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DETECTION OF STAPHYLOCOCCUS AUREUS WITH MULTI-WALLED CARBON NANOTUBE FIELD-EFFECT TRANSISTOR

K. Priyadharshini[1], S. Rathinavel[1]*

Department of Electronics and Instrumentation, School of Physical Science, Bharathiar University, Coimbatore-46, India

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ABSTRACT - *The aim of the research is used to demonstrate a Multi-walled Carbon nanotube (MWCNT) based biosensor to identify the selective determination of bacteria like Staphylococcus aureus. It is based on the (FET) field-effect transistor in which a linkage of multi-walled carbon nanotube acts as a conductor channel. Staphylococcus aureus antibodies are analyzed by the indirect ELISA method. CNT sensor is immobilized on pAbs after the hybridization of (pBASE) 1-pyrenebutanoic succinimidyl ester. The resistance variation can be measured by a potentiostat. Our proposed transistor was uncovered to develop a high-level concentration of the staphylococcus at least 100 CFU/ml. It was absorbed by the Scanning electron microscope (SEM). The sensitivity and selectivity of the transistor can be analyzed. The concentric value of MWCNT can be determined to be 0.1 mg/mL. This device is used to detect bacteria by suitable antibodies, bacteria, and viruses. The resulting interaction between pAbs with Staphylococcus aureus is increased significantly in the resistance value of the biosensor (P<0.07). The MWCNT-based biosensor can detect Staphylococcus aureus with a detection limit is 2log CFU/mL.*

Key Words: **Staphylococcus aureus, Multi-wall carbon nanotubes, Field effect transistor, Antibodies, pBASE.**

1. INTRODUCTION

Nanotechnology is a field of nature that belongs to all features of human life [1]. A Carbon nanotube has its allotropy property with a structure of cylindrical nanotubes that classifies as single-walled carbon nanotubes (SWCNT) and Multi-walled carbon nanotubes (MWCNT). The types could be functionalized or non-functionalized due to the more cohesion for the diverse substrate [2]. The properties of MWCNT are large surface area, thermal conductivity, or tensile strength is used for the applications of thermal and chemical processes by post-synthesis techniques [3]. Annealing is the process to reduce structural defects and remove the catalytic metals that are used in the synthesis process. The dispersity is increased in an aqueous solution and improves the compatibility with composite materials due to the functionalization with strong oxidizing [4]. The infection of microbial causes serious risks to humans with different types of diseases that may cause death [5]. CNT plays a significant role in the growth control of microbial so the CNT will apply in different applications like tissue engineering, biosensing application, drug delivery, and dressing the wound and it helps to enhance the strong antimicrobial activity [6].1-Pyrenebutyric acid N-hydroxysuccinimide ester (PBASE) covalent bonding with CNT helps the antimicrobial activities of Staph aureus. CNT contact directly will cause cell damage and cell death [7, 8]. The size and surface area of the CNT are the main parameters that affect the antibacterial activity to interact with the bacteria [9]. The enzyme immunoassays merge the antigen-antibody reaction with the signal amplification and sensitivity for the catalyzed materials and the conventional electrochemical methods that can be operated in the crude samples without the filtration or separation methods. The non-specific binding of bacteria or protein can be avoided by the PBASE. Staph aureus is detected by the standard plate count method with the selective enrichment in the broth prepared. The resistance of the organism increased towards the antibiotic which can be led to severe health problems and cause bacterial infection was resistant to the antibodies to remove the infection [10]. The MWCNT-based sensor is synthesized based on the field-effect transistor that nanotubes are used as an electron channel between the sources and drain electrodes. MWCNT-FET biosensor is applied for the detection of aureus due to its ability to detect the interface from absorption of charged species [11]. The MWCNT will be connected to an electrochemical electrode based on a surface modification to improve the electron transfer rate and surface area [12]. The interaction of antigen-antibody connected with the sensing layer of the CNT to transduce the charge transfer of the FET can be used to detect the bacteria. The goal of our research is to evaluate the sensor and characteristics of the devices to detect Staphylococcus aureus. The objectives of the research are to analyze the antibodies present in staph aureus for biosensors, develop a fabrication step for MWCNT-based biosensors, and also characterize the properties of CNT-based sensors to detect staph aureus.

