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Surface plasmon resonance based biomimetic sensor for urinary tract infections

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ABSTRACT

Tailor-made *Escherichia coli* (*E. coli*) receptors were created with microcontact imprinted technique and binding events of *E. coli* were carried out by a surface plasmon resonance (SPR) sensor in aqueous solution and in urine mimic in real time and label-free. *N*-methacryloyl-(L)-histidine methyl ester (MAH) was selected as a functional monomer to design tailor-made *E. coli* receptors on the polymeric film and during the formation of the polymeric film on a chip surface, Ag nanoparticles (AgNPs) were entrapped into the polymer mixture in order to lower the detection limit of biomimetic SPR based sensor. The polymeric film was characterized with atomic force microscopy (AFM), scanning electron microscopy (SEM), ellipsometer and contact angle measurements. Limit of detection (LOD) was found 0.57 CFU/mL and feasibility of the biomimetic sensor was investigated in urine mimic.

1. Introduction

Urinary tract infection (UTI) is a common bacterial infection of urinary system, annually affecting nearly 150 million people around the worldwide [1–4]. Epidemiology of UTI varies with age, anatomic abnormalities, gender, pregnancy and other factors such as catheterization and sexual intercourses [2]. Gram positive and gram-negative microorganisms are capable of causing urinary tract infections (UTIs) but *E. coli* a gram-negative bacterium, is the most isolated microorganism causing community acquired UTI and hospital associated UTI [5,6]. Symptoms of UTIs range from non-life treating symptoms e.g. pelvic pain and vomiting to severe complications including multi organ failure and even deaths; thereby, diagnosis of UTI is a great importance to prevent the unwanted conditions and decrease the incidence rates of UTI for public health [7,8].

UTI is generally diagnosed by conventional methods including urine culturing, urine microscopy and other methods for instance; ELISA, PCR, instrumental devices and biosensor-based detection [9]. In clinical studies, urine culturing is a standard, an economic approach for diagnostic of uropathogens but is time consuming, laborious and needs a selective growth media for only selected microorganisms [9,10]. Urine microscopy by gram staining is a rapid method for identification of microorganisms via cell morphology and allows to start the empirical antibiotic theraphy; however, quantification of bacteria in urine is not possible by this approach [5,11]. ELISA, PCR and instrumental devices including MALDI-TOF mass spectroscopy, Raman spectroscopy are alternatively used in diagnostic of UTIs and have shorter analysis time than urine culturing; however, use of them are needed well-trained person, labelling and costly equipments [5,12–14].

The requirement of rapid, reliable and sensitive methods for pathogen detection have promoted the emergence and development of biosensor platforms [15–19]. Among them, SPR based biosensors measure the change in refractive index at the sensor surface depending on analyte concentration and allow to investigate the binding events of molecular interactions in real time and label-free [20–23]. Meanwhile, SPR platforms are highly selective against the target molecules which are used in many fields for instance food quality [24–26], environmental safety [27] and as well as pathogen detection [28–30].

Lately, biomolecules including phages [19), carbohydrates [31], antibodies [32–35] and tailor-made receptors [36–40] are implemented on SPR platforms as biorecognition units in an attempt to construct microbial biosensors. Tailor-made receptors are in the position of biological counterparts thanks to capable of recognizing template molecules with high selectivity in addition, are easy-prepared, cheaper, reusable and withstand harsh conditions (pH, temperature) than biomolecules. So, easily integration of these synthetic receptors on SPR

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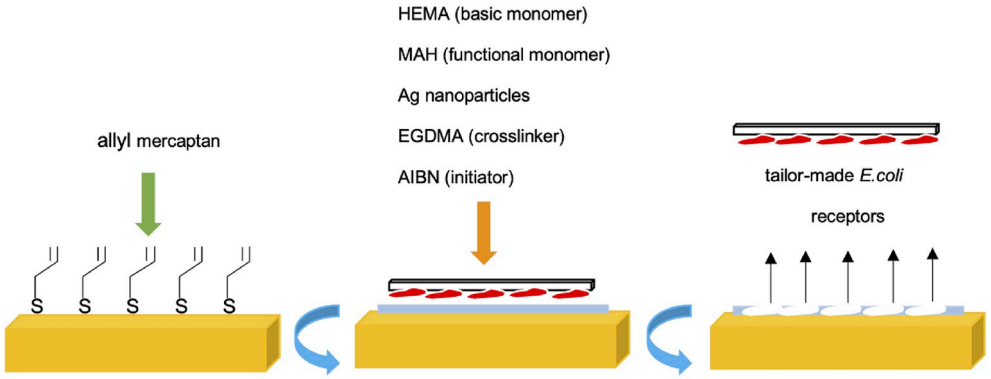

Fig. 1. Schematic representation of *E. coli* imprinted polymeric film synthesis via microcontact imprinting technique.

platforms allows to sense various analytes including clinical of interest [41,42].

