using fluorescence microscope.

Synthesis of gold-coated magnetic nanoparticles: The magnetic core-shell Fe_3O_4–Au nanoparticles were synthesized according to the procedure developed by Tamer et al. (2010).

Immunomagnetic separation: Fig. 1D illustrates the overall strategy of the immunomagnetic separation. In this step, the parameters of the separation process including the antibody concentration, the incubation times during antibody immobilization and bacteria capturing were chosen based on the previous study by Guven et al. (2011). Here, the optimum antibody concentration is 25 μg antibody/μg nanoparticle and the optimum incubation time for antibody immobilization and the bacteria capturing is 30 min. For immunomagnetic separation, the gold-coated magnetic nanoparticles were used at a concentration of 1 mg/ml. Firstly, the surfaces of the nanoparticles were modified with 50 mM 11-MUA in absolute ethanol overnight to form SAM monolayer. Nanoparticles were collected using a permanent magnet and washed with 0.05 M MES (pH 6.5) buffer. For the activation of SAM, nanoparticles were treated with the solution of 0.2 M EDC/0.05 M NHS in MES buffer for 30 min. At the end of the activation period, nanoparticles were separated magnetically and washed with MES buffer. Nanoparticles were then incubated in avidin solution (0.1 mg/ml) for 30 min to form covalent bonds between avidin and carboxyl groups. For the removal of unbound avidin, nanoparticles were rinsed with MES buffer. The avidin-coated nanoparticles were mixed with biotin-labeled antibody (0.1 mg/ml) for 30 min. The antibody-coated particles were removed magnetically from the solution and washed twice with MES buffer to remove unconjugated and loosely conjugated antibodies. The antibody-coated nanoparticles (40 μl) were mixed with 250 μl of *E. coli* and incubated at room temperature for 30 min on a vortex mixer. After the incubation, the complex was collected magnetically and washed 3 times with PBST to eliminate the non-specific interactions. Then the complex was washed 3 times with PBS for removing Tween 20 from the solutions. Lastly, the final volume of the solutions was brought to 1 ml. After washing steps, the capture efficiency was determined by plating of unbound *E. coli* cells in the supernatant onto TSB agar.

Fluorescence imaging: To visually see that *E. coli* cells were magnetically captured, both the modified particles and *E. coli* captured by nanoparticles were fluorescently labeled with fluorescamine and examined under the fluorescence microscope. For this purpose, a stock solution of 50 mg/ml fluorescamine prepared in acetone was mixed with *E. coli* (3×10^3 cfu/ml) labeled with gold-coated magnetic nanoparticles and mixed with antibody-modified nanoparticles at a ratio of 1:1 (v/v). After waiting 10 min, fluorescence images were taken.

SPR measurements: In the last step of the assay, RU changes of particles were determined by using SPR device after capturing of *E. coli*. Baseline of the SPR system was established by continuous flow of PBS buffer, and the flow rate was set to be 20 μl/min. The SPR responses of the sample solutions were obtained with 500 μl injection of sample solutions as illustrated in Fig. 1D. The complete regeneration of sensor surface was achieved by using 0.1 M NaOH containing 1% Triton X-100.

2.6. Selectivity test and real sample analysis

The selectivity test and real sample analysis were implemented by following the same assay procedure explained in Section 2.5.4. For determining the selectivity of the developed immunoassay, coliform group bacteria *E. aerogenes* and *E. dissolvens* were used and the RU changes obtained for each type of bacteria were compared with the RU changes obtained for *E. coli*.

Real sample analysis was carried out to test the ability of developed immunoassay. The samples collected from a lake, a river, a puddle and *E. coli* inoculated tap water were used, and the results were compared with the results obtained from plate counting method. For the plate counting method, the serial dilutions of water samples were prepared in PBS, and 100 μl of the dilutions were plated on SMAC agar and incubated at 45 °C for 24 h. The colonies were counted to determine the amount of *E. coli* in water samples. A statistical comparison of the results was done with t-test software written in MATLAB (MathWorks, Natick, MA, USA). The results are considered to be statistically different at $p < 0.05$.

