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Electrochemical sensing and fluorescence imaging of *E. coli* O157:H7 based on aptamer-conjugated semiconducting nanoparticles

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E. coli O157:H7 sensing, Electrochemical detection, Fluorescence imaging, Quantum dots

Abstract: Aptamers are selective molecules against to various sizes of targets from small molecules to mammalian cells. Here, we reported QDs containing electrochemical aptasensor for the detection of *E. coli* O157:H7. The electrode surfaces were modified by cysteamine (CysN), which has amine and thiol groups, via self-assembled monolayer formation. The carboxyl-functionalized quantum dots (QD) and aptamers (Apt) were conjugated to cysteamine modified gold electrodes. Linear range for *E. coli* O157:H7 was from 1 to 10^2 CFU/mL after incubation on CysN/QD/Apt modified Au surfaces. QDs provide fluorescence surface, so that adhesion of cells was followed using fluorescence microscope. Adhered cells were also imaged by scanning electron microscopy. Finally, cell analysis was carried out in real samples.

Aptamer konjuge edilmiş yarı iletken nanoparçacıkları temel alan patojen bir mikroorganizmanın elektrokimyasal belirlenmesi ve floresans görüntülenmesi

Anahtar Kelimeler
E. coli O157:H7 belirlenmesi, Elektrokimyasal dedeksiyon, Floresans görüntüleme, Kuantum noktalar

Özet: Aptamerler, küçük moleküllerden memeli hücrelerine kadar çeşitli boyutlardaki hedeflere karşı seçici moleküllerdir. Burada, *E. coli* O157:H7 dedeksiyonu için QD içeren elektrokimyasal aptasensör rapor edilmiştir. Yüzeyler, amino ve tiyol grupları içeren sisteamin (CysN) ile kendiliğinden oluşan tek tabaka meydana gelişi ile modifiye edildi. Karboksil ile fonksiyonelleştirilen quantum noktalar (QD) ve aptamerler (Apt), sisteaminle modifiye edilmiş altın elektrotlara konjuge edildi. CysN/QD/Apt modifiye edilen Au yüzeylerde inkübasyon sorasında, *E. coli* O157:H7 için doğrusal aralık 1 to 10^2 CFU/mL idi. QD floresans yüzeyler sağlar, böylece hücrelerin adezyonu floresans mikroskop kullanılarak takip edildi. Adezyon sonrası hücreler aynı zamanda taramalı electron mikroskobu ile görüntülendi. Son olarak, hücre analizi gerçek örneklerde gerçekleştirildi.

1. Introduction

Aptamers (Apts) are synthetic nucleic acids and can be selected using SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method. [1-3]. Their unique properties against to targets make them useful for the fabrication of aptasensors. In different configuration such as electrochemical or optical aptasensors can be designed. Also they are selective against to various types of molecules from small compounds such as glucose [4], illicit drugs [5], bacteria [6] to mammalian cells such as cancer cells [7]. Aptamer-based biosensors (aptasensors) are able to detect pathogens with high selectivity [8-10]. Compared to antibody or enzyme based biosensors, aptasensors have many advantages such as higher selectivity, higher stability, affinity and lower cost [11-14].

Escherichia coli (*E. coli*) is a diverse group of bacteria. *E. coli* O157:H7 which is commonly identified pathogenic strain produces toxins that cause several diseases such as gastrointestinal problems and bloody diarrhea [15, 16]. *E. coli* O157:H7 has been found in food stuffs such as meat, fruit juice, milk and products, salami, lettuce [17]. The detection, identification, and quantification of *E. coli* O157:H7 is critical in clinical diagnosis, disease control, environmental monitoring and food safety.