In this work, we designed tailor-made *E. coli* receptors and incorporated with SPR platform for diagnostic of UTI in real time and label-free. For this purpose, MAH was selected as a functional monomer to create tailor-made *E. coli* receptors on SPR chip surface by using microcontact imprinting method. During the fabrication of polymeric film on sensing surface, AgNPs were entrapped into polymer mixture to increase the sensitivity of developed bacterial biosensor. After the deposition of polymeric film that was characterized with SEM, ellipsometer and contact angle measurements. Selectivity and feasibility studies of developed biomimetic sensor were investigated in aqueous solution and in urine mimic [43], respectively. The same chip was consequtively used 3 times to test the repeated use studies.

2. Materials and methods

2.1. Materials

E. coli (A.T.C.C. 25922) as a model organism was obtained from Refik Saydam National Publish Health Agency, 2-hydroxyethyl methacrylate (HEMA), $AgNO_3$, sodium citrate, sodium borohydride ($NaBH_4$), 3-(Aminopropyl)triethoxysilane (APTES), glutaraldehyde, lysozyme and nutrient broth were purchased from Sigma-Aldrich (Germany). Allyl mercaptan, $Na_2C_2O_4$ and ethylene glycol dimethacrylate (EGDMA) were supplied from Sigma Chemical Co. (St. Louis, USA). ∝, ∝′-Azoisobutyro-nitrile (AIBN) was purchased from Fluka A.G. (Buchs, Switzerland). Other chemicals, NH_4CI, Na_2HPO_4, NaH_2PO_4, urea were supplied from Merck and $MgSO_4$ was obtained from Codex Carlo Erba (France). All water was used during the whole experimental studies, which was purified with using Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit system.

2.2. Synthesis of AgNPs

AgNPs were synthesized according to previous study and $NaBH_4$ and sodium citrate were used as a reducing agent and a capping agent, respectively [44]. Firstly 0.3 mM sodium citrate and 0.25 mM $AgNO_3$ and the same molar ratio of $NaBH_4$ were prepared in deionized water. Secondly, the same volume (100 mL) of sodium citrate and $AgNO_3$ were mixed in flasks and stirred 10 min at 700 rpm. Afterwards, reducing agent (6 mL) was added to the first solution and the temperature was increased to 115 °C and allowed to stir at 350 rpm for 90 min. Then the solution containing AgNPs was kept at room temperature overnight for cooling then centrifuged and supernatant were discarded to obtain AgNPs thereafter AgNPs were kept in dark at room temperature until used.

2.3. Culturing of microorganism and sample preparation

E. coli was cultured in nutrient broth medium (10 mL) at 37 °C for overnight under sterile conditions, after culturing of *E. coli* samples, which were kept at +4 °C during used. Stock bacterial sample (0.5 McFarland) was prepared according to McFarland standard by using a spectrophotometer at 625 nm and stock bacterial solution was serial 10 fold-diluted with the pH of urine mimic (pH 6.2) in order to adjust the *E. coli* solutions in the ranges of 10^1–10^6 CFU/mL.

2.4. Preparation of tailor-made receptors on SPR sensor chip surface

Preparation of the functional monomer and the surface modification of the SPR sensor chip were performed according to previous studies [45,46]. The gold surface of sensor chip was firstly cleaned with piranha solution (3:1 H_2SO_4/H_2O_2 v/v) for 1–2 min and rinsed with ethyl alcohol, following that was dried in a vacuum oven (200 mmHg, 40 °C). After the cleaning step, 3.0 M allyl mercaptan solution (5 µL) was dropped onto the gold surface of the SPR chip and incubated for 12 h in a sealed container for introducing the allyl groups on the gold surface. After that, pure ethyl alcohol was used to remove the unbounded allyl mercaptan molecules on the gold surface, then modified chip was dried in a vacuum oven (200 mmHg, 25 °C).

