Journal of Microbiological Methods 161 (2019) 96–101

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Electric field assisted deposition of *E. coli* bacteria into the pores of porous silicon

Sevinç Güler[a], Çiğdem Oruç[b,*], Ahmet Altındal[b]

[a] Nisantası University, Vocational School, Department of Medical Services and Techniques, 34398, Maslak, Istanbul, Turkey
[b] Yıldız Technical University, Faculty of Science & Art, Department of Physics, 34722, Esenler, Istanbul, Turkey

ARTICLE INFO

Keywords:
Porous silicon
E. coli
Impedance spectroscopy
Electric field

ABSTRACT

The effect of the electric field strength on the *Escherichia coli* (*E. coli*) bacteria deposition into the pores of porous silicon was investigated by impedance spectroscopy technique. The main idea behind this approach is that negatively charged *E. coli* bacteria can be deposited into the pores available on the surface of porous silicon upon the application of a high electric field. For this purpose, the influence of the *E. coli* concentration on the impedance spectra of the anodically formed porous silicon under various electric fields between 0 and 10 kV/cm was investigated. In addition, the effect of the application time of a constant electric field of 12 kV/cm on the impedance spectra of porous silicon exposed to living and dead bacterial cells was also investigated. The results reported in this study indicate that the number of live *E. coli* bacteria deposited into the pores of porous silicon can be controlled by the applied electric field strength. On the other hand, it was found that there is no considerable effect of the dead *E. coli* cell concentration on the recorded impedance spectra of the porous silicon based sensor platform.

1. Introduction

Because of the threat to public health care, the detection of pathogenic bacterial cells is an ongoing issue for various fields. Up until now, various detection methods based on different transducer mechanisms, such as potentiometric [Zelada-Guillén et al., 2009], optical [Sivakumar et al., 2009; Tripathi et al., 2012] and microgravimetry [Bao et al., 1996; Gabl et al., 2004] have been developed for this purpose. It is well known that these types of sensing devices require properly immobilized special sensing units, such as antibodies, bacteriophages and aptamers, on the transducer's surface [Escamilla-Gomez et al., 2009; Cho et al., 2007, Ibii et al., 2009; Joung et al., 2012; Neufeld et al., 2003; Zelada-Guillén et al., 2009, 2012]. Although these surface modification methods offer some advantages, such as selective and high precision detection of target bacteria, most of them still require skilled users and appropriate immobilization techniques. Recently, many attempts have been made to develop sensing unit free strategies for the detection and deposition of bacterial cells. The role of grain size impact and the properties of bacteria (*E. coli*, *Klebsiella oxytoca*, and *Rhodococcus rhodochrous*) on bacteria deposition in porous media were studied by Bai et al. (2016). It was reported that straining is an important mechanism affecting *E. coli* deposition. The effect of the applied electric field on bacterial deposition onto glass substrate was investigated by Busscher et al. [Poortinga et al., 2001]. They conclude that the application of an electric field results in only a small change in potential at the glass surface with no significant effect on bacterial adhesion. As a result, a simple, fast, inexpensive and antibody free device for detecting bacterial cells such as *E. coli*, which is accepted as an indicator of fecal pollution of water resources, is highly desirable.

In this respect, porous silicon (PS) as a sensor platform for the design of biosensors has some advantages over conventionally used transducers. The particularly attractive features of PS are the possibility to yield large surface areas which enable large amounts of biomolecular interactions, and compatibility with existing CMOS technology [Tang et al., 2016; Jane et al., 2009; Weiss et al., 2009; Bonanno and Segal, 2011; Alvarez et al., 2007; Archer et al., 2004; Shtenberg et al., 2013]. After the work by Lin et al. (1997) on PS as a biosensor platform, there has been extensive work on investigating the utilization of PS in biosensors. A biosensor based on specific antibody functionalized PS for optical detection of *E. coli* in the food industry was developed by Massad-Ivanir et al. (2016). It was reported that the biosensors investigated were capable of selectively identifying and quantifying the target cells. An optical sensor based on *E. coli* antibody immobilized PS was developed by Tang et al. (2016). They reported that the nanopore array coupled with Fourier Transformed Reflectometric Interference Spectroscopy can act as a sensing platform to detect the pore blockage

* Corresponding author.
E-mail address: oruc@yildiz.edu.tr (Ç. Oruç).

https://doi.org/10.1016/j.mimet.2019.04.018
Received 30 August 2018; Received in revised form 26 April 2019; Accepted 26 April 2019
Available online 29 April 2019

effect. More recently, an impedance based sensor for the detection of pathogenic bacteria in small volumes by using antibody-conjugated gold nanoparticles as a sensing unit was reported by Pal et al. (2016). Antibody immobilized macroporous silicon with various pore thicknesses was fabricated and the sensing performance toward *E. coli O157* was tested using impedance spectroscopy technique [Dev Das et al., 2011]. It was reported that high performance *E. coli O157* sensing can be achieved by using macroporous silicon with 8 μm pore depth. A good review of porous silicon chemical and biosensors has recently been published by Harraz (2014). However, most of them require surface modification of PS with a biorecognition element such as antibody and aptamer, and the binding efficiency of the antigens to antibody is low.