PCR (Polymerase chain reaction) [18], colony counting [19], antibody-based detection technologies

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[20] and optical assays using organic dye molecules [21] are conventional identification methods for *E. coli* O157:H7. Although standard microbiological techniques allow the detection of bacteria, these processes are relatively slow and lack of high performance detectability and specificity analysis of target structure. Opposed to conventional assays, electrochemical sensing offers several benefits over traditional techniques such as short time, high-yield screening, simultaneous analysis, advanced detectability and unlabelled detection methods and devices [22].

We described here a novel electrochemical assay combining with a specific aptamer against to *E. coli* O157:H7 outer membrane proteins. Furthermore, carboxyl functionalized QDs were used together with aptamer to prepare aptasensors via EDC/NHS (1-ethyl-3- (3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide) chemistry [23] and the designed surfaces were applied to image and determine *E. coli* O157:H7.

2. Material and Method

2.1. Chemicals and reagents

Escherichia coli O157:H7 cells were retrieved from DSMZ (DSM 19206, German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany). 2- (N-morpholino) ethane sulfonic acid (MES) and 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) were purchased from Sigma and EDC/NHS was dissolved in MES Buffer, pH 6.0. Carboxyl Quantum Dot was from Invitrogen. Specific aptamer with amine groups against to *E. coli* O157: H7 was obtained from IDT and the sequence of aptamer is [24];

5'-/5AmMC6/ATCCGTCACACCTGCTCTACGGCGCT
CCCAACAGGCCTCTCCTTACGGCATATTATGG
TGTTGGCTCCCGTAT-3'

E. coli O157:H7 cells were cultivated in 50 mL Luria Bertani (LB) medium at 37 ˚C with 175 rpm [25]. LB medium consists of tripton: 10 g/L; yeast extract: 5.0 g/L and NaCl: 10 g/L. During storage of cells for a month, they were seeded on agar; 15 g/L. To test the specificity of aptasensor, *Gluconobacter oxydans* (Gram (-) bacteria) were added to the aptamer modified surfaces instead of *E. coli* cells. *G. oxydans* cells was cultivated in 50 mL of growing medium. It consists of yeast extract (5.0 g/L) and glucose (5.0 g/L) (at 28 ˚C with 175 rpm) [26]. Cultivated cells were used in late logaritmic phase. *E. coli* K12 cells were from Microbiology Department Culture Collection of Ege University, Faculty of Science and were cultivated in 50 mL LB medium.

2.2. Apparatus

The basic equipments used in the project are listed as incubator (New Brunswick Scientific, USA), autoclave (Hirayama, Japan), centrifuge (Experiment, England), pH meter (Han Instrument, USA). Electrochemical measurements were acquired from PalmSens electrochemical measurement unit. Gold electrodes were utilized as working electrode, a platinum electrode as counter electrode and Ag/AgCl as reference electrode during measurements.

2.3. Gold surface modification with the QD and aptamers

The pathway to modify gold surface with QD and aptamer is as follows: Firstly, 10 µL of 100 mM cysteamine (dissolved in 500 µL of Milli-Q water) was placed on Au electrode and incubated for 1 hour. Then, 5 µL of 0.5 M EDC, 5 µL of 0.125 M NHS, 12.5 µL of 2.5 µM Apt and 2,5 µL QD were retained on to the Au surface. The mixture was incubated for 2 hours. Furthermore, *E. coli* cells in different numbers were added and incubated for 45 minutes. In Fig. 1, the schematic representation of aptamer and QD modified Au surfaces was illustrated.

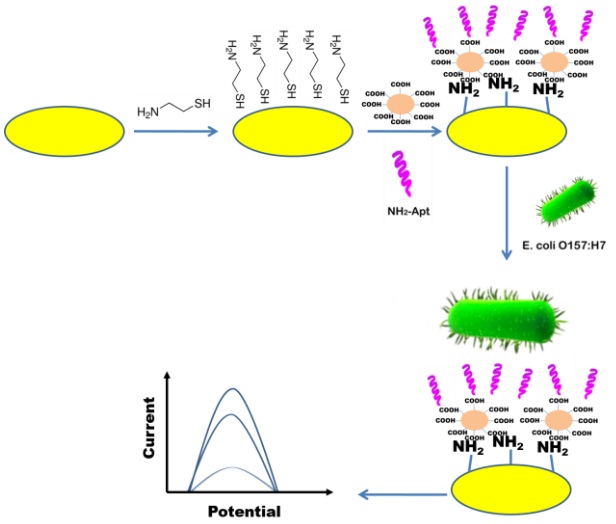

Figure 1. Schematic representation of steps for modification of the gold electrode surfaces