Before the covalent attachment of *E. coli* cells on glass surfaces, which were firstly immersed in %2 (v/v) APTES solution for 2 h to introduce glass slide surfaces amine groups. Following this, the activation of amine groups on glass slides were treated with %3 (v/v) glutaraldehyde solution in PBS buffer for 2 h. Thereafter, cultured live *E. coli* cells were dropped and coated on the activated surfaces and kept at +4 °C for 12 h. Subsequently, *E. coli* attached glass slides were washed with deionized water to remove the unbounded *E. coli* cells from the modified glass surfaces.

Before the formation of polymeric film on the modified gold surface of SPR chip (Fig. 1), polymer mixture containing 20 mg MAH as a functional momoner, 400 µL HEMA as a basic monomer, 100 µL EGDMA as a crosslinker, 3.0 mg AgNPs, 5.0 mg AIBN as an initiator were prepared as a stock monomer solution and 2.5 µL solution was taken from a stock solution and was dropped on the modified gold surfaces. The preparation of *E. coli* imprinted surfaces or designing of synthetic receptors were prepared by using micro-contact imprinting technique and the glass slide surface where *E. coli* bounded was brought into contact with the monomer solution which was drop casted on the gold surface of SPR chip and pressed like a sandwich.

Afterwards, the polymerization was continued nearly 30 min at room temperature by using UV light (100 W, 365 nm). Following that, the preparation of *E. coli* imprinted (MIP) sensor, glass slide was stripped from the modified gold surface in order to create the tailor-made *E. coli* receptors. After the design of synthetic receptors, MIP sensor surface was washed with %10 (v/v) ethanol solution and 1.0 M

lysozyme solution (in PBS buffer, pH 7.4) for the removal of possible bacterial residues during the micro-contact imprinting process. Finally, the surface of SPR chip was rinsed with deionized water.

The same micro-contact imprinting process was applied for the preparation of non-imprinted (NIP) sensor. However, the immobilization of *E. coli* cells onto the glass slide was not conducted.

During the formation of NIP polymeric film onto the modified gold surface, monomer solution containing; HEMA, EGDMA and AgNPs were used with the same ratio during the preparation of MIP sensor.

2.5. Characterization studies

Before the SEM analysis of AgNPs, AgNPs were suspended in ethanol solution and sonicated for 1 h at room temperature, then 2.5 μL AgNPs were dropped on a stamp and dried at room temperature. Afterwards, AgNPs sample was analyzed with SEM without gold coating. SEM analysis were carried out using Gaia 3 microscope (Tescan, Czech Republic). The size distribution of AgNPs were measured by zetasizer (Malvern Instruments, London, U.K.) and light scattering method was performed at incidence angle 90° and 25 °C. Before the measurement of size distribution, AgNPs were suspended in deionized water and were sonicated for 30 min at room temperature. Before the data analysis, refractive index and density of deionized water were used as 1.33 and 0.88 mPa s, respectively. AFM analysis were carried out in tapping mode with ambient AFM (Nanomagnetics Instruments, Oxford, UK) and data processed with Gwyddion software (ver:2.5.4.). The thickness and surface wettabilities of polymeric films on MIP and NIP sensor surfaces were investigated with ellipsometer (Nanofilm EP3, Goettingen, Germany) and contact angle (Kruss DSA 100, Hamburg, Germany) measurements. The thickness of the polymeric films on both sensors were measured with ellipsometry at a wavelength of 658 nm with incidence angles of 62°. For understanding the surface wettabilities of the polymeric films, sessile drop method was used and different parts of the surfaces were chosen and images were taken.

2.6. Kinetic analysis

Prior to kinetic analysis, stock *E. coli* solution (0.5 McFarland) was prepared according to McFarland standard by using a UV spectrophometer at 625 nm and serial dilutions were made with pH 6.2 buffer solution. Afterwards, the kinetic analysis of *E. coli* detection was carried out in aqueous solution (pH 6.2) and in urine mimic in real time and label free at room temperature.

Before the binding events of *E. coli*, MIP sensor surface was cleaned with ethanol solution and was rinsed with deionized water. After that, the surface was equilibrated with pH 6.2 buffer solution and different amounts of *E. coli* solutions (1.5×10^1–1.5×10^6 CFU/mL) were separately (5 mL) sent to SPR system. When the stable signals were monitored, 1 mg/mL lysozyme solution (0.1 M PBS buffer) was used to remove *E. coli* from the surface. Then MIP sensor surface was washed with ethanol and was rinsed with deionized water for the next cycle. The LOD of proposed sensor was calculated (Supplementary data) with previous study [47].

