Digest Journal of Nanomaterials and Biostructures Vol.13, No.4, October-December 2018, p. 1165-1171

NANOSTRUCTURED SILICON TRAPPING FOR SINGLE ESCHERICHIA COLI BACTERIA DETECTION

A. RAMIZY[a,*], M. H. EISA[b,c], M. A. ALALOUSI[a], I. M. IBRAHIM[d], I. M. ALI[d]
[a]*Department of Physics, College of Science, University of Anbar, Anbar, Iraq*
[b]*Physics Department, College of Science, Sudan University of Science Technology, Khartoum 11113, Sudan*
[c]*Department of Physics, College of Sciences, Al Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia*
[d]*Department of physics, College of science, University of Baghdad, Baghdad, Iraq*

The detection for Single Escherichia Coli Bacteria has attracted great interest and in biology and physics applications. A nanostructured porous silicon (PS) is designed for rapid capture and detection of Escherichia coli bacteria inside the micropore. PS has attracted more attention due to its unique properties. Several works are concerning the properties of nanostructured porous silicon. In this study PS is fabricated by an electrochemical anodization process. The surface morphology of PS films has been studied by scanning electron microscope (SEM) and atomic force microscope (AFM). The structure of porous silicon was studied by energy-dispersive X-ray spectroscopy (EDX). Details of experimental methods and results are given and discussed. The values obtained were compared with the published data.

(Received October 1, 2018; Accepted December 4, 2018)

Keywords: Nanostructured silicon, Escherichia coli, Trapping, Detection

1. Introduction

Detection of single cell analysis has primarily been explored using different methods such as "optical, magnetic, or acoustic forces" in order to trap target particles. Trapping and detection techniques for single cell bacteria (SCB) are attracted much research interest for public health [1]. Today various techniques are now available for the analysis of a single cell; with the aid of these techniques, many biological questions can be answered [2]. Therefore, trapping and detection technique of single cell bacteria continues to find applications in both physics and biology. There is an intense research activity going on to develop bacteria possess extremely favorable simplest living organisms with rigid cell walls. However, single cells Escherichia coli (E-coli) bacteria are well-characterized organisms, which can be easily grown, cheap and rapid reproductively [3].

Moreover, E-coli bacteria are divided into positive and negative types with rod-shaped without distinct nucleus [4-11]. Furthermore, there are several excellent reviews and studies concern with the tapping and the detection of e coli bacteria now available [1- 10].

Nanostructured porous silicon (PS) has been widely studied due to its attractive properties [11]. Nanostructured porous silicon (PS) is a sponge-like structure, composed of silicon skeleton pinheaded by a network: of pores. The porous silicon is attractive material for controlled porosity, pore size and can be tailored as a function of the certain application [12-16]. Porous silicon has unique properties can be easy prepared by electrochemical etching popularly known as anodization with many different applications [12]. Although porous silicon has many potential applications, we will focus on the optical application for bacteria. The detailed information on the porous silicon was given elsewhere [11-16].In this work, a silicon substrate was enticing to generate a thin layer of porous silicon on the silicon substrate with pores as large as possible in diameter.

*Corresponding authors: asmat_hadithi@uoanbar.edu.iq

The surface morphology, layer thickness, pore diameter, pore shape, wall thickness and etching rate were studied.

A large surface area of single-cell *E. coli* bacteria inside the micropore for light trapping and molecular interaction inside the porous layer were reported.

2. Materials and methods

2.1. Sample preparations

A sample of E. coli bacteria germ-negative culture (strain) was provided by "School of Biology" (Universiti Sains Malaysia, Pinang, Malaysia). E. coli cultures were grown in Petri ditch at 37°C with shaking (125 rpm).The E. coli bacteria germ negative was collected from culturing fresh sample Petri ditch. These samples were prepared under the sterile condition to reduce contamination from outsider's bacteria. To count the number of bacterial cells, cultures were serially diluted with 500µl of the chosen dilution was mixed with warm tryptic soy agar (TSA) and poured into plates in triplicate. The cultures were taken from growing cultures and diluted into 3000µl of water for sampling. Plates were incubated at 37°C for 24 h [15].