2. MATERIALS and METHODS

2.1 Materials

DMF, TFA, and PBASE were purchased from (Loba Chemic). The culture of the staphylococcus aureus was purchased from Sigma-Aldrich Solutions. pAb was obtained from Thermo fisher scientific and diluted to a concentration of 4 mg/ml with carbonate-bicarbonate buffer. The other reagents are used in the analytical grade.

2.2 Preparation of MWNTs

MWCNT is synthesized by a CVD method and the iron nanoparticles are used as the catalyst. The nanotube was made on a silicon substrate that contained a catalyst in the thin film and the two buffer layers with 2 nm of iron, 15 nm of aluminum oxide, and silicon dioxide. Ethylene is the source of carbon. Nanotube will be grown in the oven at a temperature of 700-800℃. The silicon substrates contain the catalyst that was placed on the feeder which was in the presence of H_2 and Ar in the vacuum oven and the substrate is heated for up to 15 minutes. At the time of heating, hydrogen gas was put into the chamber and it is supplied for 45 minutes. For the growth process, the supply of hydrocarbon gases is stopped and the feeder is taken out of the oven. The system was removed with the presence of Ar poured for 5 minutes and the oven cooled slowly to 200℃ [13]. The process was completed by cleaning the whole system. The 3 mg of MWCNT powder was dissolved in 18 ml of DMF and 2 ml of TFA was sonicated for 1 hour. The mixture was centrifuged at 15,000 rpm for 30 minutes. The solvent was poured and the settled CNT dissolved again in the same process. Finally, the carbon nanotubes are diluted with toluene and the solution occurs colorless and transparent. The dispersion was analyzed by the SEM.

2.2.1 Functionalization of MWCNTs

The MWCNT is functionalized by modified Hummer's method. The 2 g of MWCNT is treated with the 1 g of $NaNO_3$ solution was mixed with 50 ml of Concentrated H_2SO_4 in a 250ml conical flask. The solution was stirred for 1 hour in an ice bath and followed by 3 g of $KMnO_4$ was append to the solution under vigorous conditions. The rate of addition is controlled carefully to keep the reaction temperature below 20℃. After removing the reaction mixture from the ice bath and the solution was stirred for 1 hr at 35℃. Then 46 ml of distilled water is added slowly and the mixture was stirred for 30 mins. Finally, 15 ml of hydrogen peroxide and 300 ml of Distilled water were added slowly to the suspension. The solution was centrifuged with 10% hydrochloric acid and distilled water several times. The washing process will be repeated until the pH is neutral. The solution was kept in the hot oven at 60℃ for 24 hrs to obtain the functionalized MWCNT as a grey [14].

2.3 Development of MWCNT-FET

The Dielectrophoresis method is used for thermally oxidized Si wafers with electrode pairs consisting of metallic electrodes like gold (Au). The electrodes are patterned by photolithography and oxidized with silicon wafers. The n-type silicon wafer is used for dry oxidation at a temperature of about 1100℃. The wafer was covered on both sides with 100 nm of silicon dioxide. The backside was etched by buffered oxide solution (BOE) with a 500nm aluminum thin film deposited on the backside gate contact using e-beam evaporation. The electrodes are prepared by e-beam lithography 50 µm long and 500 nm wide with a gap of 800 nm between the electrodes. The 100 nm gold thin films are developed by e-beam evaporation. For the dielectrophoresis position, a 6 µl drop of MWNT solution was dispersed on the device. The solvent was applied on the surface with nitrogen gas after 2 min, and the devices were annealed at 300℃. Figure 1 shows the overall process for the fabrication of biosensors.