2.4. The electrochemical determination of the electrode response

Cyclic voltammetry (CV) (between -0.4 V–0.4 V) and differential pulse voltammetry (DPV) (between -0.4 V and 0.5 V) techniques were performed after each modification in $[Fe(CN)_6]^{3-/4-}$ solution (dissolved in 50 mM pH 7.0 sodium phosphate buffer; SPB), as a redox probe (5.0 mM). *E. coli* adhesion onto the Apt modified gold electrode produced the decrease in the current signals which were related to the cell adhered onto the electrode. Changes in the sensor responses as a current were deliberated as follows; $\Delta I = I_o - I_c$ (where I_o is the current at without cell and I_c is the mean current in the presence of cells).
To test the aptansensor success for the detection of *E. coli* in real samples, *E. coli* cells were spiked to tap water and dilutions of cells were carried out using the same tap water samples. The obtained signal after

addition of tap water, including *E. coli* cells was used to calculate numbers of *E. coli* in samples using the linear graph.

SEM images of modified surfaces were taken. The samples were dropped on to gold covered glass slides. The modified slides were placed onto copper grid surface. After drying samples, they were positioned onto container and imaged using the microscope.

3. Results and Discussion

3.1. Characterization of CysN/QD/Apt surfaces

As described in the method section, after the electrode modification with Apt and adhesion of the cells, cyclic voltammogram (CV) were formed in SPB (pH 7.0, 50 mM) containing 5.0 mM $K_3[Fe(CN)_6]$ at 25 mV/sec between -0.4-0.5 V. $K_3[Fe(CN)_6]$ is a probe for the following of electron transfer mechanism on the electrode surfaces. CV graphs showed an intense fall in the peak current when CysN, QD/Apt were covered and *E. coli* O157:H7 adhered on Au electrode due to the moderately limit for transfer properties of electrons (Fig. 2). The DPV peak current decreased also after the addition of *E. coli* O157:H7 cells (Fig. 3). This proves the successful modification of Au surfaces with aptamers and the adhesion of *E. coli* O157:H7 cells. The results of CV and DPV support each other.

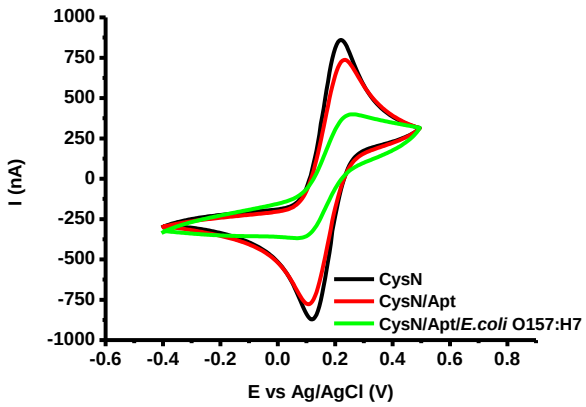

Figure 2. Cyclic voltammogram of the modified Au electrodes addition of cells showing the differentiation of the current (in SPB (50 mM pH 7.0) including 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl).

3.2. The effect of the aptamer concentration on DPV signals

The CysN/QD/Apt modified Au surfaces were prepared using various concentrations of Apt. The graphic shown in Fig. 4 was obtained using different signals of the CysN/QD/Apt modified Au surfaces after addition of *E. coli* cells. As it is shown in Fig. 4, the most appropriate aptamer concentration was found to be 2.5 µM and the surfaces were prepared using aptamers in this concentration in all subsequent experiments. Concentrations of aptamer less than 2.5 mM used to prepare selective surfaces cause lower electrochemical signals.