In this work, we focus on the development of an *E. coli* bacteria deposition strategy in order to improve the binding efficiency and sensitivity of the antigen-antibody interaction based biosensors. The other aim of this work is to demonstrate that the proper direction of the electric field (E) applied to a PS based platform can facilitate bacteria deposition on PS pores.

2. Experimental

2.1. Fabrication of porous silicon platforms

The electrochemical anodization method was employed to fabricate porous silicon sensor platforms. For this purpose, n-type silicon wafers with (111) orientation and of resistivity $1.25 \times 10^{-2}\,\Omega$ cm was used as the starting material. The anodization of the silicon wafers was carried out at a constant current density of 15 mA.cm^{-2} in solution of HF (40%) and H_2O (HF: H_2O; 1: 3% vol.) for 40 min, in which the n-type silicon works as the anode and the platinium (Pt) metal as the cathode. Before the anodization processes, ohmic contacts were formed on the back side of the silicon wafers by soldering of pure indium (In) metal at atmospheric conditions. Acid proof wax was used to preserve the back of the electrodes (ohmic contacts). Finally, the top contacts on the porous silicon (with a porosity of 60%) wafers were formed by using silver paint and the devices in In/Si/PS/Ag structures were fabricated. All the PS samples were fabricated in the same way to avoid any unwanted structural modification. Scanning electron microscopy (SEM) technique was used in order to investigate the surface morphology of the PS samples.

2.2. Deposition experiments

The bacterial strains used in this study - *Escherichia coli*, were supplied by Istanbul University, Cerrahpaşa Medicine Faculty, Microbiology Laboratory and were grown in Mueller-Hinton Agar (MHA) at 37 °C. During the deposition experiment, 30 mM NaCl aqueous solution (serum) was used as the liquid media (with bacteria taken from the top without any contact with Agar). After 22–24 h of incubation time, the desired concentrations of the bacteria to be tested were adjusted to 2×10^4, 3×10^4, 5×10^4, 6×10^4, 8×10^4, and 1×10^5 colony forming units (cfu) by way of dilution of the McFarland standard compliant optical density (OD) of 625 nm, using a UV spectrophotometer. A schematic diagram of the experimental set up is shown in Fig. 1. After immersion of the PS samples in bacteria solution in a Petri dish, two aluminium (Al) plates were placed in a parallel plate capacitor configuration. Impedance spectra measurements were carried out by using an HP 4192A impedance analyzer with a frequency range from 5 Hz to100 kHz. During the impedance measurements, an electric field between 0 and 10 kV/cm was applied to the Al plates.

3. Results and discussion

It is well known that the pore size in PS strongly depends on the etching process including current density and the concentration of HF in etching solution [Kobayashi et al., 2007]. The surface morphology of the samples was studied using a scanning electron microscope (SEM). Fig. 2 shows a top view SEM image of a typical PS sample which is prepared under constant current conditions of 15 mA cm^{-2}. A porous micro structured morphology with typical pores in the range of 5–12 μm is clear.

One of the main purposes of this study is to investigate the effect of the applied electric field on the deposition of the bacterial cells onto micro pores, as a new sensing strategy. The PS based devices are characterized electrically to estimate the amount of *E. coli* deposited with different *E-coli* concentration. During the characterization of the devices by impedance spectroscopy technique, a sine wave of 250 mV amplitude was applied between the In ohmic contact and Ag top contact to avoid any random fluctuation of the impedance at the electrode-electrolyte interface. Fig. 3 (a) shows the effect of the *E. coli* concentration on the impedance spectra of the PS based device without electric field. As is clear from the data presented in Fig. 3(a), the device exhibits maximum impedance for all frequencies in serum without *E. coli* and it decreases with the addition of *E. coli* bacteria. Most importantly, a significant decrease in the magnitude of the impedance was detected when the concentration of the bacterial cells was changed from 2×10^4 to 1×10^5 cfu. The observed impedance spectra shown in Fig. 3 (a) reveals that the capture or the deposition of the *E. coli* bacteria can be achieved by a properly designed PS surface.

The experimental medium was prepared using serum and bacteria from MHA. As no bacteria were contacted from the top and there was no contact with the agar, no nutrients were added to the experiment. The serum contains 0.9% NaCl. However, all other experiments were carried out with reference to this liquid. Therefore, impedance changes are caused by the bacteria. The fattening place is crowded and stressful. To minimize this and compare the conditions with the natural environment, bacteria were diluted with serum as in many microbiology studies. After waiting for a period of time in this environment, experiments were performed.

The other purpose of this study is to provide a method for increasing the effectiveness and sensitivity of the currently used bacterial sensors. In other words, by using an electrical field effect, existing sensors can be more in contact with the bacteria and measurement sensitivities can be increased.

