Metal Assisted Stain Etched Porous Silicon for Detecting Klebsiella Bacteria

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Doi: 10.23918/eajse.v9i1p353

Abstract: Double-layered porous silicon was prepared by Stain etching and Metal assisted chemical etching (MACE) methods and examined in the detection of Klebsiella. A P-type silicon wafer is firstly poroused by stain etching method using hydrofluoric acid (HF) and nitric acid (HNO_3). The poroused silicon is deposited by copper via the classical electrochemical deposition process then the copper-coated silicon is etched again in HF and hydrogen peroxide (H_2O_2). The stain etching process obtains micro-sized pores on the surface of the silicon; on the other hand, the copper assisted chemical etching step forms more nanopores that help to capture bacteria. The chemical constituent of the double etched porous silicon was measured by Energy Dispersive X-ray Spectroscopy (EDX). The Scanning Electron Microscopy (SEM) method is used to observe the morphology and pore dispersion of the sample. The SEM images show that the nano-sized and micro-sized pores are formed. The prepared porous silicon is applied for Klebsiella detection. The Fourier transform infrared radiation (FTIR) test of the sample illustrates the adhesion of the bacteria to the porous silicon. This can be observed through the formation of the new peaks in the FTIR graphs when the graphs are compared before and after Klebsiella introduction. The carbon-carbon double and triple bonds and proton-oxygen double bond formation proofs the adhesion of the bacteria to the porous silicon. This method of acquiring nanoporous silicon as well as the FTIR results can be used to detect bacteria generally by differentiating the types of bond vibrations of the bacteria. This is a fast, easy and affordable method to manufacture biosensor with an improved affinity.

Keywords: Nanoporous Silicon, Klebsiella, Biosensor, Copper Deposition, Stain Etching, FTIR Spectroscopy, Detection

1. Introduction

The porous silicon is assumed to be a desirable transducing substrate for biosensing reasons with applications in several scientific disciplines due to its low cost of manufacture, large internal surface area, variable pore size and optical features(Moretta, De Stefano, Terracciano, & Rea, 2021; Vercauteren, Leprince, Mahillon, & Francis, 2021). Additionally, it has a hard structure that gives a chance to use it more than one time(Yaghoubi, Rahimi, Negahdari, Rezayan, & Shafiekhani, 2020). Most of the detecting methods used to be introduced in bacterial or viral detection were expensive and complicated. Since it is necessary in the medical field and the food industry in daily life, finding simple and cheap detecting tools have become one of the most attractive and appealing field among scientists and engineers(Massad-Ivanir & Segal, 2014; Yaghoubi et al., 2020). One of the cheapest and fast analytical biosensors is manufactured by a transducer and a bioreceptor (a biological detection apparatus) this bioreceptor is attached to the transducing substrate (Lazcka, Del Campo, & Munoz, 2007; Mehrotra, 2016; Moretta et al., 2021; Yoon, 2016).

Received: December 20, 2022
Accepted: February 12, 2023
Ali, H.A.D, & Hassan, U.M. (2023). Metal Assisted Stain Etched Porous Silicon for Detecting Klebsiella Bacteria. *Eurasian Journal of Science and Engineering*, *9*(1),353-362.

According to voluminous literature the demand for quick-response, low-cost, and user-friendly technologies is gradually replacing the expensive, time-consuming, and specialized laboratory equipment needed for the present methods of bacteria identification; moreover, biosensors which are particularly interesting study subjects, have seen significant advancements in both technology and applications over the past 20 years(Moretta et al., 2021).

Among the numerous available nanomaterials like quantum dots, metallic nanoparticles, and carbon nanotubes, nanoporous silicon offers exceptional potential for applications in multiple scientific domains, from biosensing to drug administration, due to its well-known optical and physical characteristics (Arshavsky-Graham, Massad-Ivanir, Segal, & Weiss, 2018; Tieu, Alba, Elnathan, Cifuentes-Rius, & Voelcker, 2019; Yaghoubi et al., 2020). As indicated by the consistent volume of scientific publications on porous silicon-based sensors published every year in peer-review journals, it has been an active issue for research investigations since the initial tests on porous silicon as a biosensor platform decades ago(Moretta et al., 2021). Most of the sensors can be classified into optical and electronic sensors. The most effective constituent of these sensors is the nanostructure part which can be a porous silicon that has nanowires or nano-sized pores(Zheng, Patolsky, Cui, Wang, & Lieber, 2005), nanotubes(Baltog, Baibarac, & Lefrant, 2005) and graphene(Mannoor et al., 2012). These nanostructures increase the sensitivity of the sensors to a high level due to their large surface area(Sailor & Link, 2005).