2.4 Preparation of Bacterial cultures

The culture was prepared in a test tube by inoculating BHI broth with the bacteria moved from the plate to the test tube by a cotton wipe. Staphylococcus aureus was kept in broth by incubating for 16 hrs at 37℃. The culture was washed 5 times with 15 mM PBS by centrifuging at 6000 rpm for 10 min at 4℃. pAbs were diluted with a concentration of 4 mg/mL with carbonate bicarbonate buffer.

2.4.1 Indirect ELISA for the binding specificity of pAbs with Bacteria

The bacteria were collected and washed several times at 15 mM PBS buffer by centrifuging at 6,500 rpm for 15 mins at 5℃. A chemical reaction of 100µL anti-Staph aureus antibodies was varied with PBS buffer and incubated with the microplate. The growth of color change is measured using a microplate reader and the result was calculated using the below equation (1) [15].

Difference of = Resulted value – Resulted Value

Absorbance after 30 min after 0 min (1)

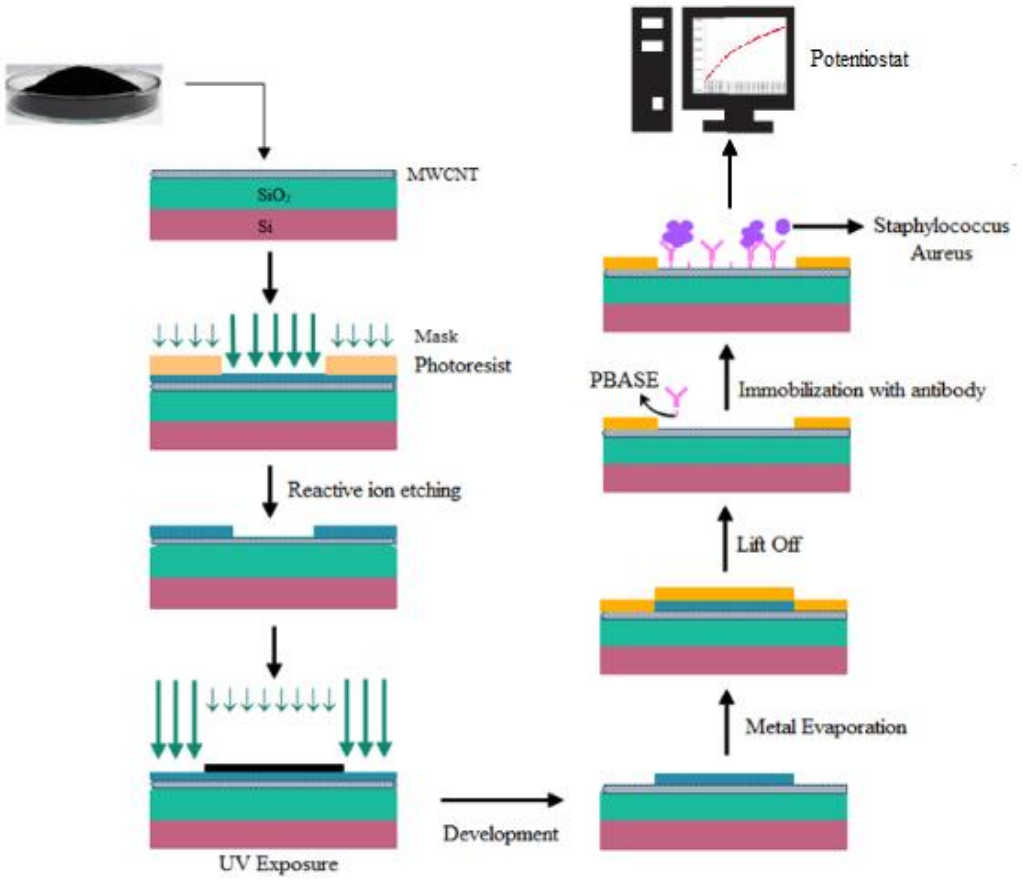

Fig -1: Fabrication of proposed MWCNT-FET biosensor to detect the Staph aureus bacteria.