All the experiments in this study were performed in duplicates, and the average values and standard deviations were calculated.

3. Results and discussion

An ideal biosensor should have high specificity, high sensitivity, rapid response, and flexibility of use (Bakker and Telting-Diaz, 2002). One of the factors that affect the success of an SPR biosensor is the immobilization of antibodies to the sensor surface (Dudak and Boyaci, 2009) and there are two significant points related to the surface modification; antibody orientation and the exponential dependence of the electromagnetic field intensity on the distance away from the interface. In this study, we have investigated the sensing strategies in SPR biosensor, and the obtained results are shown below.

3.1. Non-specific adsorption of E. coli antibodies onto the SPR surface

Physical adsorption is the simplest and most rapid immobilization method, and it does not require chemical modification of biological components. Despite all, it has some limitations including random orientation, inactivation of the antibodies upon direct contact with the metal surface and less stability (Shankaran and Miura, 2007). In order to test the performance of the simplest method, *E. coli* antibodies were immobilized to the sensor surface and *E. coli* cells were analyzed. RU was increased during the binding of antibody to the sensor surface as shown in Fig. 1S, but the sensor response was only 110 ± 14 RU for *E. coli* at a concentration of 5.5×10^7 cfu/ml. Response curve for the sample solutions with different concentrations of *E. coli* (5.5×10^1–5.5×10^7 cfu/ml) is presented in Fig. 2A. The linear range of the calibration curve ($y = 25.8x - 70.8$) is between 5.5×10^3–5.5×10^7 cfu/ml with the coefficient regression (R^2) 0.936. Nevertheless, the slope of the line is very small and LOD value found by using three times of the noise is 4.8×10^5 cfu/ml. Physical adsorption is the simplest method used to immobilize sensor surface. However, the efficiency and the reproducibility of this method are insufficient for the sensitive detection of bacteria.

3.2. Specific adsorption of E. coli antibodies onto the SPR surface via the avidin–biotin interaction

Avidin was used for specific immobilization of biotin-labeled antibodies to the sensor surface, and the changes in the RU were investigated with respect to the binding of avidin to the sensor

surface via direct physical adsorption and immobilization of biotin-labeled antibodies (Fig. 2S). After the surface preparation, *E. coli* solutions at various concentrations were injected to the SPR system. Fig. 2B shows the response curve for the sample solutions with different concentrations of *E. coli* (3.5×10^1–3.5×10^6 cfu/ml). The linear range of the calibration curve ($y = 98.8x - 290.8$) was found to be between 3.5×10^3–3.5×10^6 cfu/ml with R^2 0.946 and the limit of detection was 6.2×10^3 cfu/ml. An assessment of the slopes of the two dose-response plots indicates that the response from this method is approximately 4 times stronger than that for first method.

3.3. Immobilization of E. coli antibodies onto the SPR surface via self-assembled monolayer formation

The formation of SAM is a widely used fabrication method because of several advantages, including terminal functionality, prevention of non-specific adsorption, re-use and the good stability (Shankaran and Miura, 2007). In this method gold-thiol monolayer of 11-MUA was deposited to the gold surface and then avidin was immobilized over the SAM surface via covalent binding. In the last step of the surface preparation method biotin-labeled antibody was adsorbed via avidin–biotin interaction. SPR sensogram of immobilization of *E. coli* antibodies onto the SPR surface via self-assembled monolayer formation is shown in Fig. 3S. The calibration curve of the method was obtained by injecting the *E. coli* solutions at different concentration to the modified sensor surfaces. The calibration curve that is shown in Fig. 2C has a linear range ($y = 92.7x - 106.0$) between 5.2×10^1–5.2×10^5 cfu/ml for *E. coli* with a detection limit of 35 cfu/ml.

3.4. Method based on the combination of immunomagnetic separation and SPR spectroscopy

3.4.1. Synthesis of gold-coated magnetic nanoparticles

Fig. 3 shows representative TEM images of spherical shaped gold-coated iron nanoparticles. It is found that large quantities of well-defined nanoparticles are obtained, and the products consist of a large amount of monodisperse nanoparticles. The shape distributions were obtained by measuring individual particles from TEM images. According to TEM results of Tamer et al. (2010), the iron nanoparticles have an average diameter of 9.5 ± 3 nm and after coating with gold, the average particle diameter is increased from 9.5 ± 3 to 12.5 ± 3 nm.