For those sensors that are based on porous silicon, there are two main detecting methods known as direct and indirect methods. In the indirect detection method, detection is accomplished by focusing primarily on bacterial cell fragments like proteins or DNA excreted by the bacteria. Therefore, the target bacteria must be taken to pretreatment processes like heating and lysis in order to get cell fragments. Biosensors of this type of detection can be furthermore classified into subgroups according to the kind of capture probe molecule(Massad-Ivanir & Segal, 2014). On the other hand, in the direct detection method, the complete bacterial cell is directly bound to the porous silicon transducer. The whole bacterium is captured using proper bio recognition components(Massad-Ivanir & Segal, 2014). This recognition component in porous silicon-based biosensors is the meso and nano porous layers. Microbes and other biomolecules must be detected quickly in the medical and food industries in order to monitor air and water quality or perform urgent clinical tests. Traditional testing, on the other hand, is based on time-consuming laboratory analysis, which occasionally necessitates the use of highly qualified experts(Gongalsky, Koval, Schevchenko, Tamarov, & Osminkina, 2017). Thus, porous silicon guarantees the desired sensor due to the features mentioned above. There are many ways to achieve a porous surface of silicon such as stain etching, metal assisted chemical etching, anodic etching, etc. Some of these etching methods (like stain etching) result in micro sized pores in high resistive silicon and some of them (such as metal assisted chemical etching) produce nano-sized pores(Karbassian, 2018). Moreover, some of the etching methods are complicated and expensive, whereas some of them are cheap and easy.

The effective bacterial attachment to the porous silicon surface is a problem. Several solutions have been presented to overcome this issue such as coating the porous silicon with a substance that has an affinity to bacteria. However, this makes the sensor more expensive and less stable. Another solution is to make changes on the porous surface itself, whereas; this may oblige us to compromise the optical performance of the sensor(Gongalsky et al., 2017). The modifications on the porous surface by using two etching methods allow us to improve the sensitivity of the sensor. Stain etching and metal assisted chemical etching of silicon are the two preferred processes in this study in order to perform double

porousing process on the silicon and use it to detect Klebsiella bacteria. It belongs to the tribe Klebsielleae in the family Enterobacteriaceae, Klebsiella is the second most common enteric genus discovered in the human gastrointestinal tract(Ristuccia & Cunha, 1984).

Different suggestions are to investigate the formation of new bonds via FTIR graphs which show the molecular vibrations. One of the most applicable and easy spectroscopic methods that guarantees the researchers collect data easily and affordably is FTIR spectra. The spectra give a chance to observe the varying molecular vibrations of the sample that is tested in the infrared range (Al-Holy, Lin, Cavinato, & Rasco, 2006; Naumann, Helm, & Labischinski, 1991). Therefore, by observing the change in the molecular vibrations it is possible to see whether the bacteria are attached or not. In this study the double poroused silicon is used for detecting Klebsiella bacteria using FTIR spectra to observe the bonds related to the bacteria.

2. Experimental Method

A P-type silicon wafer (Silicon Quest International, SQI) with crystallographic orientation 100 and resistivity 1-10 ohm.cm is cut into 1.5 cm by 1.5 cm pieces and then is cleaned by RCA method via the following steps: first, 65 ml of NH_4OH is poured into 325 ml of deionized water in a beaker. Then, the solution is heated to about 70^0C on the heater. After that, the beaker is taken off the heater and filled with 65 ml of H_2SO_4. Vigorous boiling of the solution can be seen after only several minutes so at this time the silicon substrate is put into the boiling solution and waited for about 15 minutes. Later the substrate is etched in 50% HF solution for 5 minutes. Then the substrate is ready for use.