In order to clarify the effect of the bacteria concentration on the impedance of the PS samples, the variation of the measured impedance with bacteria concentration at selected frequencies (*f*) is shown in Fig. 3 (b).

It was observed that the impedance of the sample decreases with increasing bacteria concentration because a nearly linear relationship between the bacteria concentration and logarithmic value of the impedance is clear. To date, all reported passive bacteria detection devices with PS are based on optical methods. In this work, detection and cell growth on PS based structures were monitored by intensity changes in the PS reflectivity spectrum [Naik and Ghosh, 2009; Vorontsov et al., 2007]. The authors found a linear relationship between bacteria concentration and the optical intensity changes in the PS. Although precise determination of bacteria concentration in suspensions was not necessary for our purpose, UV–visible absorption spectra of the serum with and without *E. coli* addition was taken in order to be sure that the concentration of the bacteria in serum solution was different. The variation of the UV–visible spectra (Fig. 4) of the test samples indicated that the concentration of the bacteria was different.

It should be mentioned here that the impedance of the device is a decreasing function of the frequency for all bacterial cell concentrations (see Fig. 3 (a)). A rapid decrease in impedance of the device at low frequencies and a nearly frequency-independent behavior in the high frequency region is also clear. For example, while the magnitude of the impedance of the sensing platform in serum for the lowest frequency (5 Hz.) was 82.4 kΩ, it was 1.1 kΩ for the highest frequency (100 kHz.).

From the significant decrease of the impedance of the PS platform in the low frequency region, it can be concluded that the alternating

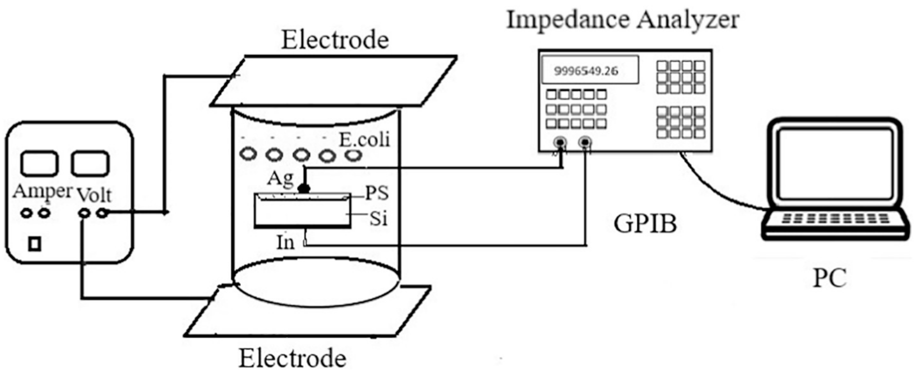

Fig. 1. Schematic diagramme of the experimental set up.

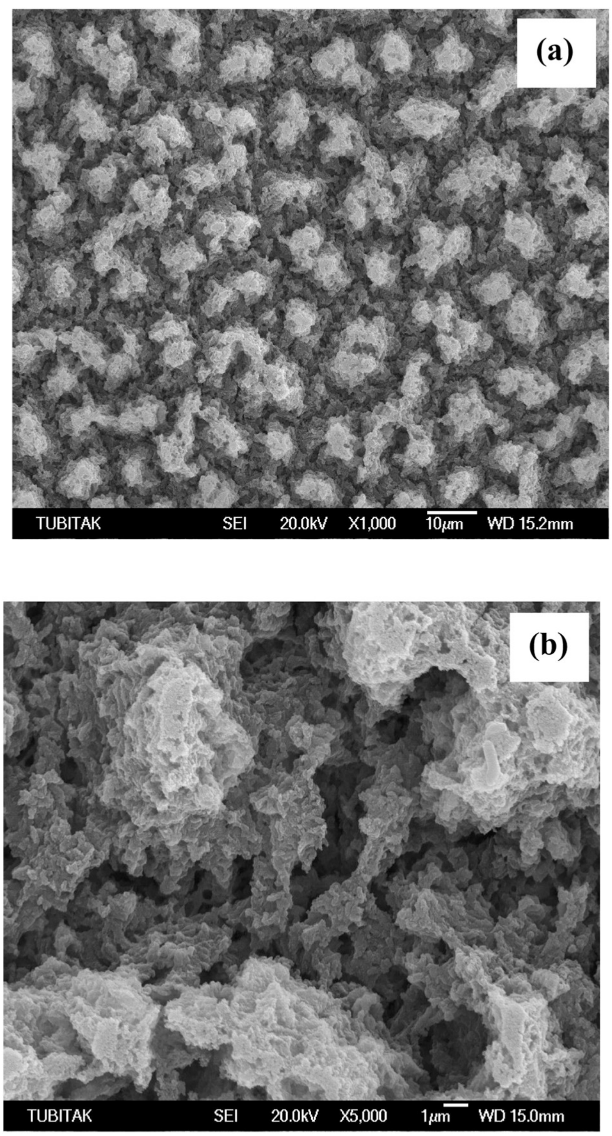

Fig. 2. Top view of the scanning electron microscope image of a typical PS sample.

