RESEARCH ARTICLE

DOI: 10.34256/famr2315

Frontiers in Advanced Materials Research

Study of Staphylococcus aureus Adhesion on Surface Modified Silver Coated Non-Woven Polyethylene Fabric subjected to Atmospheric Plasma treatment

Mahalakshmi Sundarapandian [a], Anaswara [b], B. Nandhini [b], B. Suresh [b, *]

[a] Division of Biotechnology, School of Life Sciences (Ooty Campus), JSS Academy of Higher Education and Research, The Nilgiris, Tamil Nadu, India.

[b] Division of Microbiology, School of Life Sciences (Ooty Campus), JSS Academy of Higher Education and Research, The Nilgiris, Tamil Nadu, India.

* Corresponding Author: sureshb@jssuni.edu.in

Received: 19-02-2023, Revised: 12-05-2023, Accepted: 20-05-2023, Published: 30-05-2023

Abstract: This study reveals the adhesion of *Staphylococcus aureus* on the modified surface of silver-coated non-woven disposable polyethylene fabric used in hospitals to cover the patient's bed. The bacteria *Staphylococcus aureus* is responsible for many Nosocomial infections. Therefore, we should take action to reduce the spread of *Staphylococcus aureus*. The present study is focused on a nonwoven polyethylene fabric used as a bedspread that has been plasma-treated and coated in silver to prevent the adhesion of *S. aureus* and its growth. Non-woven polyethylene fabric is plasma-treated for quick silver adherence before being coated with silver and treated with *S. aureus*. Tests for fabric characterization were performed. It includes contact angle, FTIR, and SEM.SEM, FTIR, and contact angle measurements are made on the control, plasma-treated, silver-coated, and *S. aureus* samples. The plasma treatment will cause the fabric to enhance its surface properties. The increased surface roughness will cause the silver to adhere rapidly. The Silver will also prevent the bacteria from multiplying. Silver's antibacterial characteristics, guarantee the destruction of the germs. A bedspread made of nonwoven polyethylene fabric with a silver coating is possible. so that the sufferers can rest comfortably. The number of nosocomial infections spread by the clothing will decline. It is possible to prevent bacterial infections in the patients and medical staff.

Keywords: *S. Aureus*, Nosocomial infections, Plasma treatment, Non-woven Polyethylene fabric

1. Introduction

Any desired alteration in a material's qualities brought on by an organism's essential functions is referred to as biodeterioration [1]. A significant role in microbial adherence and transmission is played by textile surfaces [2-4]. Microorganism kind, surface features, and other environmental conditions (physical and chemical) all play a role in how microbes interact with textiles. The Bacterial species, conditions for development, surface organisation, and age are all factors that affect the zeta potential on their surfaces [5]. On the surfaces of materials, bacterial adhesion and biofilm growth can result in a number of health issues [6]. Microbial attachment on textiles is also affected by the surface of the substance properties such as roughness, chemical composition textile surface tension, and so on. Another parameter is zeta potential that describes the character of the dissociation of a functional group in the case of soft surfaces such as textiles. The surface charge of a fibre is determined by its molecular and supramolecular structure. The surface charge is also affected by the composition and concentration of the adsorbate [7]. Under physiological conditions, bacterial species have a negative charge on their surface [8]. Antiviral qualities can be added to textiles during processing, assisting in the reduction of viral transmission [9]. Antimicrobial compounds have been applied to textiles using a variety of techniques. A practical method to finish products with an antibacterial finish is to use the finishing agent in the dope solution while spinning the textile fibres using a spinneret and coagulation bath [10]. This method creates a finish that is incredibly durable since the finishing chemical turns into an essential component of the fibre itself. Another typical finishing technique that employs water as a medium and necessitates additional drying is exhaustion or impregnation [11]. Additionally, some cutting-edge antimicrobial finishing techniques, such sol-gel, have been documented. However, this technique cannot be used on natural fibres; it can only be used with synthetic or regenerated fibres. A common approach for adding finishes to fabrics made of natural or synthetic fibres is the pad-dry-cure process. Although this is a practical strategy with industrial-scale use, it has a number of important limitations. For instance, padding results in a 70%–100% wet pickup, necessitating the energy-intensive drying procedure to evaporate the water. In addition, as the finishing chemical evaporates, it migrates across the cloth's surface, leaving an uneven finish [12]. Deposition of layers one by one and another method [13,14]. Layer-by-layer deposition is a method for depositing various polymer layers on fabric by gradually dipping the cloth into cationic and anionic solutions of polymers [13].