Double layered porous silicon is accomplished via stain etching of mirrored surface and then metal assisted chemical etching. The latter is done by simple electrochemical deposition of Cu onto the substance.

2.1 Stain Etching

The chemical solution for stain etching is mainly HF and an oxidant like HNO_3. Firstly, for the stain etching, the solution was prepared from HF (Scharlau 48%) and HNO_3 (Scharlau min 69%) with a volume ratio of 400:1 respectively (see Fig.1). The silicon sample was covered with paraffin then it was rinsed in the solution and left for 20 minutes. After that, it was taken out and washed with distilled water then dried by hair drier and sent to SEM and EDX study.

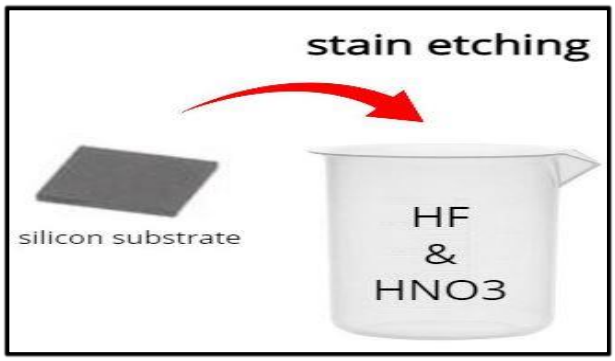


Figure 1: Stain etching process.

2.2 Cu Deposition Processes

Now, for the second etching process which is metal assisted chemical etching the surface of the porous silicon is coated with Cu thin layer via the classical electrochemical deposition process. The deposition is done by immersing a Cu bar as an anode and the porous silicon as a cathode into a solution containing 5g of copper sulfate ($CuSO_4$) and 200 ml of deionized water. 2 mA DC electric current is applied for 15 minutes (see Fig.2).