3.4.2. Immunomagnetic separation

The magnetic nanoparticles were modified with SAM and coated with avidin and antibody, respectively. The nanoparticles were then incubated with different concentrations of *E. coli*. Here, the capture efficiency did not change substantially until $\sim 10^5$ cfu/ml and the percentage of captured *E. coli* was found to be approximately 55%. In other words, the bacteria concentration is the limiting factor up to 10^5 cfu/ml and after this concentration; the amount of the nanoparticles becomes a limiting factor. The upper limit of the working range of the proposed strategy is below 10^5 cfu/ml.

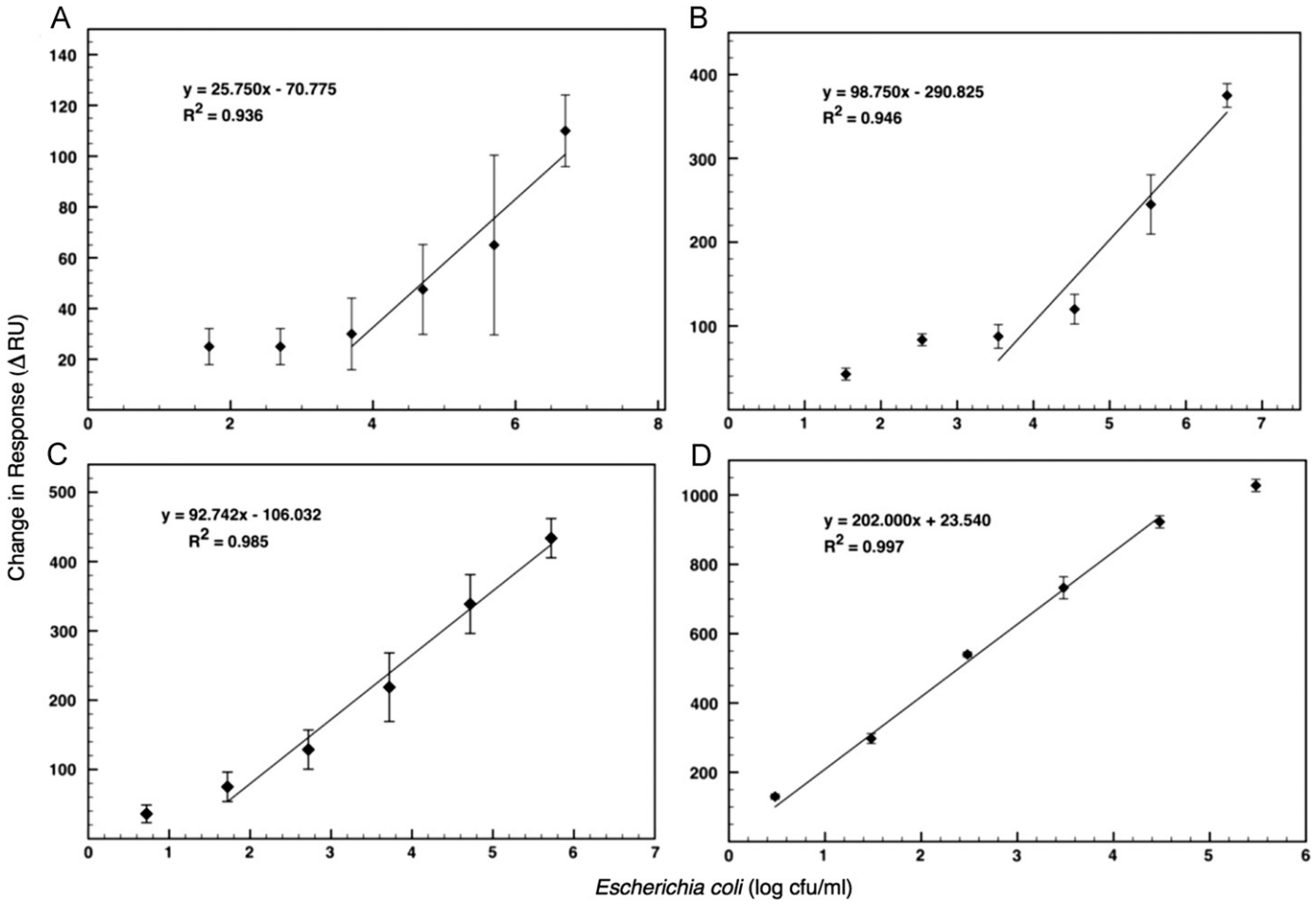

Fig. 2. Calibration curves for *E. coli* obtained with SPR systems based on the (A) non-specific adsorption of antibodies, (B) specific adsorption of antibodies via avidin–biotin interaction, (C) SAM formation and (D) using of magnetic gold nanoparticles.

3.4.3. Fluorescence imaging

Before the SPR analysis, to visually see the achievement of the IMS, both the modified nanoparticles and *E. coli* captured with nanoparticles were fluorescently labeled and examined under the fluorescence microscope. Here, fluorescamine was used as a fluorescent label. Fluorescamine reacts readily