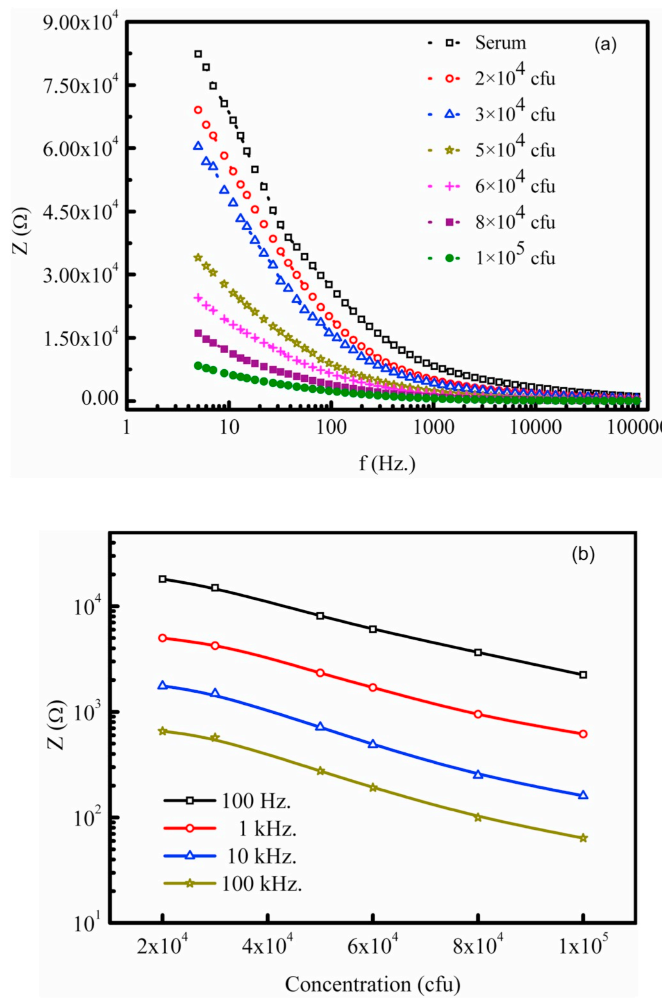

Fig. 3. (a) The effect of the *E. coli* concentration on the impedance spectra of the In/Si/PS/Ag structures (b) Concentration dependence of the impedance at selected frequencies.

current conductivity increases with frequency and the single relaxation process [Yüzüak et al., 2015]. It can be considered that the overall impedance of the device composed of the series combination of the double layer impedance at the electrode-electrolyte interface, double layer at the PS-electrolyte interface and the solution resistance. The observed decrease in impedance can be attributed to the variation of the double layers' impedance with frequency. Similar low frequency dispersion in impedance spectra were recently reported for antibody immobilized macroporous silicon by Dev Das et al. (2011). They concluded that the observed decrease in impedance is primarily due to a decrease in double layer impedance at the electrode-electrolyte and SiO_2 electrolyte interfaces. It was also reported that the ions in the solution have enough time to cover the crests and follow the variation of the applied AC signal at low frequencies.

In this study, we aimed to extend the idea of the deposition of

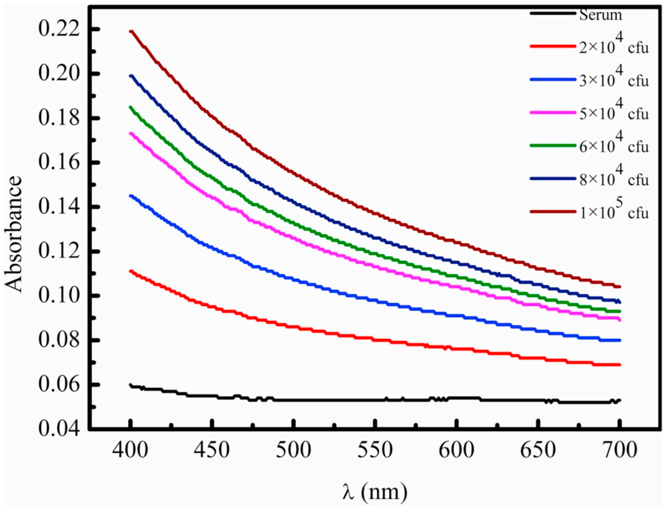

Fig. 4. UV–visible absorption spectra of the suspensions with various bacterial concentrations.

