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Whole cell imprinting based *Escherichia coli* sensors: A study for SPR and QCM

Erkut Yilmaz [a], Daryoush Majidi [b], Erdogan Ozgur [b], Adil Denizli [b,*]

[a] Department of Chemistry, Aksaray University, 68100 Aksaray, Turkey
[b] Department of Chemistry, Hacettepe University, 06800 Ankara, Turkey

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ABSTRACT

The detection of pathogenic bacteria is essential for a sustainable prevention of water quality and by this way prevention of life threatening illnesses. It is important to use rapid methods for pathogenic bacteria detection but traditional detection methods may take up to a whole week. In this report, we describe a molecular imprinting based bacteria sensor via whole cell imprinting for rapid detection of bacteria from water sources. Here, *Escherichia coli* (*E. coli*) is selected as model bacteria because of it is one of the most abundant pathogenic bacteria in ground water sources and may cause severe illnesses. The presence of *E. coli* in the water sources is an indicator of urban and agricultural runoffs, so monitoring *E. coli* contamination is important to preserve quality of the water sources. Traditional detection methods for *E. coli* include genomic analysis, antibody based assays, culture methods, fluorescence and microscopy. Disadvantages of traditional bacteria detection methods induce many researches on other detection methods like biosensors. In this study, a new label-free rapid and selective detection method was developed via micro contact imprinting of whole cell on both optical and mass sensitive devices. The amino acid based recognition element, N-methacryloyl-L-histidine methylester (a polymerizable form of histidine) was used in this study to obtain similar recognition as in natural antibodies.

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1. Introduction

Early detection of pathogenic bacteria has great importance. Here, *Escherichia coli* (*E. coli*) is selected as model bacteria because of it is one of the most abundant pathogenic bacteria in ground water sources and may cause severe illnesses [1]. *E. coli* O157:H7 is a type of fecal coliform bacteria and acts as an easy indicator for fecal coliform contamination. Recreational and drinking water supplies have been reservoirs for *E. coli* allowing for the transmission of the pathogen and outbreaks of infection [2]. As little as 10–100 *E. coli* cells are sufficient to cause disease [3]. Ultimately, contamination with this organism can result in lowered water quality and increased human fatality [4]. It is important to use rapid methods for pathogenic bacteria detection but traditional detection methods may take up to a whole week. Traditional detection methods for *E. coli* include genomic analysis via PCR, antibody based assays, culture methods, fluorescence, and microscopy [5]. Among classic detection methods of microorganisms the most commonly used procedures are colony counting and culture [6]. Culture and colony counting based methods are more long term than PCR but both of them afford decisive explicit result. Also newly developed PCR technologies like real-time-PCR, results are retrieved in short periods [7]. Developments in biosensors of technology bring more acceptable results in a short time period and this is the reason for the increase of interest in these devices.

One drawback to rapid detection methods is that they usually require many steps including a lengthy enrichment process. This enrichment process may include separation and extraction techniques, and sample growth in media selective for *E. coli.* Disadvantages of traditional bacteria detection methods cause many researches on other detection methods like biosensors. However, biosensors are usually known to provide real-time measurements and allow rapid analysis time. Though biosensors offer many advantages to current rapid detection methods, there is still a lot of room for growth in this field [8].

There are many types of biosensors currently being explored for bacteria detection. Among these biosensors Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance (QCM) based biosensors have many advantages including real-time detection capabilities and total detection (preparation and detection) of 1 h

* Corresponding author at: Hacettepe University, Department of Chemistry, Biochemistry Division, 06532 Ankara, Turkey. Tel.: +90 312 2977983; fax: +90 312 2992163.
E-mail address: denizli@hacettepe.edu.tr (A. Denizli).

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or less and low organism detection limits. Both SPR and QCM based biosensors are label free technologies and they are known for their ease of use, sensitivity, and quick response time. These advantages include real-time detection capabilities and low detection limits. Use of SPR and QCM based biosensors to detect bacteria may offer a fast, reliable, and cost effective way to quantify the organism. This will allow not only researchers, but also monitoring stations to obtain quick and reliable results, and in effect produce quicker solutions to the contamination problems in field works.

In this study, we combined advantages of SPR and QCM based sensors with molecular imprinting based synthetic receptors. Molecular imprinting is a method for producing cavities in/on polymeric structure that fits to the target molecule. Because of these cavities that have shape and chemical recognition memory (similar to the recognition mechanisms in living organisms) the resulting structure known as biomimetic or synthetic receptors. There are many biosensor applications of molecular imprinting for the detection of variety of biological molecules like proteins [9–12], hormones [13], but only a few studies on whole cell imprinting. In a study conducted by Dickert and Hayden a selective detection of *Saccharomyces* was emphasized using cell imprinted polymers with a detection ranges within 10^4–10^9 cells/mL in a QCM system [14]. In another study, Tokonami et al. [15], effectively developed a label free and selective *E. coli* identification by surface imprinting the whole cell imprinting of bacteria on the surface of overoxidized polypyrrole, which could detect the bacteria within the range of 10^3–10^9 CFU/mL. Findeisen et al. [16] studied biological agent detection via MIP based artificial receptor layers, and used *E. coli* as model agent. They used spin coated polymeric film with 300–400 nm thicknesses and achieved a lower detection limit of 0.1 mg/mL *E. coli*.