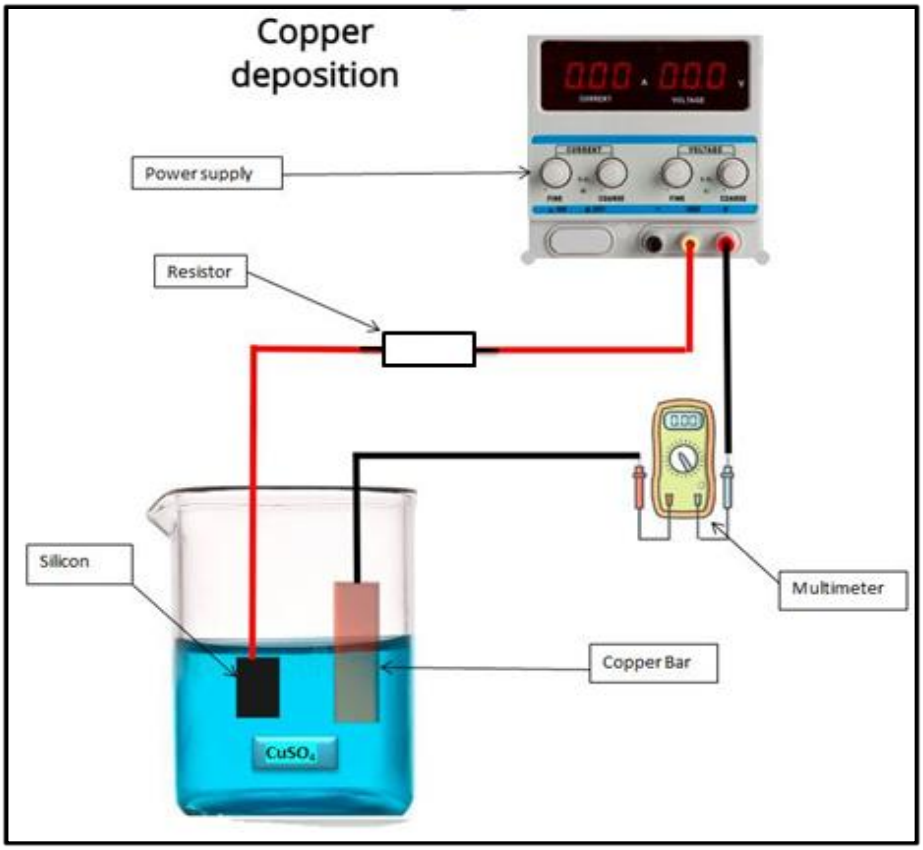


Figure 2: Cu deposition process by electrochemical method

2.3 Metal assisted chemical etching (MACE) process

Figure 3 shows the cell setup of (MACE) process which is composed of two pieces of Teflon and a washer between them. The Cu deposited poroused silicon sample was fixed on the lower Teflon part. Then a solution of HF and H2O2 (Scharlau 50%) with a volume ratio of (1:10) respectively, was poured into the cell, some amount of ethanol was mixed with the solution in order to decrease surface tension, and the sample was left for 20 minutes in order to get nanoporous layer. EDX spectroscopy was used to investigate the chemical composition of the sample surface and to see whether the Cu particles exist or not.

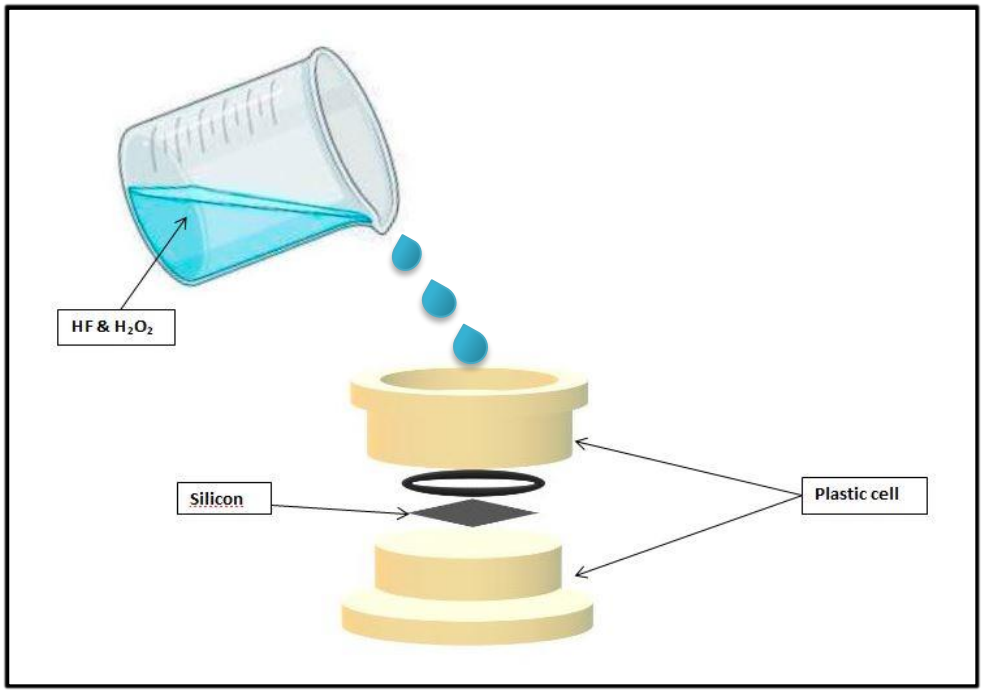


Figure 3: Schematic diagram of metal assisted porousing process of the second layer

After that, the double poroused silicon was washed with HNO_3 in order to remove Cu metal remnants in the pores as they may play an antibacterial role (Fellahi et al., 2013; Gongalsky et al., 2017). Then the double etched porous silicon is heated at 250°C for 5 minutes in order to increase its hydrophilicity (Gongalsky et al., 2017) and make it more sensitive. Then FTIR spectrum was taken using (SHIMADZU IR Affinity-1) for the double etched porous silicon. All samples were taken to the Scanning electron microscope (SEM) in order to take SEM image and to observe the porous structure of the surface morphology of samples.