with free amino groups to form a fluorescent product easily detectable by both fluorescence microscopy and spectrofluorometry (Pihlajamaki et al., 2012). Fig. 4A shows the fluorescence image of the gold-coated magnetic nanoparticles immobilized with avidin and antibody, whereas Fig. 4B shows the fluorescence image of the *E. coli* cells captured with modified nanoparticles. As it can be seen in Figs. 4A and B, there is an increase in fluorescence due to the capture of *E. coli* because of the increase in the number of free amine groups. However, there is another main reason of the fluorescence increase. Gold is a fluorescence quencher and quenching effect depends on the distance between the molecule and the metal surface (Lokesh, et al., 2009). In Fig. 4A, fluorescamine is close to the gold surface and thus gold quenches the fluorescence intensity. On the other hand, the distance between the gold surface and fluorescamine gets bigger and so the fluorescence intensity increases. As a result of these, it could be said that *E. coli* cells had been successfully separated in a liquid media through the magnetic nanoparticles.

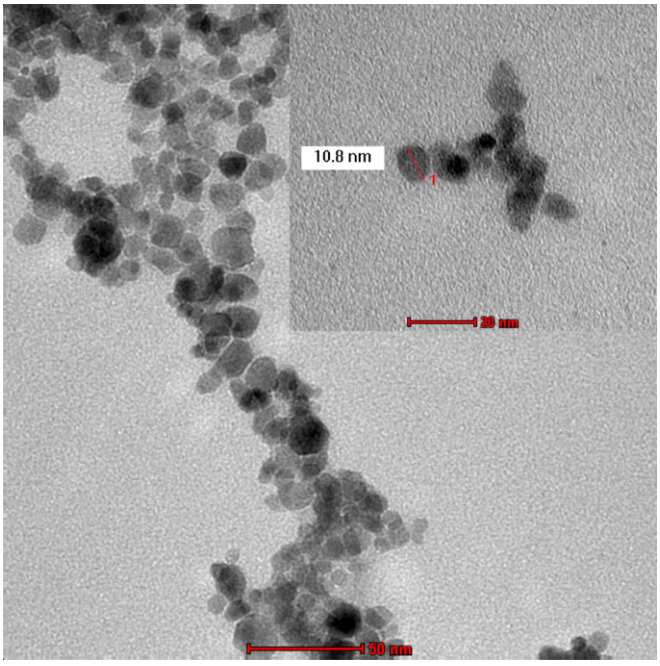

Fig. 3. Representative TEM images of spherical shaped gold iron nanoparticles.