Laboratory plasma treatment can be used to demonstrate how it alters the surface, chemical, and physical properties of fibres. Future progress and various strategies are applied to solve plasma scaling problems [15]. Low pressure plasma techniques have been utilised to modify the surfaces of textiles and polymers. Low pressure plasma has advantages such as homogeneous glow, low breakdown voltage, high concentration of reactive species, and non-thermal plasma formation.

The present study aims to determine the determination of the inhibitory concentration of silver on Staphylococcus aureus, the Effect of plasma treatment on surface modification of the fabric and to study the adhesion property of S.Aureus on non-woven polyethylene coated with silver.

2. Materials and Method

Staphylococcus aureus pure culture collected from EMS Hospital Perambra, Kozhikode, was used for this work.

2.1. Tests for identification of Staphylococcus aureus

2.1.1. Gram Staining

1. Preparation of bacterial thin smear
2. Air dry and heat fixing
3. Apply a primary stain (crystal violet).
4. Add a mordant (Gram's iodine).
5. Rapid decolorization with ethanol
6. Counterstaining by safranin.
7. Observe the result under oil immersion, using a microscope.

2.1.2. Cultural characteristics tests

a) Nutrient Agar (NA)

b) MacConkey agar

c) Blood agar

Prepare the nutrient agar media, MacConkey agar media, and Blood agar media, and then the quadrant streaking of the bacterial culture and incubate at 37°C for 24 hours.

2.1.3. Coagulase test

a) Slide coagulase test

The slide test is performed by preparing a suspension of bacterial cells mixed into a drop of plasma on 3 slides. If bound coagulase is present in the bacterial cells, then the presence of plasma will cause the bacterial cells to clump.

b) Tube coagulase test

The tube coagulase test is performed by mixing bacterial cells into a larger volume of plasma in a small test tube. As the bacteria multiply in the plasma, they secrete staphylocoagulase. Staphylocoagulase initiates blood coagulation by activating prothrombin.

2.2. Fabric Treatments

The non-woven polyethylene fabric is cut into 15 X 2.5 cm measurements.

2.2.1. Plasma treatment

The CAP plasma treatment was accomplished by atmospheric pressure AC excited dielectric barrier discharge plasma reactor as shown in Fig. 1 which consists of a square type of plasma chamber with the dimension of 40 cm L X 40 cm B X 20 cm H. Two parallel electrodes with dimensions of 30 cm X 30 cm were placed within the plasma chamber. Moreover, a polypropylene sheet of 3 mm was fixed on the inner surface of the two electrodes which act as a dielectric layer to avoid arcing and passage of high current. The distance between the electrodes was fixed at 6 mm during the plasma processing. The plasma was generated between the two electrodes using a high-voltage AC power supply (V_{max} = 40 kV, I_{max} = 40 mA, and f= 50 Hz). The upper electrode is a live electrode, and the lower electrode is grounded. The sample was kept on the lower electrode. In this reactor, the active plasma zone displays a symmetrically square. The three electronic mass flow controllers (MFCs) are used to control the flow of processing gas during the surface treatment. In the beginning, the ultrasonically cleaned fabric was placed on the surface of the ground electrode, and the chamber was closed carefully. After that, plasma processing gas was filled between two electrodes which were controlled by gas flow controllers. An AC voltage was applied between two electrodes and the same was adjusted till a stable glow discharge was produced. Ultimately the samples were treated in uniform atmospheric pressure glow discharge plasma as a function of various operating parameters.

2.2.2. Preparation of $AgNO_3$ and impregnation of $AgNO_3$

$AgNO_3$ (2.0g) and $Na_3C_6H_5O_7$ (0.64g) were dissolved in deionized water (1.0mL) and 6.0mL, respectively. Following that, under the circumstances of magnetic stirring at room temperature, silica spheres (0.2 g), $AgNO_3$ solution, and $Na_3C_6H_5O_7$ solution were added to the mixture of deionized water (25 mL) and ethanol (25 mL). After being gathered and purified by repeated centrifugation in deionized water and 100% ethanol numerous times, the NPs were dried in a vacuum oven at 60 degrees Celsius two and a half hours later.