Klebsiella bacteria is prepared by putting the Klebsiella bacteria from an agar plate to a culture solution media suitable for its growth in a tube and then incubated for 24 hours in an incubator at 37°C in order to let the bacterial colonies grow since this temperature is the optimal temperature for Klebsiella growth. After that, the centrifugation process is applied to remove Klebsiella bacteria from its culture solution. Klebsiella bacteria solution was centrifuged at 3000 rpm for 10 minutes. The whole bacteria accumulated at the bottom of the tube. Then the cultural solution was removed, and the remained bacteria were washed several times to guarantee the culture removal then the Klebsiella bacteria mixed with 40ml deionized water. Three drops of the bacterial mixture were poured onto the surface of the double poroused silicon surface and left for 20 minutes to be dried. Then the FTIR spectra of the sample was taken and compared to the previous one in order to see whether new vibrational bonds on FTIR spectrum are formed or not. After preparing of any porous, the sample was taken to the Scanning electron microscope (SEM) in order to take SEM image and observe the porous structure of the surface morphology of the sample.

3. Results and Discussion

The chemical composition of the prepared double poroused silicon surface was analyzed by Energy Dispersive X-ray (EDX) spectroscopy in order to see whether the copper particles do exist or not to guarantee the completeness of the copper deposition, the process of metal assisted chemical etching

and the formation of the nano-ranged pores. This analysis was done before washing the double poroused silicon in the HNO_3 and applying the bacterial solution on to the surface of the porous silicon. Figure 4 is the EDX graph and image of the chemicals of the sample. The existence of gold particles is due to the gold sputtering to increase the conductivity of the sample required for the scanning electron microscopy (SEM) device. The existence of copper particles can be seen clearly with a small ratio as desired. The colors of the surface chemical elements in the EDX image are as follows: pink represents Cu particles, yellow represents the silicon, the green color is the gold particles that were sputtered onto the sample before taking the image and orange is representing the silicon oxide resulted from the oxidation of the porous surface.

Figure 4 reveals that the Cu particles exist in the porous silicon; that means, it proves that the deposition of Cu has taken place via the electrochemical deposition process; moreover, it shows that these particles have participated In the MACE process as well.

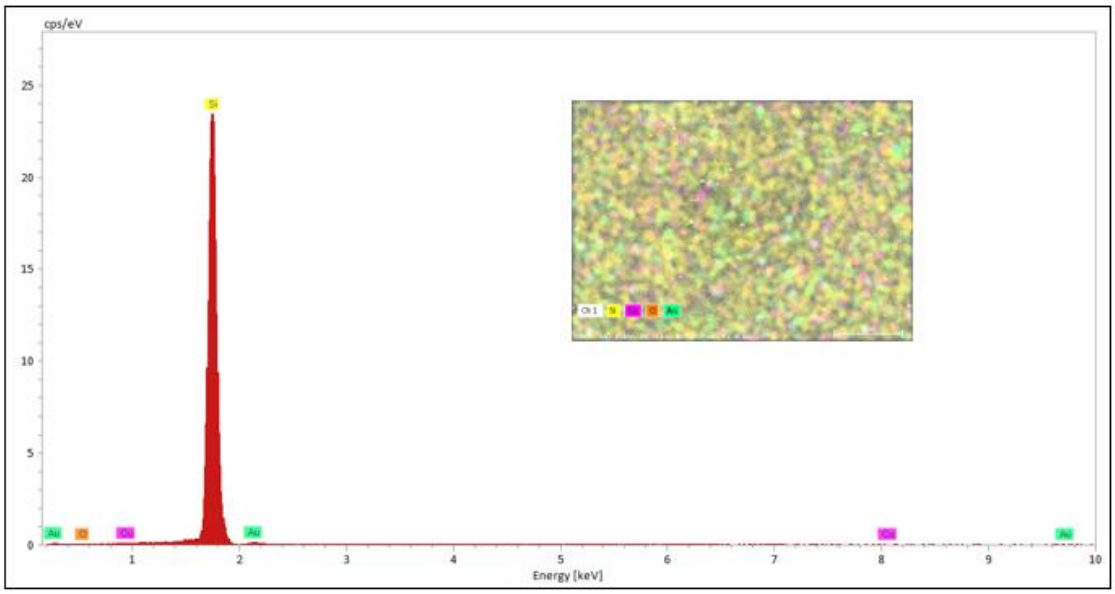


Figure 4: EDX spectrum and image of the double etched porous silicon before washing by HNO3.