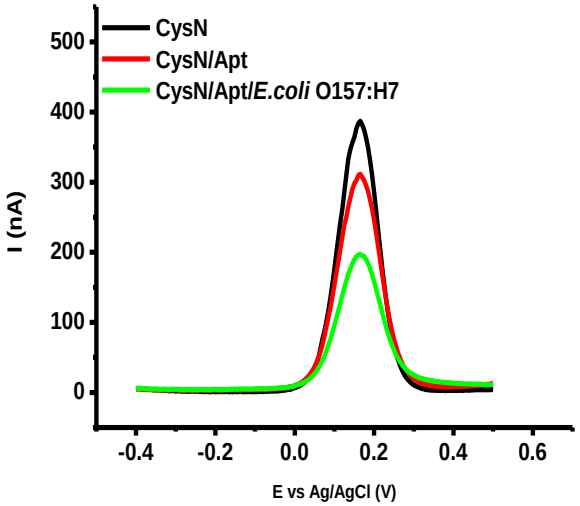

Figure 3. Differential pulse voltammogram after the addition of cells showing the differentiation of the current (in SPB (50 mM pH 7.0) including 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl).

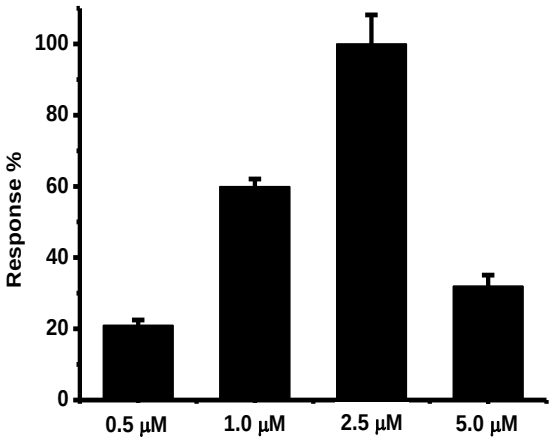

Figure 4. The effect of aptamer concentration on DPV signals of CysN/QD/Apt modified Au surfaces (in SPB (50 mM pH 7.0) including 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl).

3.3. Linear range for *E. coli* O157:H7

Altered numbers of *E. coli* O157:H7, from 10 to 10^2 CFU/mL, were prepared by serial dilution in % 0.9 NaCl solutions. A linear graph was obtained using the fall in the current depending on *E. coli* O157:H7 concentration within DPV measurements. The linear correlation was defined by the equation of y=-0.00435x+0.931 (R^2=0.998) and y shows ΔI (µA) and x shows the logarithm of the numbers of *E. coli* from 1 to 10^2 CFU/mL (Fig. 5). This linear range is suitable for *E. coli* O157:H7 detection in various samples and is compatible with the literature [27, 28].

Fluorescence microscope images of CysN (Fig. 6A), CysN/QD/Apt covered (Fig. 6B) and adherent cells (Fig. 6C). As shown in Fig. 6C, the presence of *E. coli* cells on CysN/QD/Apt covered Au surface was imaged by fluorescence microscopy.

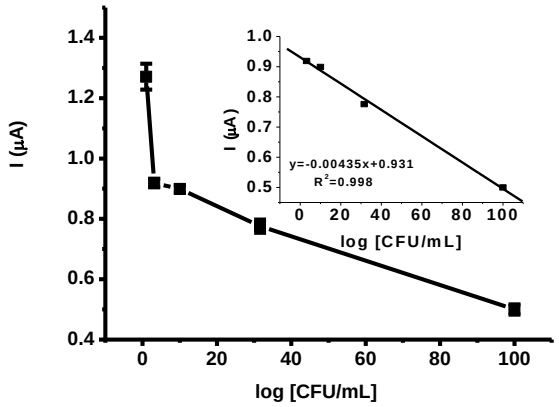

Figure 5. The effect of numbers of *E. coli* on DPV signals of CysN/QD/Apt modified Au surfaces (in SPB (50 mM pH 7.0) including 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl).