2.7. Selectivity studies

E. coli is the member of Enterobacteriaceae family, a gram negative, rod shape bacteria and in order to evaluate the recognition ability of MIP sensor, two bacterial strains *Salmonella* sp. and *Staphylococcus* sp. were selected. The concentration of competitive bacterial samples were prepared according to the McFarland standart (0.5 McFarland) by using a spectrophotometer and were adjusted to 1.5×10^6 CFU/mL with 10 fold serial dilutions. Before the selectivity studies, MIP sensor surface was firstly cleaned with ethanol solution, equilibrated with pH 6.2 buffer solution, then bacteria samples (5 mL) were separately sent to SPR device for the monitoring of kinetic analysis. After the stable signals were monitored, MIP sensor was firstly cleaned with 1 mg/mL lysozyme solution (0.1 M PBS buffer) nearly for 30 min and then ethanol solution was used to sterilize the surface. After the sterilization, MIP sensor was rinsed with deionized water for the next experimental studies.

2.8. Urine mimic studies

Urine is a clinical analyte used for diagnostic of UTI. So, the feasibility of developed biomimetic sensor was investigated in urine mimic as a clinical analyte. Urine mimic solutions were spiked with *E. coli* cells. Before the feasibility studies, the surface of MIP sensor was equilibrated with urine mimic solution after that the different amounts of *E. coli* solutions (1.5×10^4–1.5×10^5 CFU/mL) were sent to SPR system in order to evaluate the binding events of *E. coli*. After the signals were stable, firstly 1 mg/mL lysozyme solution was used to remove the *E. coli* from the surface, then MIP sensor surface was cleaned and rinsed with ethanol solution and deionized water, respectively.

2.9. Reusability studies

Reusability studies of biomimetic sensor were made in aqueous solution by using repeated three adsorption-desorption-regeneration cycles. For this purpose, the same chip was used and 1.5×10^4 CFU/mL solution was selected to examine the reusability of developed sensor. After reusability studies, MIP sensor surface was firstly treated with lysozyme solution (1 mg/mL) thereafter, sterilized with ethanol and rinsed with deionized water.

3. Results and discussion

3.1. Characterization studies of AgNPs and the sensor surface

Particle sizes of AgNPs were found 31.55 nm with a standard deviation of 3.859 nm and the measurement results were illustrated in Fig. 2A. The particle size of AgNPs is a suitable size for the plasmon formation [48] and it was easier to detect change of reflective index that can enhance the sensitivity of SPR biosensing owing to getting the smaller sizes of AgNPs [49]. As illustrated in Fig. 2B and C were showed that AgNPs were successfully synthesized and distributed in the polymeric film.

Polymeric films on gold sensor surfaces were investigated with SEM, ellipsometer and contact angle measurements. As seen in Fig. 3A, whole *E. coli* cells were successfully imprinted onto the modified gold surface by using micro-contact imprinting approach and during the imprinting process, the specific rod shape of *E. coli* can be protected by this imprinting process. Additionally, the same results of SEM image could be supported with AFM (3B) image. So, in the light of both images, specific rod shape binding cavities were successfully created on the MIP sensor surface.

The surface wettabilities of MIP and NIP sensors were investigated with contact angle measurements and results were given in Fig. 4. As seen in Fig. 4, the surface wetteability of MIP sensor was more hydrophilic than NIP sensor and the measurement results of were found 65.7° ± 2.23 for MIP and 72.5° ± 0.45 for NIP sensors. As shown in contact angle images, MIP sensor surface was more hydrophilic than NIP sensor, because during the preparation of MIP sensor, MAH is as a functional monomer, which was used during the formation of MIP sensor. So, a functional monomer MAH can increase the surface wettability of MIP sensor; meanwhile, the increased hydrophilicity of MIP sensor surface supported that the functional monomer was successfully jointed into the polymer chain.