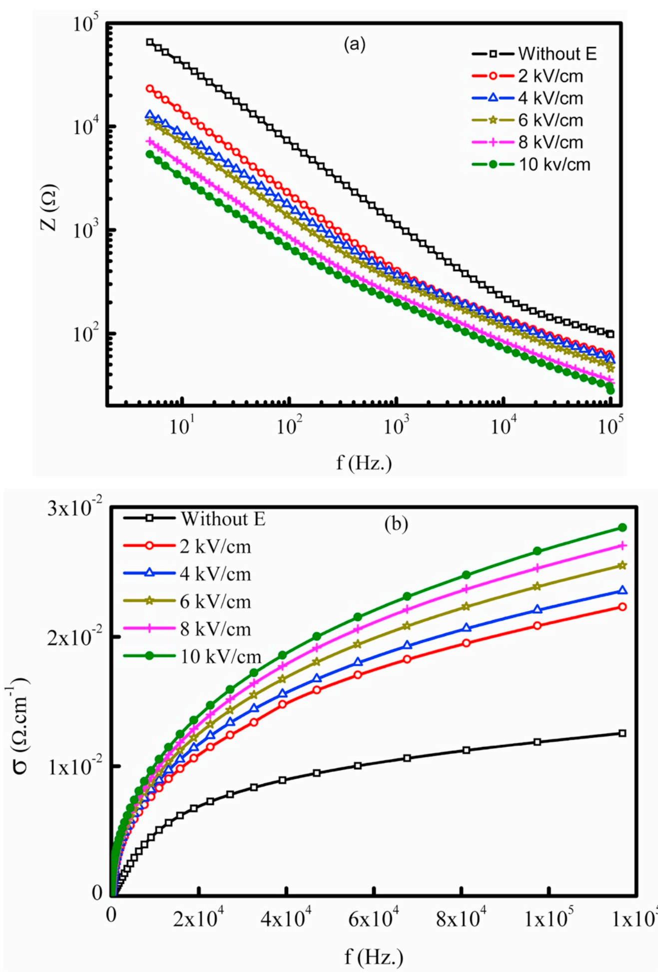

Fig. 5. (a) The effect of the electric field on the impedance spectra of the PS based sensor immersed in a bacteria solution of 5×10^4 cfu mL^{-1} (b) The variation of the conductivity with frequency under the indicated applied electric field.

bacterial cells to investigate an impedance based sensor for non specific detection of *E. coli* in liquid samples with In/Si/PS/Ag based structures. As mentioned before, the other aim of this work was to demonstrate that an applied electric field with a proper direction improves the bacteria deposition into the pores. In order to see the effect of the electric field on the impedance spectra of the device, the PS based device was immersed in a bacteria solution of 5×10^4 cfu and impedance spectra were taken under various electric field values between 0 and 10 kV/cm (Fig. 5(a)). It should be mentioned here that a positive voltage was applied to the bottom electrode in order to push negatively charged *E. coli* bacteria toward the PS surface. Although the concentration of the bacteria is the same, a decrease in the observed impedance spectra clearly shows the effect of the applied electric field, and the impedance of the device decreases with applied electric field for all frequencies investigated. It is well known that pore size is an important factor for deposition applications which limit the species that can be captured into the pores [Harraz, 2014]. This observation reveals again that a properly directed electric field can facilitate the deposition of *E. coli* bacteria into the pores of a PS surface which is prepared under constant current conditions of 15 mA cm^{-2}. More recently though, it was shown by Anany et al. (2011) and Han et al. (2014) that the number of phages and virions deposited increases with increasing electric field. In order to be sure that the observed decrease in impedance of the device is due to the applied electric field, conductivity variations of the devices were also measured under the same conditions (Fig. 5 (b)). The measurements of the effect of the applied electric field on the conductivity of the In/Si/PS/Ag in 5×10^4 cfu *E. coli* suspension indicate that the conductivity of the device increases with increasing electric field, thereby supporting the premise that the number of deposited *E. coli* increases with increasing electric field.

In order to check the reversibility of the observed electric field dependence in impedance spectra, the same measurements were also carried out on the same device and for bacteria concentration under reverse biased electric field (a positive voltage was applied to the upper plate of Fig. 1). The impedance spectra obtained are presented in Fig. 6. To make the effect of the reverse electric field on the impedance spectra clearer, the measured impedance spectra are presented in the inset of Fig. 6 using semi-logarithmic scale. A close investigation of Fig. 6 indicates that the measured impedance of the device increases with the increase in applied electric field. This suggests that deposition of the *E. coli* into the pores can be suppressed by reversing the applied electric field.

In another set of experiments, the effect of application time of constant forward and reverse electric field on the deposited number of bacterial cells was also investigated. Application time varied between 0.5 and 3 h, and a bacterial cell concentration of 5×10^4 cfu was

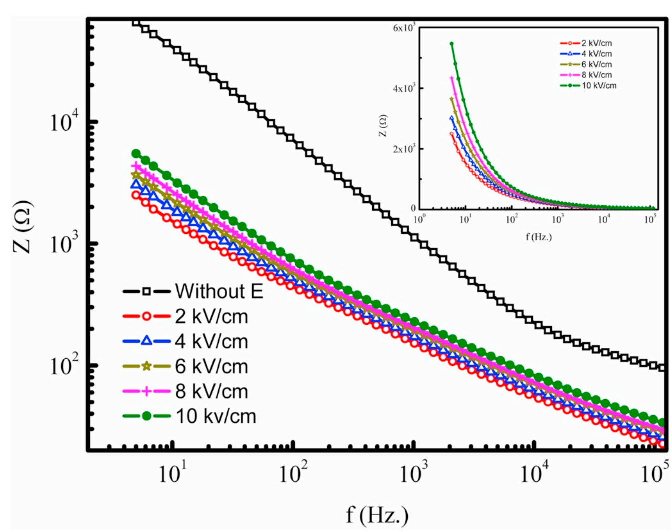

Fig. 6. The effect of the direction of the applied electric field on the impedance spectra of the In/Si/PS/Ag structures immersed in 5×10^4 cfu *E. coli* suspension.