FTIR in the range of 500 cm^{-1} to 4000 cm^{-1} was performed before and after putting the Klebsiella bacteria on the surface of the double etched porous silicon in order to observe whether the bacteria is captured or not. It is known that all bacteria have some kind of receptors on their body. This receptors are in the nano range which allow the bacterium to be captured via Vander Waals principle(Gongalsky et al., 2017). The process of investigating the bacterial capture is done by studying the variation in the spectral pattern before and after Klebsiella introduction to the porous surface. Figures 5 & 6 illustrate FTIR spectra of the double layered porous silicon before and after putting the Klebsiella bacteria. The peak around 600 cm^{-1} indicates the Si-Si bond and the peak at 900 cm^{-1} represents the $Si-H_2$ bond of a nano range porosity structure. Moreover, the big peak near 2340cm^{-1} again indicates the $Si-H_n$ bonds in the nanoporous structure of silicon as shown by Raul J. and Miguel M(Martin-Palma, Manso-Silván, & Torres-Costa, 2010). The existence of the bacteria in the pores can be indicated by the peak around 1600cm^{-1} and 2150 cm^{-1} which is the vibrational bonds of the carbon-carbon double bond and triple bond of the Klebsiella structure respectively. This is in agreement with ref.(Gongalsky et al., 2017). The peak near 1250 cm^{-1} is due to the stretching mode of vibration of the Proton=Oxygen double bonds in the cell membrane of the bacteria(Al-Holy et al., 2006).

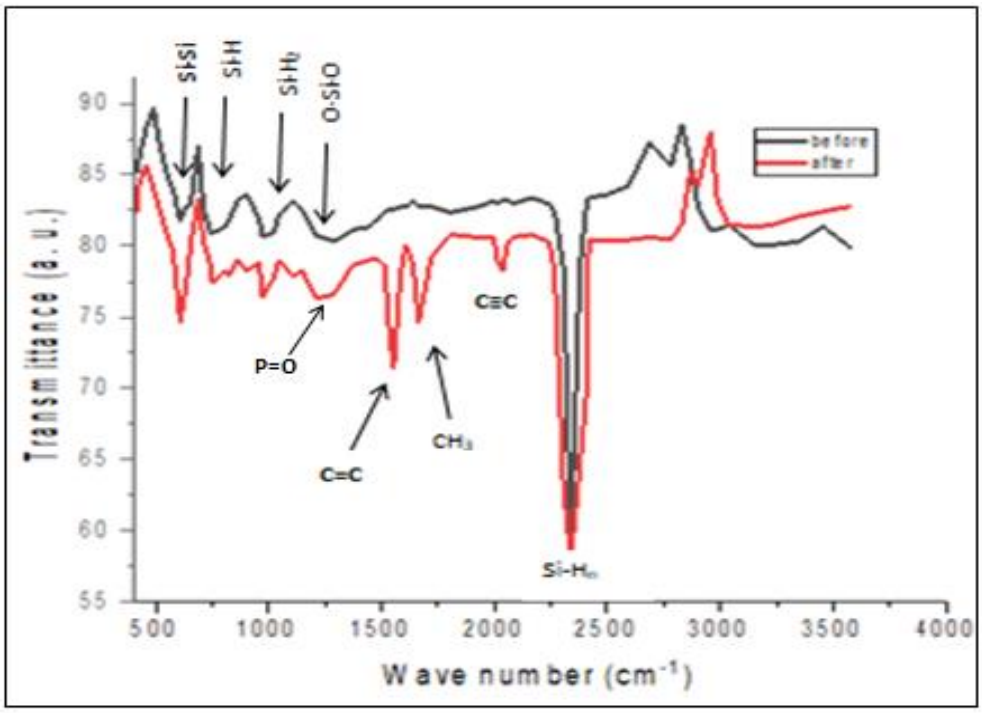


Figure 5: A. FTIR images of the double etched etched porous silicon before (black) and after (red) putting Klebsiella on it (the noise due to the device is eliminated).