The thickness of the films on MIP and NIP sensors were evaluated with ellipsometer measurements and thicknesses of the polymeric films on MIP and NIP sensors were calculated 50.0 nm ± 11.7 and 50.0 nm ± 8.2, respectively and ellipsometric measurement results

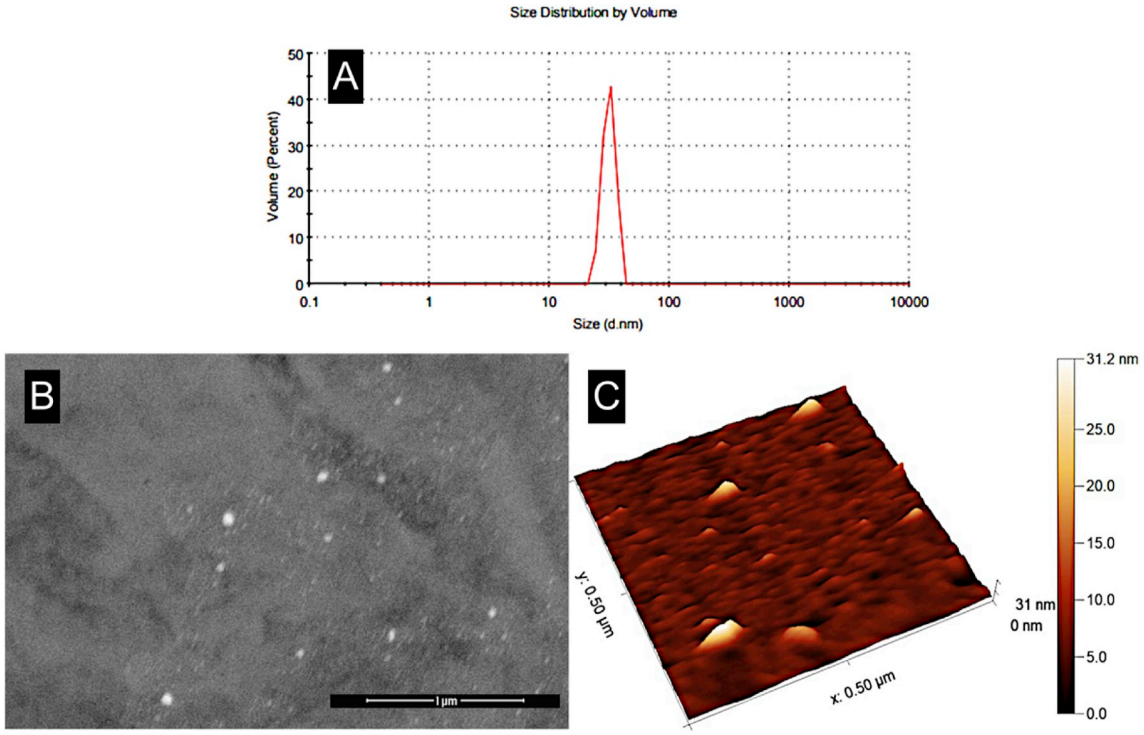

Fig. 2. Zeta-sizer measurement (A), SEM (B) and AFM (C) images of AgNPs.

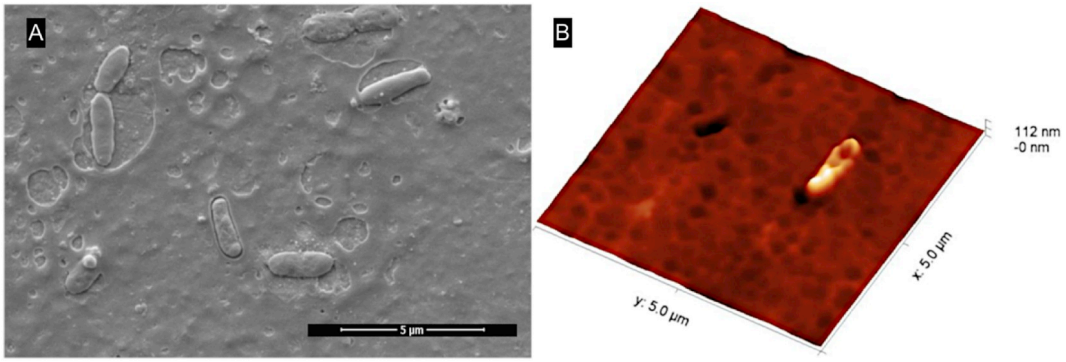

Fig. 3. SEM (A) and AFM (B) images of the specific binding cavities of *E. coli* formed on the polymeric film of the MIP sensor surface.