2.5 Immobilization of PBASE on the MWCNT

The concentration of PBASE is used as a linker on the biosensor for the Sensing event that affects the biosensor signal. The fabricated MWCNT-FET biosensor was tested with the active amount of linker with the concentration known for 2 hours at normal room temperature. The resistance is used to analyze the identity of the optimal linker concentration at the connection between the receptor and the MWCNT field-effect transistor.

2.6 Fabrication of MWCNT-based biosensor

The non-covalent functionalization of the MWCNT is used to configure the biosensor with a PBASE linker (6 mM) as a linker for 2 hours at room temperature. It is followed by the cleaning of distilled water to remove the unwanted PBASE on the biosensor [16]. 4 mg/ml of antibody was centrifuged at 15,000 rpm for 10 mins and diluted with buffer solution. The antibody was attached to the surfaces of the MWCNT by showing the antibody to the linkage of PBASE with being settled overnight at 4°C, to allow the formation of covalent bonds. The MWCNT-based biosensor is attached to the pAbs to wash with PBS buffer (pH 7.4). The potentiostat is used to measure the resistance value of the antibody surface.

2.7 Detection of S. aureus using biosensor

The Staphylococcus aureus cells are collected from the full-grown culture after 3 times of washing with 10 mM PBS (pH 7.4). The solution was centrifuged at 5000 rpm for 20 mins at 5°C. The bacteria were sensed by the optical density (OD) which is measured by 650 nm using a spectrophotometer with a standard curve for log CFU/mL. 0-1 mL of aureus culture solution was dispersed on the MWCNT-based biosensor for 30 mins and incubated at room temperature to allow the bonding connection between the bacteria and antibody. The sensor was prepared and spread over the PBS buffer on the biosensor that was attached to the antibodies. The potentiostat is used to analyze the linear sweep voltammetry curves after the incubation. The current and voltage curves are calculated between the linear analyses with the resistance (R) can be measured by

inversing the values [17]. The resistance difference (ΔR) can be calculated using the below equations:

$$\Delta R = (R_1\text{-}R_0) / R_0 \quad \text{-------} \ (2)$$

Where R_0 is the linker resistance value,

R_1 is Staphylococcus aureus culture resistance value.

3. RESULT AND DISCUSSION

3.1 Bacteria binding with pAbs specificity

The indirect ELISA method is implemented to show the detailed binding of the Staph aureus with pAbs. This method consists of primary antibodies connected with the enzymes and secondary antibodies reacting with the primary antibodies that reacted with an enzyme. While the enzyme is reacted with substrates that can produce a fluorescent compound, changes in a color reaction change between the enzymes and substrate that are connected with the secondary antibodies [18]. The concentration of the bacterial species is 1 X 10^8 CFU/mL. When the pAbs are distilled several times and tested on the biosensor, the resistance of the biosensor increases gradually. This indirect ELISA method is only suitable for the specific binding of pAbs to aureus and also the antibody is used to develop the biosensor to detect the staph aureus.

3.2 Immobilization of Antibody on MWCNT

The antibody into the MWCNT involves a connection between the CNT surface and antibodies. The species can be adsorbed by noncovalent on the MWCNT surface through □-□ stacking, hydrophobic, and electrostatic interaction. The linker of the CNT surfaces is pyrenebutanoic acid, and succinimidyl ester [19]. The PBASE of the hydrophobic pyrenyl group will adsorb irreversibly into the sidewall of the CNT through a □-□ stacking interaction. Figure 2 shows the binding of PBASE with the MWCNT surface and the resistance value will not change the sensor. The succinimidyl ester will be grouped by another end of the 1-pyrenebutanoic reacted with the secondary and primary amines on the surface of the antibodies for nucleophilic substitution in the occurrence of the DMF solvent. Immobilization is achieved by the amino group of the antibody with the linker to form covalent amide bonds.