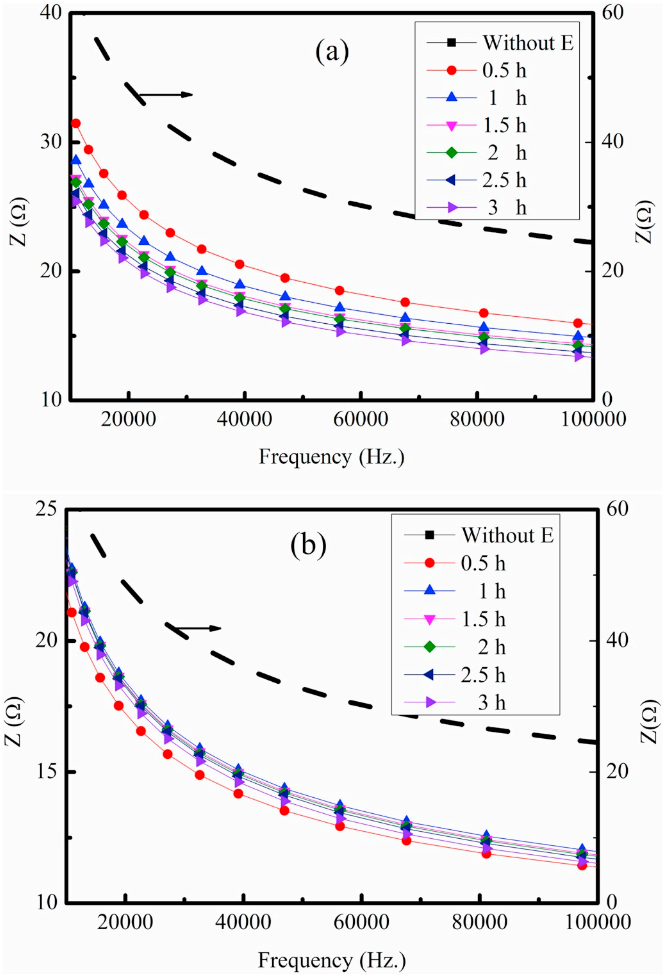

Fig. 7. The effect of the application time of (a) forward and (b) reverse electric field in the case of living cells.

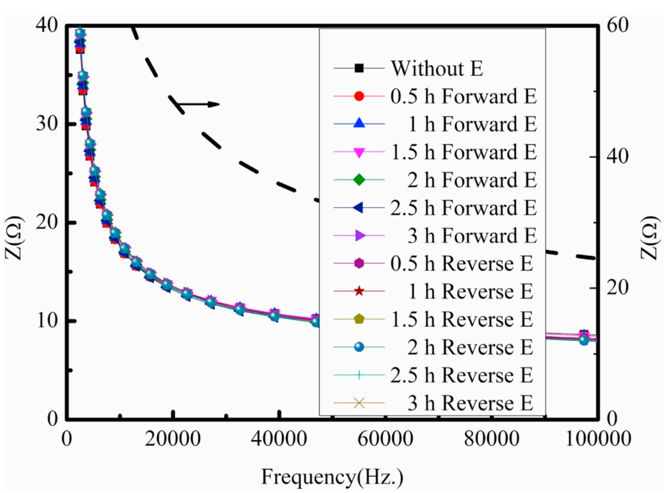

Fig. 8. The effect of the application time of forward and reverse electric fields in the case of dead bacterial cells.

selected for this set of experiments. Fig. 7 (a) shows the variation of the impedance spectra of the PS based device with a constant application time of a forward electric field of 12 kV/cm. The idea behind the choice of 12 kV/cm is to be sure that the applied electric field alone does not cause the cells to be killed. As can be seen clearly from Fig. 7 (a), the impedance of the device decreases with increasing application time of the electric field for all frequeny values investigated. The same experiment was repeated for the reverse electric field and the obtained spectra are presented in Fig. 7 (b). A comparison of Fig. 7 (a) and (b) indicates that the number of bacterial cells deposited into the pores on the PS sensor surface strongly depends on the application time of the forward electric field. The results presented in Fig. 7 (b) also suggest that the motion of the *E. coli* bacteria in a liquid can be controlled by the application of an electric field with appropriate polarity.