2.2. Trap preparation

Microstructures PS which were used as trap have areas from 1-5 micron (µm) fabricated from n-type (100) using anodization method. Approximately 103 pores were constructed at the surface of silicon with the area. For preparing microstructures PS with the variable of pore diameters, seven sample of P-type (100) wafer cleaned before anodization process was performed. After cleaned process was done, PS was dried and ready to use as a trap. Anodization process was performed in some conditions and parameters which can make the PS have the specific diameters for each sample.

For this work, n-type (100) oriented silicon wafers of 2.74 mm thickness and 6.21Ohm.cm were used to form PS structures by electrochemical anodization method under the light. With this fabrication, the parameters which were used to form the PS have the duration of 20 minutes, wafer n-type (100) and 1/4 (hydrofluoric acid/Acetone) and variable currents density from 2-14 mA was used. The electrolyte used was 12% Acid Hydrofluoric: Acetone taken in the mole ratio of 1: 4. Anodization was carried out at current densities varying between 2 and 100 mA/cm2 and for same duration 20 minutes. After anodization, the PS samples were dried under nitrogen shower.

After that, samples were imaged by using SEM and AFM to measure the diameter and thickness of porous. Table 1 shows values of pore diameters. All the processes were operating in a clean room at Nano-Optoelectronics Research Laboratory, School of Physics; Universiti Sains Malaysia.

Table 1. The diameter and thickness of PS based on variable current density applied in anodization process.

Current Density (mA/ cm^2)	***Diameter (micron)***	***Thickness (micron)***
2	0.50-0.83	2.5
4	0.83-1.00	4.0
6	1.00-1.20	4.0
8	1.00-2.00	5.0
10	0.80-1.50	5.0
12	2.00-2.50	5.0
14	3.00-5.00	5.0

Porous silicon as a prototype traps bacteria without using light and mechanical contact technique. In this work, *E. coli* bacteria were focused as a subject to trap and detect. The diameter and thickness of pores are importance for increase the trap efficiency. However, single cell trap size must be fitted or larger than the size of the individual cell. Trapping also depends on viscosity, surface tension, the radius of the pore and gravitational force.

The height h of the capillary rise with respect to the reference level (height of liquid column in the first tube) is given according to [17]

$$h = \frac{2\gamma\cos\theta}{\rho g r} \quad (1)$$

Where, g is the acceleration due to gravity (9.8 m/s^2), ρ is the density of the liquid, r is the radius of the capillary tube, γ is the surface tension and θ is contact angle between the fluids and the capillary tube. From this trapping technique, there are no applications of electrical, chemical and optical for trapping *E. coli* bacteria. Mostly like that filled the vacancy volume at the PS. To solve the high viscosity effect of water, the Sonic Clean was used to increase the temperature and gave vibration to the solution to reduce the viscosity of water. However, a drawback of optical trapping has been the damage induced by the intense trapping light. In practice, such damage limits the exposure time for trapped specimens and has proved to be a significant problem for some optical trapping studies, particularly those in vivo.

3. Result and discussion

3.1. Results of the pore diameter and thickness of PS

In this study, the values of the diameter and thickness of PS based on variable current density applied in anodization process are tabulated in Table 1. The values of the pore diameter and thickness of PS based on current density in this work have been compared with literature data [14, 18-20]. From Table 1, it is clear that the values are in good agreement. This indicates that our measurement of the diameter, thickness and current density is right. Hence the pore diameter, thickness and current density values obtained experimentally in the present work are quite good.

In this study the porosity and thickness of PS determined using the equations [19]

$$P\,(\%) = (m_1 - m_2)/(m_1 - m_3),\ T\,(\mu m) = (m_1 - m_3)/A_{\delta Si} \quad (2)$$

Where, "m_1 and m_2 are the masses of Si-wafer before and after PS layer formation, respectively, and m_3 is the mass of Si wafer after PS removal by etching in 1% KOH solution. *A* is the area of the PS layer and δSi the density of silicon wafer". the result showed the pore diameter generally increases with increasing potential and current density which agreement with [19].