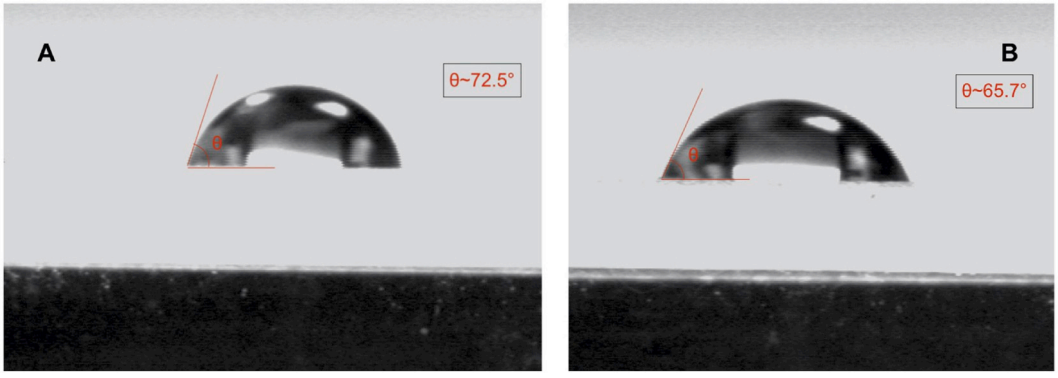

Fig. 4. Surface wettabilities of the polymeric films on NIP (A) and MIP (B) sensor surfaces.

were illustrated in Fig. 5. According to measurement results, both the polymeric films on MIP and NIP sensor surfaces were successfully formed.

3.2. Kinetic analysis

Binding events of *E. coli* were examined with SPRiLab (France) at room temperature and before the kinetic analysis, sensor surface was equilibrated with pH 6.2 buffer solution. After the equilibrated of

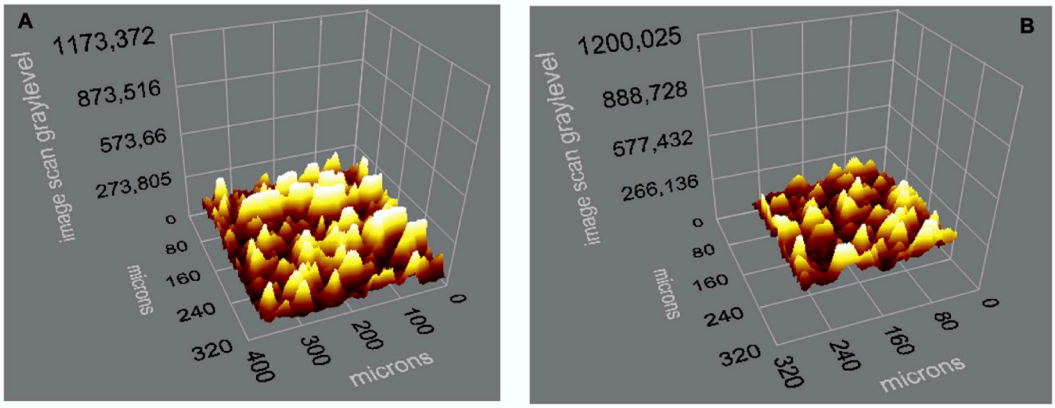

Fig. 5. Ellipsometer measurements of the MIP (A) and NIP (B) polymeric films on the sensor surfaces.

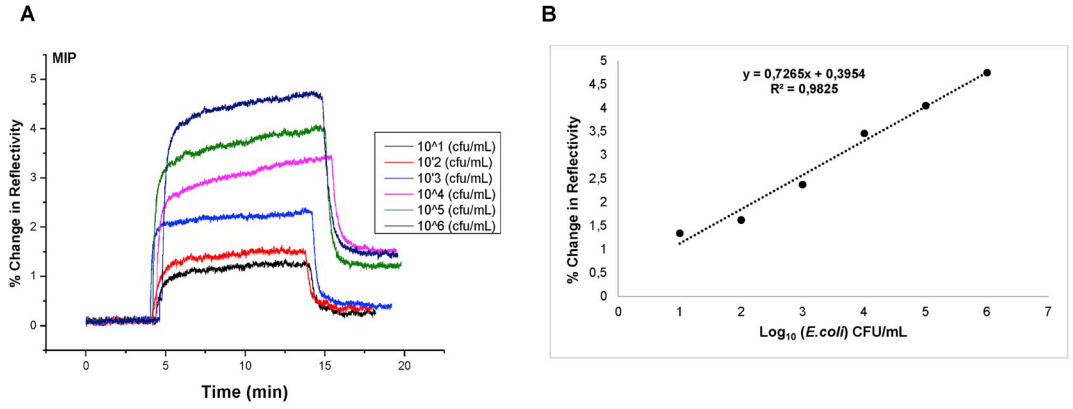

Fig. 6. Dynamic response of MIP sensor versus the *E. coli* concentrations (A) the linearity MIP sensor (B) in aqueous solution (pH: 6.2 and T: room temperature).