3.4.4. SPR measurements

In the last step of the method, various concentrations of *E. coli* captured with nanoparticles were injected to the SPR surface in multiple cycles. Here, the complete regeneration of sensor surface was successfully achieved by using 0.1 M NaOH containing 1% Triton X-100 as shown in Fig. 4S. As the amount of *E. coli* cells captured with nanoparticles increased, the SPR responses also increased. Fig. 2D depicts the calibration curve obtained by calculating the RU changes for various concentrations of *E. coli*. The calibration curve was constructed by subtracting RU value of the blank solution (include only modified nanoparticles) from each of RU values obtained from the *E. coli* solutions at various concentrations. The linear range of the calibration curve is between 30–3 $\times 10^4$ cfu/ml and with the coefficient regression (R^2) 0.997. The typical equation for the calibration curve is $y=202.0x+23.5$ and LOD for *E. coli* was found to be as 3 cfu/ml. The total analysis time, which includes capturing the bacteria by modified nanoparticles (30 min), washing procedure (approximately 10 min) and SPR measurement (25 min), was less than 70 min.

3.5. Comparison of the methods

In the beginning of this study, it was thought that quantification of *E. coli* cells would be much sensitive if *E. coli* cells come close to the sensor surface. However, as it can be seen in Table 1, the least sensitive method is the non-specific adsorption method, probably because of the random orientation, conformational changes and steric hindrance of the antibody molecules and also inactivation of the antibodies upon direct contact with the metal surface. The sensitivity of the methods is also given in Fig. 5S that indicates the sensograms obtained for the binding of *E. coli* ($\sim 10^4$ cfu/ml) to the antibody immobilized surfaces. In the case of the specific adsorption of antibody molecules to the sensor surface, quantification of *E. coli* cells becomes more sensitive than the non-specific adsorption method as a result of controlling the antibody orientation. The sensitivity also increases with the monolayer formation on the gold surface. However, because of the limitations such as limited penetration of bacteria to the electromagnetic field, the low refractive index difference between the bacterium cytoplasm and the aqueous environments in which detection was performed, and the accessibility of bacterial epitopes to the antibody, the sensitivities of these three methods are not enough to enumerate the low levels of *E. coli*. At this point, a different approach should be developed to increase the SPR

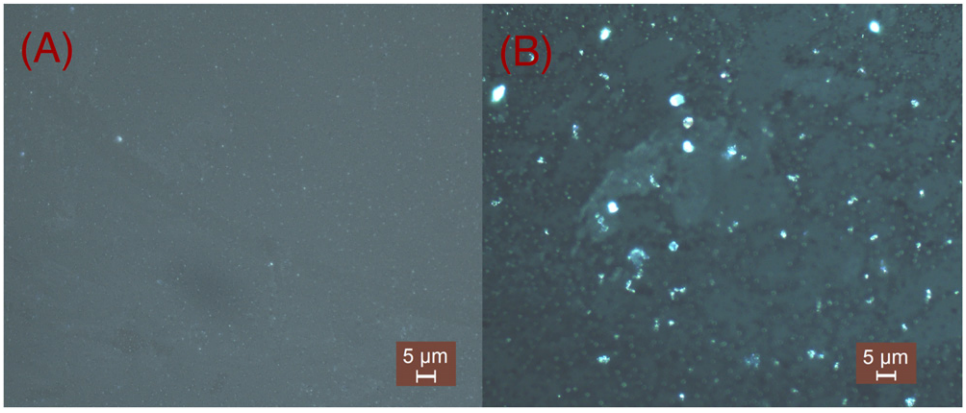

Fig. 4. Fluorescence images of gold-coated magnetic nanosphere particles: (A) coated with avidin and biotin-labeled antibody, (B) after the immunomagnetic separation of *E. coli*.

Table 1
Comparison of the methods.

Principle of the strategy	Working range (cfu/ml)	Slope of the calibration curve	LOD (cfu/ml)
Non-specific adsorption	5.5×10^3–5.5×10^6	25.3	4.8×10^5
Specific adsorption	3.5×10^3–3.5×10^6	98.8	6.2×10^3
SAM formation	5.2×10^1–5.2×10^5	92.7	35
Using of nanoparticles	3.0×10^0–3.0×10^4	209.1	3