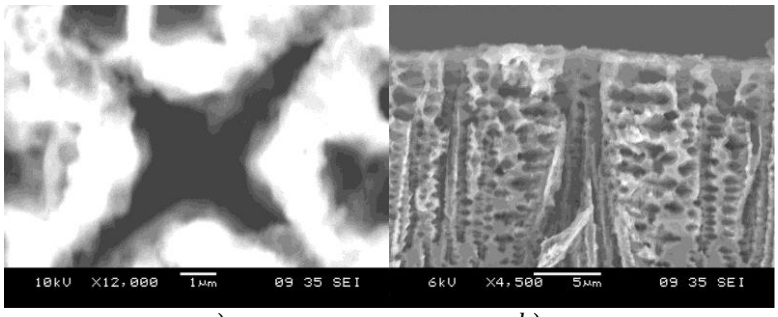
$$d_{pore} = s_{pore}\left[\frac{J}{J_{PSL}}\right]^{\frac{1}{2}} \quad (3)$$

Where, J, J_{PSL} and S_{pore} are the current density, the critical current density and the center to center spacing respectively.

3.2. Characterization of PS by SEM

The surface morphology of PS films has been studied by scanning electron microscope (SEM). Fig. 1: (A) and (B) shows surface morphology, as seen by SEM. In this study, the largest diameter pore of porous silicon was chosen for trapping *E. coli*, because the dimension of E. coli bacteria is around 2 micron lengths and 0.5micron wide. Firstly, the solution contained bacteria *E. coli* are pipette at the surface of PS. To reduce contamination, 2 µL dilution contained E. coli bacteria was pipette at the surface of microstructures PS sterilization condition. As we know, sample of PS are hydrophobic on the surface and Wise Clean equipment can produce the vibration with 40KHZ and heat 60 ºC was used after solution was pipette on the PS surface to increase the trapped E. coli bacteria for 5 minutes until the solution spread on PS surface. Sample was put on oven for 10seconds and coated with platinum for SEM imaging procedure. Detection of single cell E. coli bacteria by imaging can be done easily by using SEM, but some of the dust and medium

can gave some error to eyes as shown Fig. 1. To eliminate eyes error, EDX analysis has been done to detect single cell E. coli bacteria with PS as a background.



a) *b)*

Fig. 1. (a) SEM micrograph diameter of 4µm PS. (b) the thickness of 4µm PS was have approximately 10µm thickness.

3.3. Characterization of PS by EDX

The surface morphology and size of the Ps were examined using a Scanning electron microscope (SEM) and Energy dispersive X-ray (EDX) on INCA X-SIGHT Oxford Instruments. The EDX elemental analysis or chemical characterization of a sample composition is shown in Fig. 2. For this work, the target spot was single cell located and background spot has been analyzed by EDX for comparison detection between both of result. The point spot analysis EDX mode was used for this detection and other parameters which were used are 54 spot-size, 10kv and acquisition time of 100sec for every spot analysis.

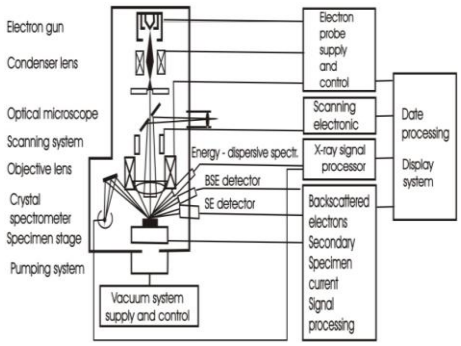


Fig. 2. EDX analysis instrument. diagram

The target cell was located by using SEM and EDX which was used for detection as depicted in Fig. 3. From the results which have been collected by EDX, the single cell E. coli bacteria have some different weight and atomic percentage (%) of atomic content. The result in Tables 2 and 3show the data collected from EDX.