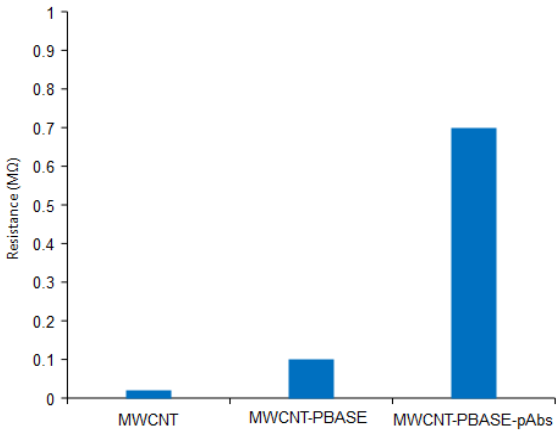

Fig -2: Resistance after the immobilization

3.3 Detection response of MWCNT-based biosensor

Microbial metabolism generally resulted in an increasing type of conductance and capacitance because of reduced impedance. In our analysis, the bacteria bind with antibodies to increase their resistance and can be monitored by the potentiostats. The resulting current flow is increased in the resistance value due to the interaction between the antibody and microorganism [20]. Figure 3 shows the detection values of the sensor with Staph aureus analyzing the fabrication of the individual steps of the biosensor. There is no difference in the resistance values between the buffer solution of PBS and bacterial culture. The biosensor is immobilized with the MWCNT, MWCNT with PBASE, and pAbs. There is a major difference in resistance between the PBS and bacteria after the sensor immobilization with antibody ($P < 0.07$). The resistance will be increased from the specific binding between the antibody and Staph aureus.

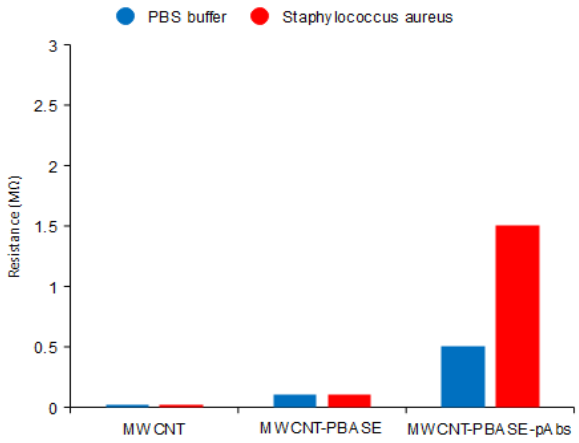

Fig -3: Resistance of MWCNT-based biosensor

3.5 Sensitivity of the biosensor

Sensitivity analysis was shown to define the biosensors with a limit of detection. A Series of 10-fold serial dilutions was prepared from the stock culture using 0.1% of peptone water [21]. The serial dilutions of Staph aureus were analyzed with the sensor and the concentration of Staph aureus cultures from 10^1 to 10^8 CFU/mL was shown in figure 4. The measurement of electrical current showed a linear characteristic among the current (ΔI) and concentration of the aureus suspension within the range of 10^8 CFU. The cell culture is increased and the current becomes decreases. While the current is increased, the increased bacterial is loaded to the MWCNT-FET surface. The occurrence of antibodies will react with the junction to become saturated with the concentration of the antigen with the high changes in the electrical properties of the MWCNT. The detection limit of the proposed sensor is 10^2 CFU/ mL with less detection time of 5 mins.

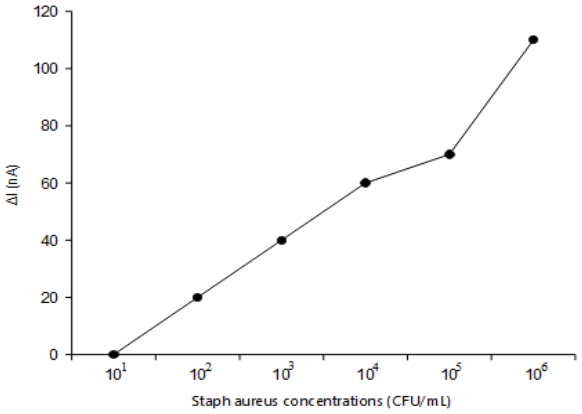

Fig -4: Relationship between the current changes and concentration of Staph aureus