Table 1
Comparison results of the present study towards previous studies according to method, bioreceptor type, linear range, LOD analysis time.

Method	Bioreceptor	Linear Range	LOD	Analysis Time	Ref
SPRi	antibody	10^3–10^9 CFU/mL	10^2 CFU/mL	35 min	[1]
Electrochemical	prob (DNA)	4–4 × 10^8 CFU/mL	4 CFU/mL	70 min	[6]
Electrochemical	phages	15–1.5 × 10^8 CFU/mL	1 CFU/mL	140 min	[17]
Microfluid	antibody	10^3–10^7 CFU/mL	1.2 × 10^2 CFU/mL	< 7 min	[50]
Microfluid	antibody	10–10^7 CFU/mL	10 CFU/mL	30 s	[51]
Electrochemical	–	7 × 7 × 10 cell/mL	7 cell/mL	–	[52]
SPR	tailor-made receptor	1.5 × 10^1–1.5 × 10^6 CFU/mL	0.57 CFU/mL	20 min	this study

Table 2
Selectivity results of the devepoled sensor.

	MIP		NIP		
	%R	k	%R	k	k'
E.coli	4.75		0.42		
Staphylococcus sp	0.19	25	0.49	0.85	29.41
Salmonella sp	0.45	10.56	0.40	1.05	10.05

sensor surface, MIP sensor was characterized in the range of 1.5 × 10^1–1.5 × 10^6 CFU/mL *E. coli* samples in aqueous solution. As illustrated in Fig. 6A binding events of *E. coli* were gradually increased with the increasing amount of *E. coli* concentrations and correlation coefficient (R^2) of MIP sensor was calculated 0.9825 (Fig. 6B). When desorption solution went to MIP sensor surface, which removed the bound *E. coli* cells from the specific binding cavities caused a decrease in the signal responses.

LOD of proposed sensor was calculated (Supplementary Data) as 0.57 CFU/mL and analysis time of our developed biomimetic sensor was found approximately 20 min, which is shorter than the urine culturing of bacteria in selective media as a standard method and some of reported studies for *E. coli* detection by using different biosensing platforms [1,6,17]. Comparison results including bioreceptor type, linear range, LOD and analysis time of our study and other sensing approaches were given in Table 1.

3.3. Selectivity

Selectivity is the main criteria for molecularly imprinted polymers (MIPs), so; to test the selectivity of developed sensor, *Staphylococcus* sp. and *Salmonella* sp. strains were chosen because *Staphylococcus* sp. is a gram positive, grape-like bacteria and has different cell wall and morphology when compared with *E. coli*. The reason of *Salmonella* sp. selection is its rod shaped and the same family with *E. coli*. For these reasons, we selected them to determine the shape recognition and the memory abilities of MIP. To calculate the selectivity of MIP sensor, we used selectivity coefficient (k) and relative efficiency coefficient (k')