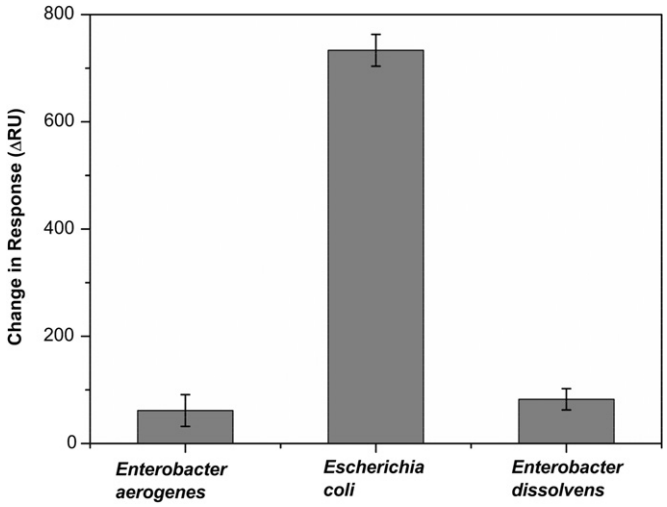

Fig. 5. Changes in RU induced by the binding *E. aerogenes* (5×10^3 cfu ml^{-1}), *E. dissolvens* (6.7×10^3 cfu ml^{-1}) and *E. coli* (3×10^3 cfu ml^{-1}).

responses and to decrease the analysis time. It is known that the response of the SPR biosensors could be enhanced using gold nanoparticles (Jung et al., 2011).

Using gold nanoparticles in SPR systems could be an effective method of enhancing the detection of low molecular weight or low concentration analytes (Fu et al., 2007; Yuan et al. 2007). Whereas the detection of large molecular weight analytes (e.g., proteins, bacteria) by SPR spectroscopy based on the use of gold nanoparticles have been also studied (Ko et al., 2009). In recent studies gold nanoparticles have been generally used in two different approaches in SPR-based assays; either as a signal enhancer in the sandwich format assays (Kim et al. 2010), or as a coating material of the sensor surface for increasing the sensitivity (Grunwald et al., 2009). However, in this study the gold nanoparticles were used for both the separation of bacteria from the sample and enhancing the SPR responses of the bacterium cells, and based on the results given Table 1, the nanoparticle-based SPR method is the most sensitive one investigated in this study.

3.6. Selectivity test and real sample analysis

The selectivity of nanoparticle-based immunomagnetic separation was tested with other coliform bacteria *E. aerogenes* (5×10^3 cfu/ml) and *E. dissolvens* (6.7×10^3 cfu/ml). The results are shown in Fig. 5. The changes in response for *E. aerogenes* and *E. dissolvens* were 61 RU and 83 RU, respectively. These responses are lower the response that corresponds to the detection limit. However, 734 RU value was found for *E. coli* (3×10^3 cfu/ml). As a result, it was observed that the response induced by the non-specific binding was much lower than that of the response induced by *E. coli*.

To check the accuracy of the developed method, *E. coli* determination was realized for real water samples and the obtained results were compared with the plate counting method. The responses of the SPR system for lake water and puddle were smaller than the detection limit of the sensor. Furthermore, the results obtained from the plate counting method indicated that there were no fecal coliform in these samples. The sensor response for river water was 27 cfu/ml and it was 30 cfu/ml for the plate counting method. In addition to these, known concentrations of *E. coli* (4×10^1, 4×10^2 and 4×10^3 cfu/ml) were added to tap water from stock *E. coli* solution (4×10^8 cfu/ml), and enumeration was implemented with the nanoparticle-based SPR method. Results were obtained from the calibration curve as 6.5×10^1, 3.4×10^2, 4.4×10^3 cfu/ml, respectively. The results obtained from this method agreed well with those obtained from plate counting method ($p > 0.05$).

4. Conclusions

We have investigated the effect of different surface modifications on the SPR response for bacteria detection. We have shown here that the challenges of size and morphology of bacteria for SPR applications could be overcome by using gold-coated magnetic nanoparticles. It is possible to quantify 10 cfu/ml *E. coli* in an hour with using antibody-coated nanoparticles. By using the proper antibodies, it could be possible to perform simultaneous detection of pathogenic bacteria. On the other hand, this new approach could be used for detection of other large molecules like proteins, and polysaccharides.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.04.034.

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