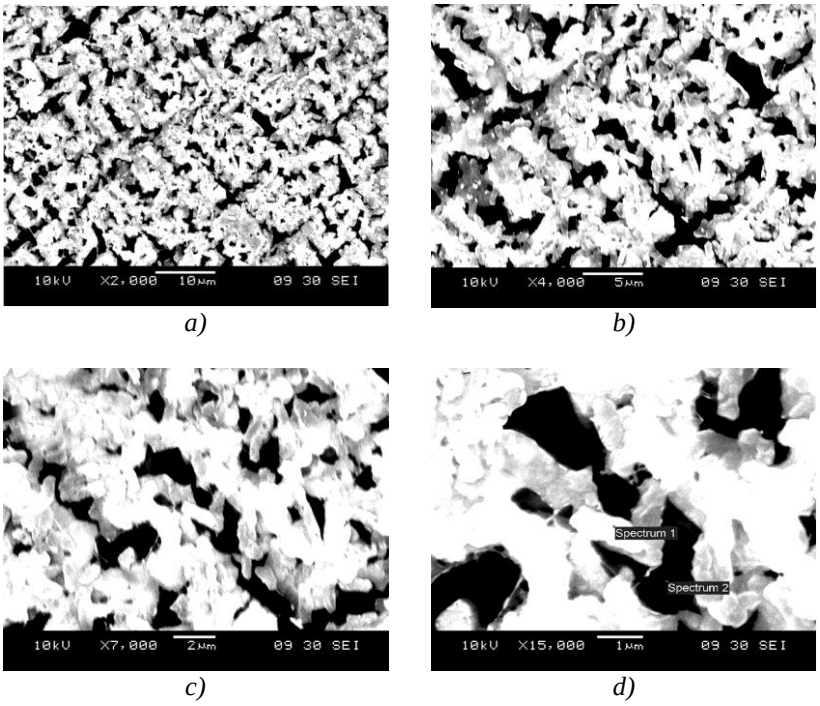


Fig. 3. SEM micrograph of single cell E. coli *bacteria targeted for EDX analysis. (a) with 2k magnification, (b) with 4k magnification, (c) with 7k magnification and (d) with 15k magnification.*

Table 2. Data collection from the single cell E. coli *bacteria by spectrum 1.*

Elements	Weight%	Atomic%
C	28.07	49.29
O	18.64	24.58
Na	0.77	0.71
Si	25.56	19.20
S	1.14	0.75
Cl	1.27	0.76
Totals	100.00	

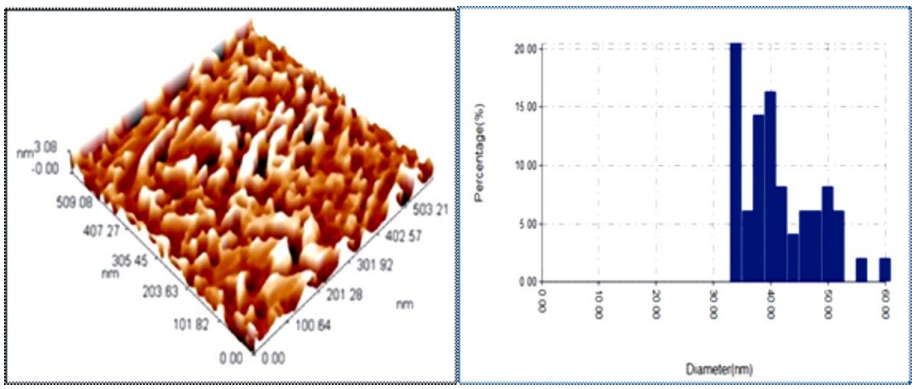
Table 3. Data collection from the background of PS by spectrum 2.

Elements	Weight%	Atomic%
C	3.43	7.45
O	9.06	14.77
Na	0.29	0.33
Si	82.20	76.35
Cl	0.73	0.54
Totals	100.00	

3.4. Characterization of PS by AFM

The pore size and morphology were determined from the Atomic Force Microscopy measurements. A sponge-like structure was produced and when current density increased some of the pores coagulate to form larger structures. Irregularly nanocrystalline silicon distribution on the surface can be recognized as shown in Fig. 4. Fig. 4 (a) shows AFM images of PS prepared by ECE technique with a constant time 20 min while Fig. 4(b) shows the schematic granularity accumulation distribution. It showed that the PS layer has sponge like structure and the as grown

sample cracked. The etched surface became rougher. Fig.5 shows the energy gap of PS is increased as compared with as grown sample [22]



a) *b)*

Fig. 4. (a) AFM images of PS prepared by ECE technique with a constant time 20 min. (b) Schematic Granularity Accumulation Distribution.