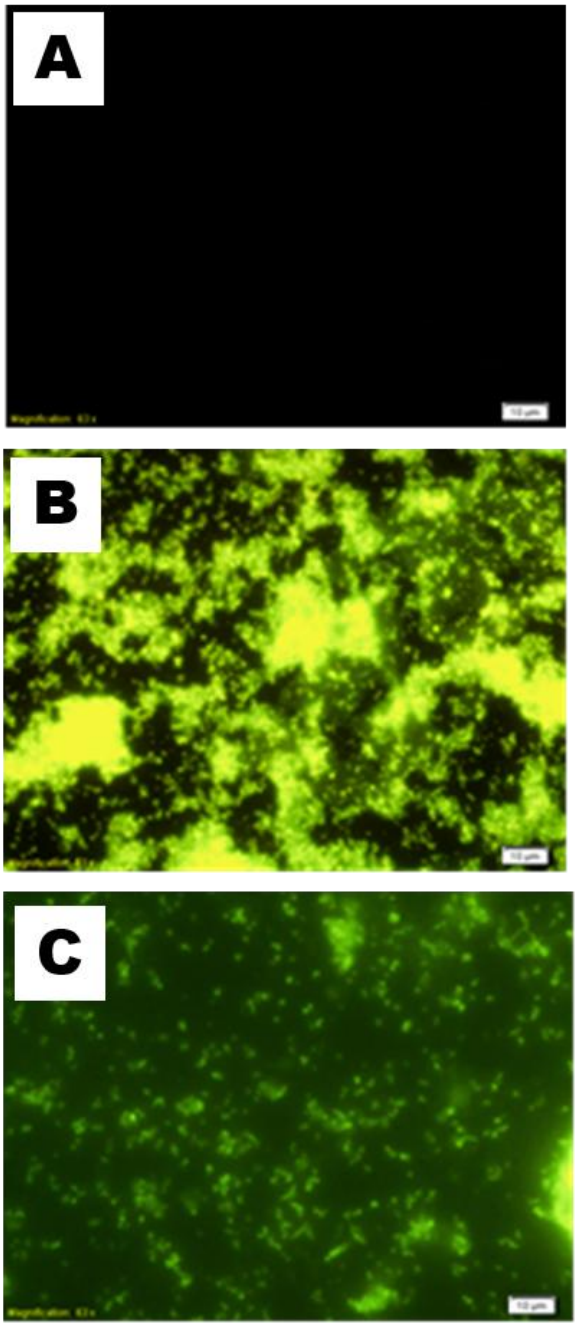

Figure 6. Fluorescence microscope images of CysN (A), CysN/QD/Apt covered (B) and adherent cells (C).