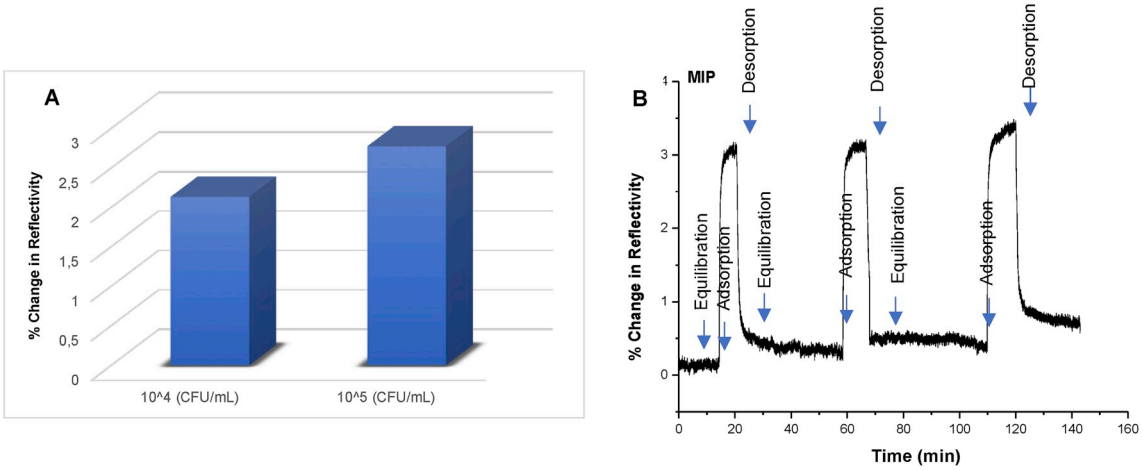

Fig. 7. Urine mimic analysis (A) and reusability studies (B) of the MIP sensor (pH: 6.2 and T: room temperature).

values. k value was calculated with %R (template/competitor) whereas, k' was calculated by using %R (MIP/NIP) value. The result of the selectivity studies was shown in Table 2 and the biomimetic sensor recognised 29.41 and 10.05 times greater then *Staphylococcus* sp. and *Salmonella* sp., respectively.

3.4. Urine mimic studies

Feasibility of the prepared MIP sensor was evaluated in urine mimic. For this aim, different bacterial solutions in the range of 1.5×10^4–1.5×10^5 CFU/mL was prepared and was sent to SPR system to investigate the usability of the biomimetic sensor in urine mimic as a clinical analyte. As seen in Fig. 7A., increasing amount of *E. coli* concentration can increase the SPR signals of develop biosensor and these results supported the usability of proposed sensor in urine mimic. In addition, the LOD of MIP in urine mimic, was found lower than 10^4 CFU/mL and this result was quite lower than the threshold value of UTI's symptoms [48] and analysis time of proposed sensor was shorter (nearly 20 min) than conventional methods especially urine culturing. So, developed biomimetic sensor could be alternatively used for diagnostic of UTIs owing to its lower LOD and shorter analysis time.

3.5. Reusability

Reusability studies play a key role for adsorption studies especially cost of analysis and durability of support materials during the whole adsorption process. To examine the reusability of the biomimetic sensor, 1.5×10^4 CFU/mL bacterial solutions were prepared and went to SPR system with 3 consecutive adsorption, desorption and regeneration cycles by using the same chip. As seen in Figure the 7B., the same SPR signals were monitored after 3 consecutive adsorption-desorption and rejeneration cycles without lost of adsorption capacities. So, it is possible to say that prepared biomimetic sensor is suitable for repeated use according to the experimental results.

4. Conclusions

UTI is a common bacterial infection and if left untreated that causes organ failure and even deaths; so, diagnostic of UTI is a great importance for public health. Herein, we tried to the develop biomimetic sensor for diagnostic of UTI with the combination of molecular imprinting technique and SPR based sensor.

We chose MAH as a functional monomer via of functional groups during the designing of *E. coli* receptors on the MIP sensor surface, because the attachment of bacteria on surface is related about some factors such as length contact time, pH, hydrophobicity, surface charge and chemical structures of bacterium can effect the attachment of surface [46]. *E. coli* is a gram negative bacteria, carries a negative charge thanks to some functional groups including phosphate, hydroxyl and carboxylate groups where its cell wall and has a moderate hydrophobicity owing to lipopolysaccharide structures on cell wall [53,54].

So, tailor-made *E. coli* receptors were designed on a gold surface of SPR chip by using MAH monomer via of functional groups and before the formation of polymeric film, AgNPs were entrapped into polymer mixture to increase the sensitivity of biomimetic sensor and kinetic analysis of target microorganism were monitored in real time and label-free in aqueous solution and in urine mimic.

The specific binding cavities that were able to recognize *E. coli* was successfully designed on the MIP sensor and was showed ib SEM and AFM images. Biomimetic SPR based sensor was capable of detecting *E. coli* with high selectivity; additionally, LOD and analysis time of proposed sensor were found 0.57 CFU/mL and nearly 20 min, respectively in aqueous solution, which were lower than threshold value of UTI's and shorter analysis time than conventional methods. Meanwhile, biomimetic sensor was made an attempt in urine mimic and LOD of developed sensor was highly predictive; so, biomimetic sensor could be alternatively used for diagnostic of UTI due to its selectivity, shorter analysis time and lower LOD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.120778.

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