In order to clarify the effect of the applied electric field and its application time on the deposition of bacterial cells, the same experiments were repeated on dead bacterial cells. A mixture of serum and bacteria was prepared at a concentration of 5×10^{4} cfu. This mixture was autoclaved at 121 °C in a 1.5 atm for 20 min. A solution containing dead bacteria was obtained by this method. That is, a porous silicon based platform was inserted into a serum containing dead bacterial cells, and a 12 kV/cm electric field was applied for various times. Under these conditions, the recorded impedance spectra of the platform for both forward and reverse electric fields are shown in Fig. 8. A close investigation of Fig. 8 indicates that there is no considerable effect of the applied electric field on the recorded impedance spectra of the porous silicon based device in the case of the dead *Escherichia coli* cells. The dead bacteria in the mixture exploded and shattered. These particles were dispersed in the liquid. Therefore, the impedance decreases when the electric field is applied. However, there is no discreted burden, as in living bacteria. Therefore, while the impedance values of the live bacteria experiment changed, the impedance values remained constant in the dead bacteria experiment. This again supports the idea that the living and dead bacterial cells in a liquid can be identified by this method.

4. Conclusion

In this work, we proposed a new electric field-assisted *E. coli* bacteria deposition strategy, which will improve the binding efficiency and sensitivity of biosensors based on antigen-antibody interactions. For this purpose, a biosensor platform was prepared based on electrochemically etched *n*-type Si wafers and the effect of the bacterial cell concentration on the impedance spectra under forward and reverse electric field conditions was recorded. Results from this preliminary analysis indicate that the impedance of the PS based device in bacterial suspension decreases with increasing forward electric field, which is attributed to the variation of the double layer impedance. On the other hand, it was observed that the reverse electric field leads to an increase in impedance of the device. In order to determine the effect of the application time of the electric field, the application time of both forward and reverse electric fields was varied from 0.5 to 3 h. From the analysis of this set of experimental data, it was found that the number of bacteria deposited into the pores can be contolled by the application time of the electric field. As a result, porous silicon formed on an n-type Si wafer has great potential for electric field assisted deposition of *E. coli* bacteria in liquid samples. In addition, it is possible to increase the interaction of the bacteria with the sensor surface and the sensitivity of the sensor by using an electric field in the existing bacteria sensors.

Acknowledgements

This work was supported by the Research Fund of Yildiz Technical University (Project no. 2015-01-01-KAP03). The authors also thank Prof. Dr. F. Köksal Çakırlar (Istanbul University, Cerrahpaşa Medicine Faculty, Microbiology Laboratory) for supplying bacterial cells.

References

Alvarez, S.D., Schwartz, M.P., Migliori, B., Rang, C.U., Chao, L., Sailor, M.J., 2007. Using a porous silicon photonic crystal for bacterial cell-based biosensing. Phys. Status Solidi A Appl. Mater. Sci. 204, 1439–1443.

Anany, H., Chen, W., Pelton, R., Griffiths, M.W., 2011. Biocontrol of Listeriamonocytogenes and *Escherichia coli* O157:H7 in meat by using phages immobilized on modified cellulose membranes. Appl. Environ. Microbiol. 77, 6379–6387.

Archer, M., Christophersen, M., Fauchet, P.M., Persaud, D., Hirschman, K.D., 2004. Electrical porous silicon microarray for DNA hybridization detection. Micro Nanosyst. 782, 385–391.

Bai, H., Cochet, N., Pauss, A., Lamy, E., 2016. Bacteria cell properties and grain size impact on bacteria transport and deposition in porous media. Colloids Surf. B: Biointerfaces 139, 148–155.

Bao, L., Deng, L., Nie, L., Yao, S., Wei, W., 1996. Determination of microorganisms with a quartz crystal microbalance sensor. Anal. Chim. Acta 319, 97–101.

Bonanno, L.M., Segal, E., 2011. Nanostructured porous silicon-polymer-based hybrids: from biosensing to drug delivery. Nanomedicine 6, 1755–1770.

Cho, I.-H., Paek, E.-H., Lee, H., Kang, J.Y., Kim, T.S., Paek, S.-H., 2007. Site-directed biotinylation of antibodies for controlled immobilization on solid surfaces. Anal. Biochem. 365, 14–23.

Dev Das, R., Dey, A., Das, S., RoyChaudhuri, C., 2011. Interdigitated electrode-less high-performance macroporous silicon structure as impedance biosensor for bacteria detection. IEEE Sensors J. 11, 1242–1252.

Escamilla-Gomez, V., Campuzano, S., Pedrero, M., Pingarrón, J.M., 2009. Gold screen-printed-based impedimetric immunobiosensors for direct and sensitive *Escherichia coli* quantisation. Biosens. Bioelectron. 24, 3365–3371.

Gabl, R., Feucht, H.-D., Zeininger, H., Eckstein, G., Schreiter, M., Primig, R., Pitzer, D., Wersing, W., 2004. First results on label-free detection of DNA and protein molecules using a novel integrated sensor technology based on gravimetric detection principles. Biosens. Bioelectron. 19, 615.