3.6 Specificity of the biosensor

Specificity testing is used to measure the ability of the sensor for the target species. The target analysis sensor was located inside the surface to evaluate the sensor to detect the Staph aureus. The E.coli was applied to the sensor, the value can be measured using the current was 7.29 nA, which can be assigned to the non-specific binding of E.coli on the surface. Figure 5 shows the relative response of the biosensor to the Staph aureus. The highest value of 66.77 nA is measured with staph aureus which shows the functionalization of the MWCNT-FET biosensor. Sterile 0.1% peptone water is used as the negative control. The specificity was carried out by the biosensor towards the concentration of bacteria with E.coli and Staph aureus.

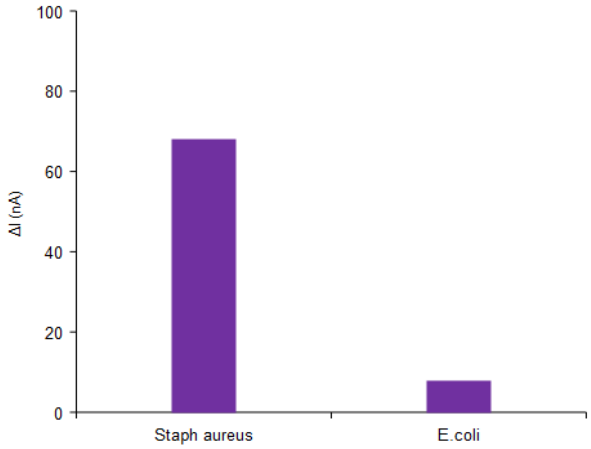

Fig -5: Specificity of the biosensor toward Staph aureus

4. CHARACTERIZATION OF MWCNTS

4.1 XRD analysis of the MWCNT

The chemical composition and structure of the MWCNT were compared with the iron catalyst and figure 6 shows the XRD pattern of the peak value. The samples showed the characteristics Bragg diffraction peak of the MWCNTs at 2θ = 26º. The peak value displays the presence of carbon and also the structure of the powder was analyzed as hexagonal regarding the JCPDS database. The intensity of the peak is decreased with the increasing loading rate of the iron ratio for MWCNTs. The XRD result shows the data about the lattice that is primitive and crystalline. The size of the crystalline is determined by the Scherrer equation [22],

$$Dp = \frac{K\lambda}{\beta \cos\theta} \qquad \text{---------- (3)}$$

Where, D_p = Average Crystallite size, β = Line broadening in Radius, θ = Bragg angle, λ= X-ray wavelength.

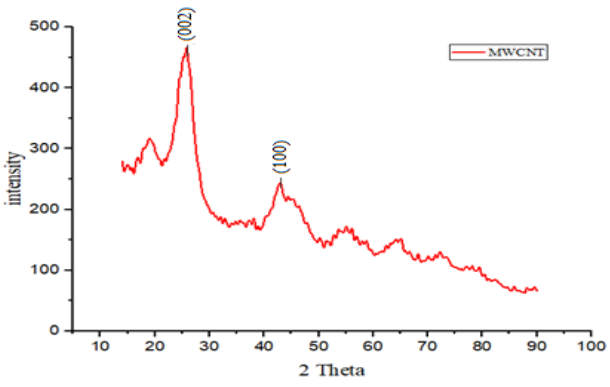

Fig -6: XRD pattern of the MWCNT

4.2 FESEM images of the MWCNT

The MWCNT is synthesized from the Chemical vapor deposition method and is analyzed for its size and morphological structure using a scanning electron microscope. The carbon nanotubes are grown on the silicon substrate with the iron catalyst. The images attained from the FESEM analysis that figure 7 shows the size and type of growth of the CNT. The diameter of the synthesized carbon nanotube was produced (approximately 10-15 nm). The specimen is placed directly in the position in the fabrication process. The growth of the carbon nanotube occurs from the iron catalyst due to high temperatures. It confirms the number of bacteria attached to the CNT was proportional to the concentration of Staphylococcus aureus.