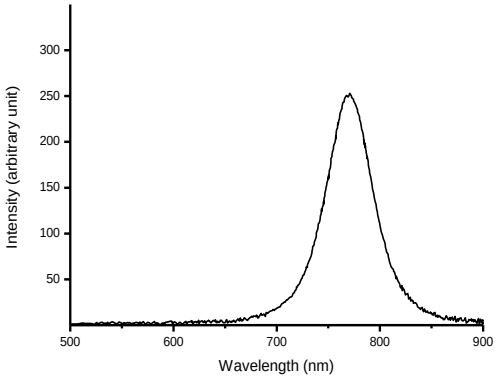


Fig. 5. PL spectrum of ps prepared by ECE technique with a constant time 20min

4. Conclusions

In conclusion, porous silicon (PS) has a large potential for applications in biosensors. However, this paper presents the first *E. coli* bacteria trap we called microstructures PS. The microstructures PS can be trapping the *E. coli* bacteria without gave damage to *E. coli* bacteria cell wall. From the results, this concept can be readily used to trap the single cell bacteria. For further achievements, the microstructures PS can develop to trap the single *E. coli* and the characteristics of a single cell can be viewed by using optical equipment with live viewed. The experiment also shows that trapping method of *E. coli* bacteria by using microstructures PS archived.

Acknowledgement

This work was supported by Nano-Optoelectronic Grant RU: 1001/PFIZIK/814010 from the School of Physics, Universiti Sains Malaysia, the Third World Academy of Science (TWAS) and the College of Science, Sudan University of Sciences and Technology (SUST).

References

[1] K. C. Neuman, S. M. Block, Rev. Sci. Instrum. **75**, 2787 (2004).
[2] A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, S. Chu, Opt. Lett. **11**, 288 (1986).
[3] T. L. Min, P. J. Mears, L. M. Chubiz, C. V. Rao, I. Golding, et al., Nat. Methods **6**, 831 (2009).
[4] T. L. Min, P. J. Mears, I. Golding, Y. R. Chemla, Proc. Natl. Acad. Sci. U. S. A. **109**, 9869 (2012).
[5] J. R. Ryan, J. F. Glarum, Elsevier: New York, (2008).
[6] A. Zaritsky, J. Theor. Biol. **54**, 243 (1975).
[7] J. E. Molloy, M. J. Padgett, Contemp. Phys. **43**, 241 (2002).
[8] M. J. Lang et al., Biophys. J. **83**, 491 (2002).
[9] E. Fallman, O. Axner, Appl. Opt. **36**, 2107 (1997).
[10] K. Ibrahim, M. H. Khalid, M. H. Eisa, M. N. Najimudin, M. A. Al Rajhi, Hajo Idriss, Journal of Nano Research **34**, 61 (2015).
[11] M. J. Sailor, First Edition. Wiley, (2012).
[12] A. Uhlir, Bell System Technical Journal **35**, 333 (1956).
[13] Xiaopeng Li, Hong-Seok Seo, Han-Don Um, Sang-Won Jee, Yong Woo Cho, Bongyoung Yoo, Jung-Ho Lee, Electrochim. Acta **54**, 6978 (2009).
[14] G. Korotcenkov, "Porous Silicon: From Formation to Application: Formation and Properties, Volume One," CRC Press, (2015), p. 83.
[15] A. Ashkin, K. M. Dziedzic, T. Yamane, Nature **330**, 769 (1987).
[16] S. B. Valerol, B. E. Logan, Langmuir **18**, 5256 (2002).
[17] S. B. Velegol, S. Pardi, X. Li, D. Velegol, B. E. Logan, Langmuir **19**, 851 (2003).
[18] C. G. Vayenas, R. E. White, "Modern Aspects of Electrochemistry 39,"Springer US, Ed. G. X. Zhang, Canada, **39**, (2006), pp.XXI-279.
[19] S. N. Sharma, G. Bhagvannarayan, R. K. Sharma, S. T. Lakshmikumar, Mater. Sci. Eng. B 127, 255 (2006).
[20] U. M. Nayef, A. H. Jaafar, Eng. & Tech. Jour. 31(3), 339 (2013).
[21] K. Rumpf, P. Granitzer, P. Pölt, H. Krenn, Phys. Status Solidi C 6, 1592 (2009).
[22] A. Ramizy, Z. Hassan, K. Omar, Sensors and Actuators B 155 (2011) 699–708