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Research Paper

Novel impedimetric aptasensor for label-free detection of *Escherichia coli* O157:H7

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ABSTRACT

Microbial safety of drinking water constitutes a major concern in countries at all levels of economic development. Thus, rapid, sensitive and cost effective methods of pathogenic bacteria detection, like common *Escherichia coli* O157:H7, which can cause important diseases, are highly required.

In this work an impedimetric transducer modified with *E. coli* specific aptamer is studied. To enhance the sensitivity a three-dimensional interdigitated electrode array (3D-IDEA) impedimetric transducer, in which the electrodes are separated by insulating barriers was used. In this sensor chemical reactions at the surface of the barrier provoke electrical charge redistribution which causes changes in the surface conductivity. A DNA aptamer, which recognizes specifically the outer membrane proteins of the *E. coli* O157:H7, was selected as the biorecognition moiety.

Here we report a novel label-free impedimetric aptasensor for detection and quantification of pathogenic *E. coli* O157:H7 with a low detection limit, good selectivity and short detection time. The developed sensor shows a linear response ($R^2 = 0.977$), proportional to the logarithm of bacterial concentration present in the sample, with the limit of detection (LOD) of about 10^2 cfu mL^{-1}. No response of the aptasensor was registered in the presence of other bacterial strains (*E. coli* k12, *Salmonella typhimurium, Staphylococcus aureus),* which confirms the selectivity of the suggested detection method. Additionally, the methodology of the aptasensor regeneration was developed, so that the detection may be performed several times with the same sensor. Moreover, suitability of the aptasensor for bacteria detection in real samples was demonstrated with a new approach involving bacteria pre-concentration.

1. Introduction

Water plays a crucial role in economic development and social welfare, being a key factor in health, food production and poverty reduction [1]. The quality of drinking or irrigation water is significant for health in both developing and developed countries worldwide.

Pathogenic bacteria in water are one of the main causes of human infection diseases and their detection with suitable, sensitive and fast methods is of great importance [2]. The presence of *Escherichia coli* bacteria in environmental samples, food or water usually indicates fecal contamination, lack of hygienic practices and storage conditions, revealing a high risk of the presence of other fecal-borne bacteria and viruses, many of which are pathogenic [3].

Among the different strains, the enterohemorrhagic *E. coli* O157:H7 can produce from slight to life-threating diarrhea and is considered the major causative agent of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) due to the production of verotoxins [2,4]. The illness is associated with the consumption of contaminated food, including unpasteurized milk or fresh products like leaf lettuce and apples, as well as water that has not been properly disinfected [5]. Therefore, the presence of these bacteria is a major concern in food industry and water treatment. The infection dose of entreohemorragic *E. coli* is around 100 colony forming units organisms, low compared with other virulence types [6], thus highly sensitive, rapid and selective detection methods of *E. coli* O157:H7 are on demand.

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Conventional methods of bacteria detection, like microbiological culturing and isolation, show lack of sensitivity, involve enrichment steps with long processing times and require trained personnel [7]. It may be noted that not all bacteria can be cultured in the laboratory. Immunological tests, like enzyme-linked immunosorbent assays (ELISA) in a 96-well microtiter plates, make it possible to simultaneously analyze a great number of samples. However, it is also time-consuming, requires trained staff and its sensitivity sometimes is insufficient. Nowadays, the most commonly used methods are based on molecular techniques, like polymerase chain reaction (PCR), in which the analysis time is shorter compared to traditional methods, but it uses a complex set-up, expensive reagents and equipment [8,9]. Therefore, new highly sensitive, fast and low cost analytical technologies are requested, permitting to avoid multiple operation steps and long-processing times. In this sense biosensors, devices that integrate a biological recognition element and a physical transducer that generate a measurable signal proportional to the concentrations of analytes [10], are promising tools for bacteria detection.

Different biomolecules may be employed as a recognition element in biosensors for bacteria detection. These include enzymes, antibodies, nucleic acids or lectins [9]. Antibodies are the most widely used owing to their high selectivity and affinity with target molecules [11,12]. However, antibodies production being based on a clone technology involves considerable cost. Additionally, antibodies show instability in a certain range of pH and temperature, short shelf life, easy degradation. These properties generate certain problems for their application in robust biosensors [12–15].

Aptamers are artificial oligonucleotide sequences of 30–100 nucleotides of DNA or RNA molecules that bind to specific ligands with high affinity and can be easily synthesized with high specificity to a certain target molecule. They are produced in vitro from pools of random nucleic acids sequences through the selection evolution of ligands by exponential enrichment, SELEX process [16,17]. Aptamers show binding affinity and specificity comparable to those of antibodies and they present important advantages, like better reproducibility in chemical production, stability under a wide range of pH conditions, resistance in harsh environments without losing their bioactivity, small size and low production cost [15]. Moreover, in the course of their chemical synthesis they can be easily modified introducing specific active terminal groups that help to immobilize effectively these molecules on different transducers, thus forming biosensors, commonly named as aptasensors [18]. In recent years a large number of publications have been focused on the development of aptamer-based biosensor for detection of pathogenic bacteria: *Salmonella* [19], *Staphylococcus aureus* [20], *Listeria* [21], and also *E. coli* [14].