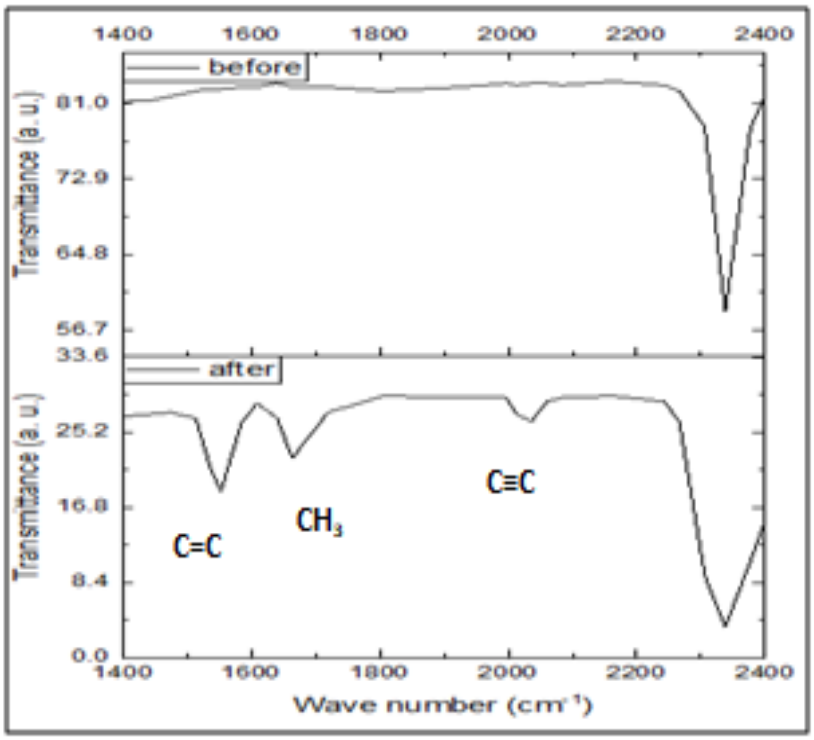
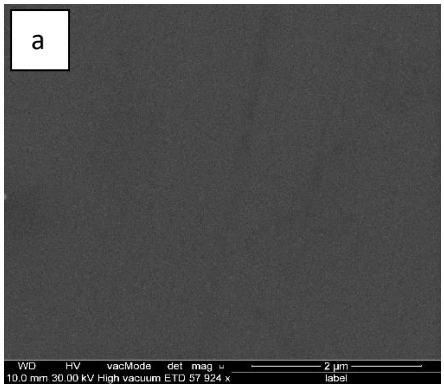


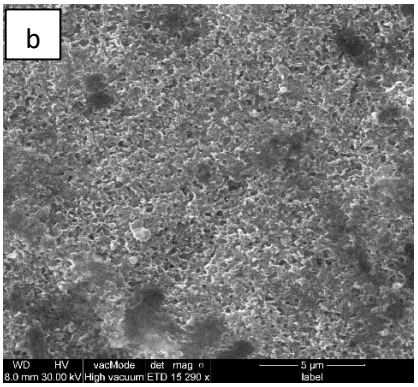
Figure 6: FTIR image with the wave number range of 1400 cm^{-1} to 2400 cm^{-1}. The formation of new peakes after Klebsiella adhesion is illustrated.

SEM images show the morphology of the porous silicon. Figure 7-a shows the virgin silicon before applying any etching processes. Figures 6-b &c are the SEM images of the porous silicon after