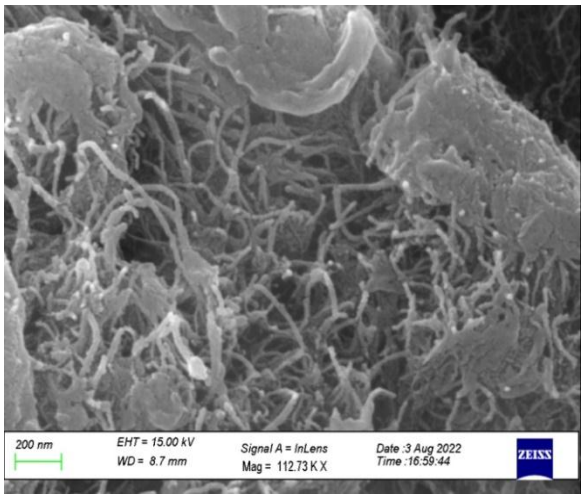

Fig -7: FESEM analysis of the MWCNT

4.3 Fourier Transform Infrared (FT-IR) analysis

FT-IR is mostly used as the qualitative method for the evaluation of functional groups. Figure 8 shows the spectra of the MWCNT from 600 to 3600cm^{-1}. It shows the carbon stability and the hexagonal structure from the MWCNT peak at 1564-1600cm^{-1} illuminating the carbon double bonding (C=C). The peak at 3776 cm^{-1} is determining the –OH stretching of MWCNT. The C=C shows the oxidation of carbon with a remarkable peak of 1696cm^{-1} as the carbonyl of the carboxyl group. The acid solution attacks the carbon double bonding at the decreasing peak at 1564 cm^{-1} and hexagonal carbon at the region 600-1300 cm^{-1}. Functional groups analyzed on the CNT surface were valued by titration.

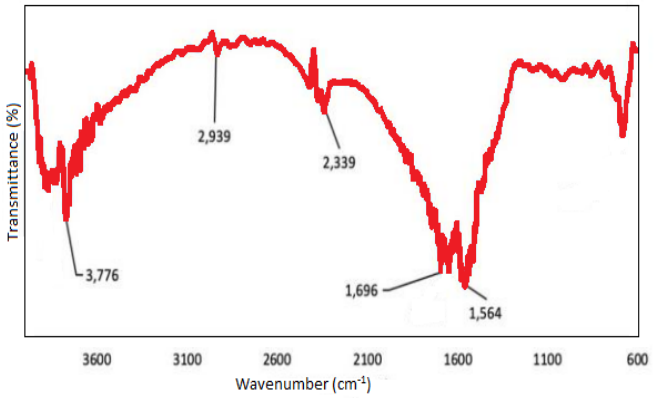

Fig -8: FT-IR analysis of the MWCNT

4.4 SEM Images of Staph aureus cell captured on MWCNT

The biosensor acted with PBS buffer that exhibited in a typical design tangled-shaped MWCNT on the surface. Figure 9 shows the SEM image exposed the aureus caught the external of the CNT-based biosensor.

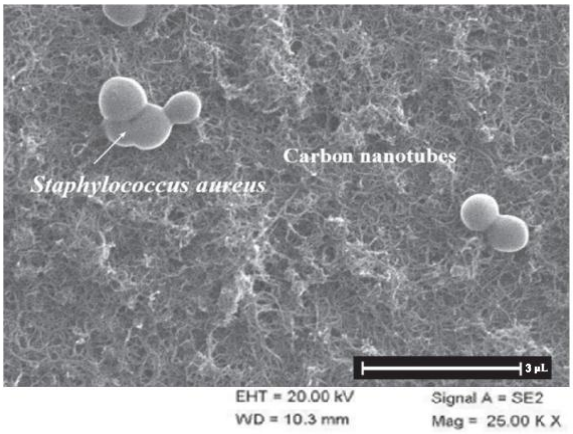

Fig -9: SEM image with antibody immobilized on the biosensor

The SEM image displays a biosensor on MWCNT that will detect the aureus cells that resulted in the resistance values obtained in table 1. The biosensor was attached with PBASE only because it cannot able to capture the cells which exhibited the resistance differences between the PBS buffer and bacterial culture. Table 1 shows the difference between the antibody attached to the PBASE and the resistance in the biosensor ($P < 0.07$). The resistance will increase after the functioning of the biosensor with the antibody for Staph aureus and the resistance will increase because of the binding of the antibody with the bacteria on the SWCNT by the Garcia-Aljaro [17].