Han, J.-H., Wang, M.S., Das, J., Sudheendra, L., Vonasek, E., Nitin, N., Kennedy, I.M., 2014. Capture and detection of T7 bacteriophages on a nanostructured interface. ACS Appl. Mater. Interfaces 6, 4758–4765.

Harraz, F.A., 2014. Porous silicon chemical sensors and biosensors: a review. Sensors Actuators B 202, 897–912.

Ibii, T., Kaieda, M., Hatakeyama, S., Shiotsuka, H., Watanabe, H., Umetsu, M., Kumagai, I., Imamura, T., 2009. Direct immobilization of gold-binding antibody fragments for immunosensor applications. Anal. Chem. 82, 4229–4235.

Jane, A., Dronov, R., Hodges, A., Voelcker, N.H., 2009. Porous silicon biosensors on the advance. Trends Biotechnol. 27, 230–239.

Joung, C.-K., Kim, H.-N., Im, H.-C., Kim, H.-Y., Oh, M.-H., Kim, Y.-R., 2012. Ultra-sensitive detection of pathogenic microorganism using surface-engineered impedimetric immunosensor. Sensor. Actuat. Sens. Actuators B 161, 824 − 831.

Kobayashi, K., Harraz, F.A., Izuo, S., Sakka, T., Ogata, Y.H., 2007. Phys. Stat. Sol. (A) 204, 1321.

Lin, V.S.Y., Motesharei, K., Dancil, K.P.S., Sailor, M.J., Ghadiri, M.R., 1997. Science 278, 840.

Massad-Ivanir, N., Shtenberg, G., Raz, N., Gazenbeek, C., Budding, D., Bos, M.P., Segal, E., 2016. Porous silicon-based biosensors: towards real-time optical detection of target bacteria in the food industry. Sci. Rep. 6, 38099.

Naik, B., Ghosh, N.N., 2009. A review on chemical methodologies for preparation of mesoporous silica and alumina based materials. Recent Pat. Nanotechnol. 3, 213–224.

Neufeld, T., Schwartz-Mittelmann, A., Biran, D., Ron, E.Z., Rishpon, J., 2003. Combined phage typing and amperometric detection of released enzymatic activity for the specific identification and quantification of bacteria. Anal. Chem. 75, 580 − 585.

Pal, N., Sharma, S., Gupta, S., 2016. Sensitive and rapid detection of pathogenic bacteria in small volumes using impedance spectroscopy technique. Biosens. Bioelectron. 77, 270–276.

Poortinga, A.T., Bos, R., Busscher, H.J., 2001. Lack of effect of an externally applied electric field on bacterial adhesion to glass. Colloids Surf. B: Biointerfaces 20, 189–194.

Shtenberg, G., Massad-Ivanir, N., Moscovitz, O., Engin, S., Sharon, M., Fruk, L., Segal, E., 2013. Picking up the pieces: a generic porous Si biosensor for probing the proteolytic products of enzymes. Anal. Chem. 85, 1951–1956.

Sivakumar, S., Wark, K.L., Gupta, J.K., Abbott, N.L., Caruso, F., 2009. Liquid crystal emulsions as the basis of biological sensors for the optical detection of bacteria and viruses. Adv. Funct. Mater. 19, 2260–2265.

Tang, Y., Li, Z., Luo, Q., Liu, J., Wu, J., 2016. Bacteria detection based on its block age effect on silicon nanopore array. Biosens. Bioelectron. 79, 715–720.

Tripathi, S.M., Bock, W.J., Mikulic, P., Chinnappan, R., Ng, A., Tolba, M., Zourob, M., 2012. Long period grating based biosensor for the detection of *Escherichia coli* bacteria. Biosens. Bioelectron. 35, 308–312.

Vorontsov, A.S., Osminkina, L.A., Tkachenko, A.E., Konstantinova, E.A., Elenskii, V.G., Timoshenko, V.Y., Kashkarov, P.K., 2007. Modification of the properties of porous silicon on adsorption of iodine molecules. Semiconductors 41, 953–957.

Weiss, S.M., Rong, G., Lawrie, J.L., 2009. Current status and outlook for silicon-based optical biosensors. Phys. E. 41, 1071–1075.

Yüzüak, M.M., Altun, S., Altındal, A., Odabaş, Z., 2015. Dielectric properties and electronic absorption: a comparison of novel azo- and oxo-bridged phthalocyanines. Dalton Trans. 44, 1397–1405.

Zelada-Guillén, G.A., Riu, J., Düzgün, A., Rius, F.X., 2009. Immediate detection of living bacteria at ultra low concentrations using a carbon nanotube based in potentiometric aptasensor. Angew. Chem. Int. Ed. 48, 7334–7337.

Zelada-Guillen, G.A., Sebastian-Avila, J.L., Blondeau, P., Riu, J., Rius, F.X., 2012. Label-free detection of staphylococcus aureus in skin using real-time potentiometric biosensor based on carbon nanotubes and aptamers. Biosens. Bioelectron. 31, 226–232.