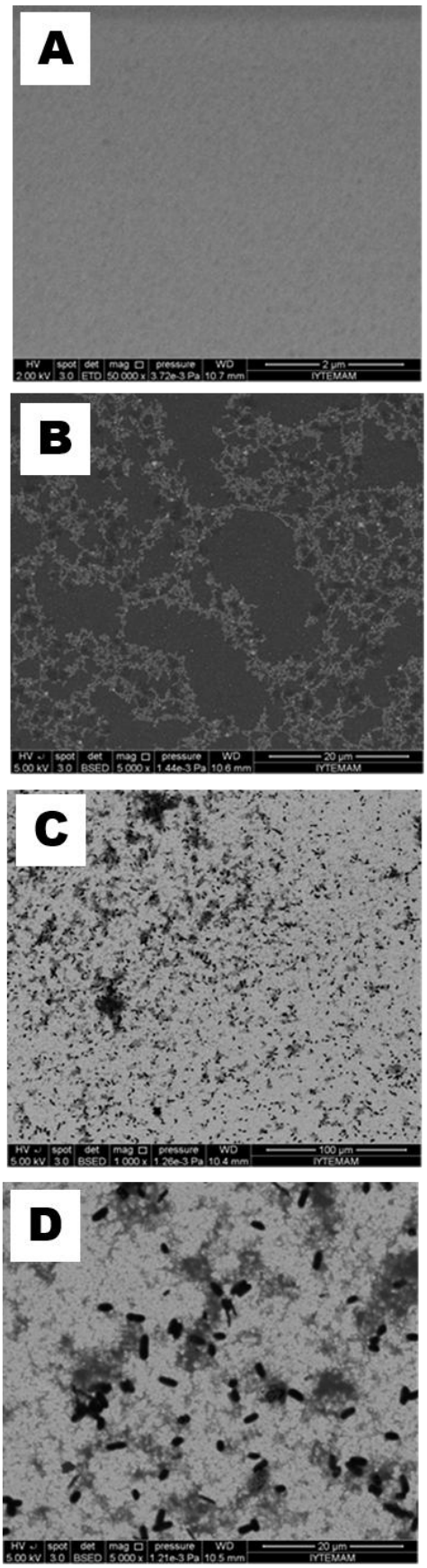

Fig. 7. SEM images of CysN (A), CysN/QD/Apt covered (B) and adherent cells in different magnification (C, D).

The surface morphology of the CysN/QD/Apt covered Au electrode, before and after *E. coli* adhesion was imaged by scanning electron microscopy. After

modification of Au surfaces with CysN, the obtained clear homogeneous surface morphology was shown in Fig. 7A. QD/Apt modified (in Fig. 7B) and the adhered cells on Au electrode were shown in Fig. 7C and 7D.

And also selectivity of the aptasensor was confirmed using *E. coli* K12 and *G. oxydans* cells which are Gram (-) bacteria as a target instead of *E. coli* O157:H7 cells. As shown in Fig. 8, there is no interference effect of other cells.

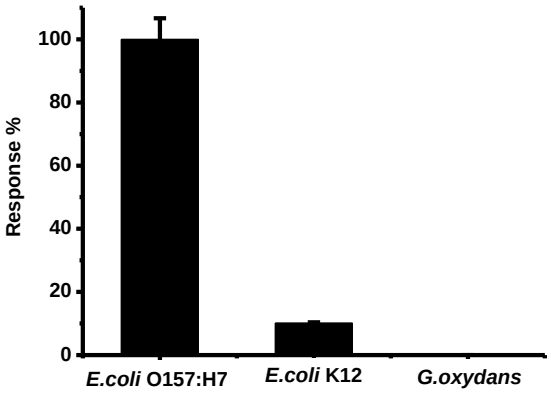

Figure 8. The selectivity tests of CysN/QD/Apt covered Au electrode aptasensor (10^2 CFU/mL of cells in SPB (50 mM pH 7.0) with the presence of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl).

3.4. Measurements on real samples

At the final step, the aptasensor was applied for the detection of *E. coli* cells in real samples. The spiked tap water samples were prepared by the addition of known numbers of *E. coli* cells with different dilutions. Using calibration graph for cells, the numbers of *E. coli* in tap water was calculated and the obtained results were compared with added numbers of *E. coli*. Added and found numbers of *E. coli* were 100 and 106.856±2.595 CFU/mL, respectively.

4. Conclusion

Semiconducting nanoparticles are useful tool for electrochemical and optical applications. Use of them in fluorescence detection is one of the important areas because of their optical features. Here, *E. coli* O157:H7 was chosen as a model microorganism and electrochemical pathogen detection was carried out using aptamer modified gold electrodes. The analysis of the pathogen in tap water was also carried out without any interference which was included in matrix of sample.

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