Immobilization Step	Resistance difference (ΔR)
PBASE	0.03 ± 0.08
PBASE and pAbs	9.76 ± 0.48

Table -1: Effect of immobilization step on the resistance difference on MWCNT-based biosensor

5. CONCLUSION

This work displays that the MWCNT-based biosensor could be suitable for the recognition of foodborne infections such as S. aureus with a field-effect transistor on MWCNT. The indirect ELISA method shows the anti-S. aureus polyclonal antibodies will target the cells. The gold electrodes fabricated on the silicon wafer were accumulated with multi-walled carbon nanotubes. The assembled MWCNT was determined to be the 1-pyrenebutanoic acid, succinimidyl ester was chosen as a linker to bind between the MWCNT and pAbs. The PBASE and pAbs were immobilized on the transistor and the resistance of the MWCNT will be increased. The captured Staph aureus on the MWCNT will be displayed using an SEM. If the number of bacteria is increased then the resistance decreased. The MWCNT-based biosensor was developed to analyze the Staphylococcus aureus with a low concentration of 10^2 CFU/mL. Therefore, the maximum detection of the biosensor was analyzed to be 2 log CFU/mL.

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REFERENCES

[1]. Liu, Jian, et al. Small 7.4 (2011):425-443.

[2]. Jung, Dae-Hwan, et al. Materials Science and Engineering C 24 (2004):117-121.

[3]. Balasubramanian, Kannan, et al. Small 1.2 (2005): 180-192.

[4]. Xing, Yangchun, et al. Langmuir 21.9 (2005): 4185-4190.

[5]. Sethi, Sanjay, et al. Clinical microbiology reviews 14.2 (2001): 336-363.

[6]. Kang, Seoktae, et al. Langmuir 23.17 (2007): 8670-8673.

[7]. Amiri, Ahmad, et al. Materials Letters 72 (2012): 153-156.

[8]. Kang, Seoktae, et al. Environmental science & technology 42. 19 (2008): 7528-7534.

[9]. Kang, Seoktae, et al. Langmuir 24.13 (2008): 6409-6413.

[10]. Rippers, R.A. edited by V. Lorian, Williams and Wilkins: Baltimore, (1980).

[11]. Villamizar, Raquel A., et al. Biosensors and Bioelectronics 24.2 (2008): 279-283.

[12]. Zhao, Guangying, et al. Analytical biochemistry 408.1 (2011):53-58.

[13]. Dobrzańska - Danikiewicz, A.D., et al. Methods 2 (2014): 3.

[14]. Karthika, Viswanathan, et al. IET Nanobiotechnology 11. 1 (2017):113-118.

[15]. Zhang, Shuqing, et al. Journal of food science 71.3 (2006): M100-M104.

[16]. Lee, Jin Young, et al. Biosensors and Bioelectronics 26.5 (2011): 2685-2688.

[17]. Garcia-Aljaro, Cristina, et al. Biosensors and Bioelectronics 26.4(2010): 1437-1441.

[18]. Park, Mi-Kyung, et al. Sensors, and Actuators B: Chemical 171 (2012): 323-331.

[19]. Ivnitski, Dmitri, et al. Biosensors and bioelectronics 14.7 (1999): 599-624.

[20]. He, Pingang, and Liming Dai. In BioMEMS and biomedical nanotechnology, pp. 171-201. Springer, Boston, MA, 2006.

[21]. Yamada, Kara, et al. PLoS One 9.9 (2014): e105767.

[22]. Arunkumar, T., et al. International Journal of Ambient Energy 41.4 (2020): 452-456.