In this report we describe a molecular imprinting based bacteria sensor via whole cell imprinting for rapid detection of bacteria from water sources.

2. Materials and methods

2.1. Materials

E. coli, a group beta *Streptococcus* and *Bacillus* sp. were supplied from the Department of Biology Hacettepe University, Turkey. Nutrient broth and Gram Staining Kit were purchased from Sigma–Aldrich Chemie, Switzerland. Gold SPR and QCM chips were purchased from GWC Tech (Madison, USA) and Maxtek Inc. (New York, USA) respectively. (3-Aminopropyl)-trimethoxysilane (APTMS), glutaraldehyde, allyl mercaptan, 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were supplied from Sigma Chemical Co. (St. Louis, USA). Lysozyme (from chicken egg white) and α,α′-azoisobutyronitrile (AIBN) were purchased from Fluka A.G. (Buchs, Switzerland). N-methacryloyl L-histidine methylester (MAH), was supplied from Nanoreg (Ankara, Turkey). All solvents (HPLC grade) were from commercial sources and used without further purification. All other chemicals used were reagent grade from Merck A.G. (Darmstadt, Germany) unless otherwise noted. All water used during the experiments was purified using a Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit.

2.2. Preparation of E. coli imprinted sensors

2.2.1. Cleaning procedures and modification of glass slides

Glass slides were cleaned with pure ethyl alcohol and deionized water for 10 min and were immersed in 20 mL of freshly prepared acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v) for 15 min to remove organic residues from glass slides. Then, they were washed with deionized water and dried in vacuum oven (200 mmHg, 37 °C) for 2 h. Acidic piranha removes organic residues due to strong oxidative character and hydroxylates glass surfaces, makes them extremely hydrophilic.

To form covalent —Si—O—Si— bond on the hydroxylated glass surface 2% (v/v) solution of APTMS in anhydrous toluene was used at room temperature for 2 h. Then, glass slides rinsed with excess of toluene to remove noncovalently bound APTMS from the surface and dried under vacuum oven. APTMS modified glass slides were subsequently immersed in 3% (v/v) glutaraldehyde solution in phosphate buffered saline to obtain aldehyde groups on the surface. For removal of unbound glutaraldehyde, glass slides were rinsed with excess of water and dried at room temperature. The 3% (v/v) glutaraldehyde solution was used as fixation solution for *E. coli*. 100 μL suspension of *E. coli* was dropped onto the glass slide surface and dried at room temperature.

2.2.2. Cleaning procedures and modification of SPR and QCM sensors

The gold surfaces of SPR and QCM sensors were cleaned with acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v), then washed with excess of deionized water and pure ethyl alcohol, respectively and dried in vacuum oven (200 mmHg, 40 °C) for 2 h.

After washing steps, 5 mL of 3.0 mM allyl mercaptan solution was dropped onto the gold surfaces of SPR and QCM chips and incubated for 12 h in a sealed container in order to form allyl groups on the gold surfaces. Unbounded allyl mercaptan molecules were removed by washing with pure ethyl alcohol and then modified chips were dried in vacuum oven (200 mmHg, 25 °C).

2.2.3. Bacteria sample preparation

Bacterial species were cultured in 25 mL of nutrient broth overnight at 37 °C. The cultures of bacterial species were centrifuged at 4000 rpm for 15 min and the precipitates were dispersed in 30 mL of sterilized water. This procedure was repeated at least 3 times to obtain purified target analyte (bacterial concentration of 10^{10} CFU/mL). Here, McFarland standards were used to adjust the number of bacteria within a desired range to standardize microbial testing [17]. Serial diluted samples from 12×10^8 to 1.5×10^8 CFU/mL (due to the McFarland standards) were prepared with sterilized water. Suspensions of bacteria with the concentration of 1.5, 3, 6, 9×10^8 CFU/mL according to the McFarland standards were used as bacterial samples.

2.2.4. Formation of E. coli imprinted polymeric film on the surfaces of SPR and QCM sensors

E. coli imprinted sensor surfaces were obtained by micro-contact imprinting through bringing both of *E. coli* attached surfaces and modified gold surfaces into contact like a sandwich. HEMA (100 μL), EGDMA (500 μL), deionized water, MAH (10 mg) and the initiator AIBN containing stock monomer solution were prepared. 3 μL aliquot was taken from the stock monomer solution and dropped onto the allyl mercaptan modified SPR and QCM chips. Then, *E. coli* attached surfaces were placed into this solution and pressed. Polymerization was initiated using UV light at room temperature (100 W, 365 nm) and was continued for 20 min at room temperature under nitrogen atmosphere. After the polymerization process, *E. coli* attached glass slides were stripped from the SPR and QCM sensor surfaces. The polymeric film coated SPR and QCM sensors were washed with pure ethyl alcohol and 1 M lysozyme solution (in pH 7.4, PBS buffer, 100 mM) for 15 min to remove possible bacterial residues during imprinting process from sensor surface. Finally, they were dried in a vacuum oven and stored in a vacuum desicator until use. With microcontact imprinting technique complementary cavities with chemical recognition ability for *E. coli* were obtained on the sensor surface (Fig. 1). The non-imprinted sensors were

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