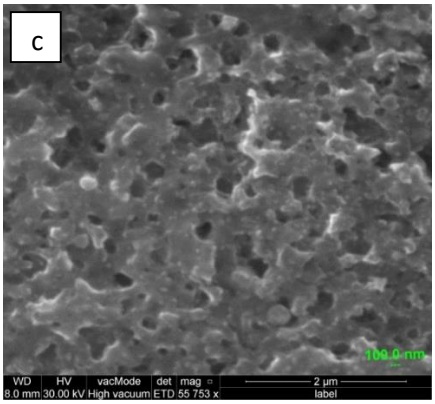
applying double etching processes with different magnifications. The pores produced by stain etching are micro sized. Several pores were produced that have micro-sized diameters. The irregularity of the pores are due to nonuniform doping distribution in the silicon wafer. Moreover, the MACE process mainly produces nano-sized pores. In figure 7-b & c. The image of the nano porous structure can be clearly seen. There is a huge number of micro and nano sized pores. The existence of nano pores is due to the copper assisted chemical deposition process. These nano pores increase the surface area to volume ratio that facilitates the bacterial adhesion and trapping process



SEM image of the virgin silicon before applying the etching process.



SEM image of double etched porous silicon with respectively low magnification



SEM image of the porous silicon after stain and MACE processes with high magnifications

Figure 7: SEM images of the virgin silicon substrate (a), and double etched porous silicon (b& c) with low (b) and high (c) magnifications.

The pore sizes are measured, and their distribution is shown in Figure 8. It is clearly shown that the diameter of most of the pores lay between 50 nm and 150 nm which indicates that the process of nano pore fabrication has taken place and the surface can be regarded as nano nature surface.

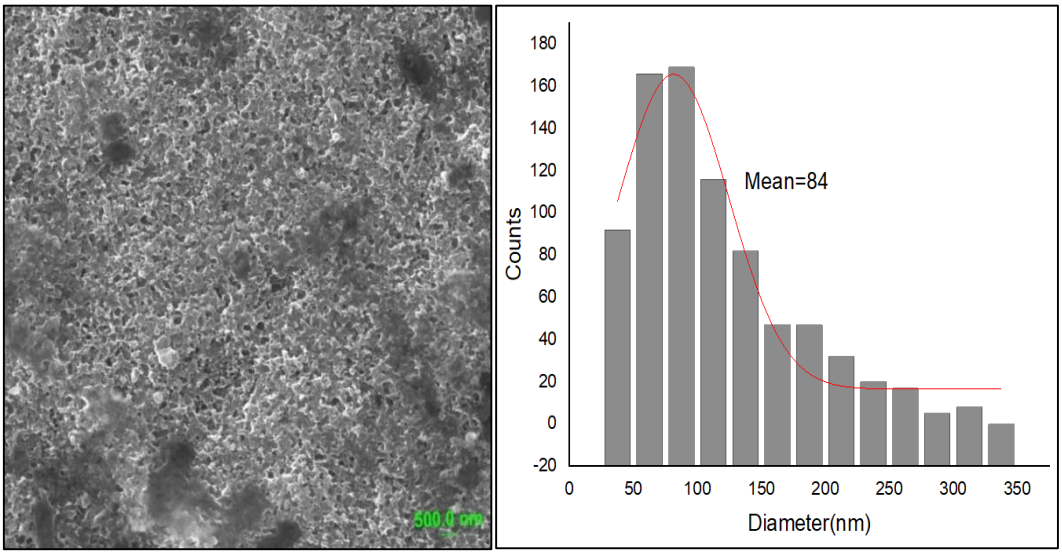


Figure 8: SEM image of double etched porous silicon and the histogram of the pore diameter distribution.

4. Conclusion

In conclusion it is clear that the copper particles on the micro porous silicon surface have an important role in metal assisted chemical etching process and produce nano pores. Measuring the pore diameters and their distribution revealed that most of the pores are in nano range with an average of 84nm. As a result, this method can be assigned as a quick and cheap method to get double layers of a mixture of nano and micro pores. This method can be used for other types of porous silicon-based sensors such as gas sensors and pH sensors. Moreover, development in the biosensors can be achieved through this process. The FTIR spectra can be considered as an important means of fabricating fast and affordable bacteria detector by testing the small differences in the functional groups of different bacterial structures. This method can also be improved upon for other types of bacteria. The variable FTIR band can be used to determine each bacterium and associated bacteria species can be determined accordingly.

5. Acknowledgments

The bacteria solution which used in this work was prepared by Mr. Jutyar Ibrahim from Biology Department /College of Education/ Salahaddin University. His support is gratefully acknowledged.

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