More concretely, in the case of *E. coli* O157:H7 a great number of optical and electrochemical biosensors have been reported in the literature [22], but only few works are focused on the use of aptamers. Zelada-Guillén et al. [23] developed a potentiometric aptamer-based biosensor with single-walled carbon nanotubes (SWCNT) to detect *E. coli* O157:H7 in different complex samples, like milk and apple juice. Wu and coworkers presented a rapid colorimetric detection systems presenting a limits of detection around 10^4 cfu mL^{-1} [24]. Recently, Demirkol and Timur [25] reported a sandwich aptamer-based fluorescent assay for analysis in a microtiter plate format that permits to detect selectively the enterohemorragic *E. coli* down to 10^2 cfu mL^{-1}. However, the majority of the reported aptasensors for this pathogenic *E. coli* strain are based on indirect methods, which complicate the detection process. Here we present a novel label-free impedance based biosensor to detect *E. coli* O157:H7 that pretends to overcome some limitations of aptasenaors presented in previous published works.

Electrochemical impedance spectroscopy (EIS) technique permits to study changes occurring on the solid/liquid interface on the surface of electrodes produced by physical, chemical or biological interactions [26]. The impedance measurements may be carried out in a faradaic or nonfaradaic mode. In the first case, the presence of a redox couple like a ferri/ferrocyanide couple that discharges on the electrode surface is required. The charge transfer resistance of this electrochemical reaction is the main parameter that is affected by the surface biochemical reactions. In the nonfaradaic mode, a transient current flows across the interface that mainly depends on the interfacial capacitance. This mode of impedance measurements is considered as a more amenable method [27] for direct biosensing applications. Different kind of electrodes can be used as impedimetric transducers for bacterial detection, but interdigitated electrode arrays (IDEA) present certain advantages, like small size, increased signal-noise-ratio and fast establishment of a steady state [28], compared to other electrodes systems.

Planar IDEA devices are formed by a pair of comb-like metal electrodes on a planar insulating substrate by conventional microfabrication techniques. In this case, impedance is measured between the two electrodes and depends on the solution conductivity and the interfacial properties of the electrodes: interfacial capacitance [29] and surface conductivity [30]. While traditional macro-electrodes with large surface area can be used to carry out measurements of interfacial capacitance, in micro-scale transducers the surface charge also plays an important role [30]. In this sense, to improve the sensitivity of standard IDEA sensors a three-dimensional interdigitated electrode arrays (3D-IDEA) impedance transducer, in which the electrodes are separated by insulating barriers, was proposed [31]. The 3D-IDEA devices are highly sensitive to changes in the surface charge at the solid/liquid interface produced by chemical and biochemical reactions, and previously have been demonstrated useful in different label-free detection processes [32,33].

In this work, we report a novel label-free impedimetric aptasensor for the direct detection of *E. coli* O157:H7. The surface of the 3D-IDEA transducer was initially modified with a mercaptosilane to covalently immobilize an *E. coli* specific 5′ disulfide-modified DNA aptamer by means of a thiol/disulfide exchange reaction [34]. The biofunctionalization strategy is schematically presented in Fig. 1. The impedimetric measurements allow registering the presence of target bacteria at different concentrations with low limit of detection in a short time. Moreover, the feasibility of the aptasensor was validated with real samples introducing a pre-concentration step with filtration system approach developed in our group. Finally, one of the main novelties of this study is the regeneration of the aptasensor after the detection of bacteria, confirming the great potential for its practical application compared with other label-free detection systems. The present study suggests a rapid and attractive method for bacterial detection especially in water quality analysis in environmental samples.

2. Experimental

2.1. Electrodes fabrication

The interdigitated electrode array was formed on a silicon wafer covered with a 2.5 mm thick thermal silicon oxide layer. As an electrode material a highly conductive tantalum silicide ($TaSi_2$) was deposited using magnetron sputtering. This layer was patterned using conventional lithography giving as a result interdigitated electrodes with 216 digits of 3 μm width and 3 μm gap between the adjacent electrodes. The aperture between the electrodes is 1.4 mm and the total length between the electrodes is 301 mm. The wafer was covered by a 4 μm thick low pressure chemical vapor deposition (LPCVD) silicon dioxide in which electrode digits and contact pads of the transducers were opened by deep reactive ion etching

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