2.2.3. Inoculation of *S. aureus* in Lb broth

First, prepare LB broth. Then Inoculate with the *Staphylococcus aureus* and kept for incubation at 37°C for 24 hours. The next day, add the $AgNO_3$ dipped and dried samples into the peptone broth. Mixed thoroughly.

2.2.4. Drying

Kept for drying in room temperature.

2.2.5. Study of antimicrobial activity of Ag-coated fabric in comparison with,

a) Ampicillin **b)** Gentamycin and **c)** Penicillin

- 3 Petri dishes of Muller Hinton Agar media were prepared for the antimicrobial activity of Ag-coated fabric.
- 0.1M, 0.2 M, 0.4 M, and 1 M $AgNO_3$ solutions were prepared.
- The untreated fabric samples were cut into small round pieces. And the samples were dipped into the different molar $AgNO_3$ solutions.
- Swab the *Staphylococcus aureus* into the 3 Petri dishes of Muller Hinton agar media.
- In the 3 Petri dishes, put the 4 different molar concentrations of $AgNO_3$ dipped samples, and put Ampicillin, Gentamycin, and Penicillin in each Petri dish. And incubate at 37°C for 24 hours.

2.3. Fabric Characterization tests

Sent samples for 3 tests. The samples are C (Control), P (Plasma treated), S (Plasma treated and $AgNO_3$ dipped and treated with *Staphylococcus aureus*.

2.3.1. SEM

Nearly 1012 CFU ml^{-1} of bacteria were suspended in 0.01 M PBS and incubated with fabric samples for 18 hours while being stirred. Samples were rinsed in buffer after incubation to get rid of non-adherent cells. The fabrics were then overnight fixed in 2% glutaraldehyde at 4°C, following two buffer rinses. By passing through a graded sequence of ethanol/water solutions at 30% (2X5 min), 50% (2X10 min), 70% (2X15 min), 90% (2X20 min), and 100% (2X30 min), samples were further dehydrated. Prior to being examined with a Jeol JSM 6100, the samples were air-dried and then gold was sputter coated on them (Jeol JFC 1100).

2.3.2. FTIR

In a Perkin Elmer hydraulic press, the samples were compressed into KBr pellets, and a Perkin Elmer spectrum RX/FT-IR system was employed to record the spectra of each sample.

2.3.3. Contact angle

Understanding drop motion on a surface requires an understanding of the contact angle hysteresis parameter. Hysteresis is caused by a variety of factors including surface roughness, pollution, chemical heterogeneity, drop size, molecule orientation and deformation, and liquid molecular transit.

3. Results and Discussion

3.1. Gram staining

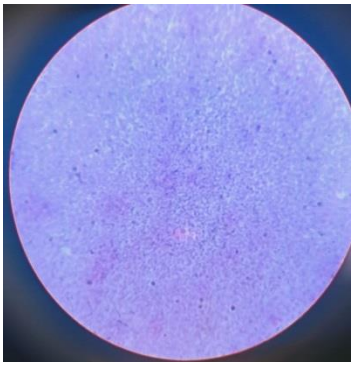

Figure1. Gram staining

The bacteria are seen by round shaped purple colour (Gram positive cocci) arranged in clusters.

3.2. Cultural characteristics test

Figure 2. Nutrient agar

Figure 3. MacConkey agar

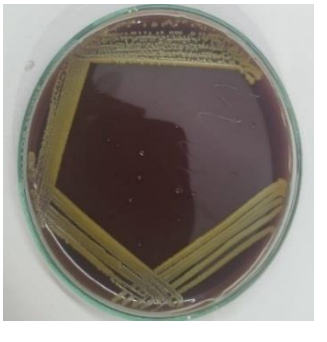

Figure 4. Blood agar

a) Nutrient agar media

Large, Circular, Smooth, Shiny surface and are pigmented (golden-yellow)

b) MacConkey agar media

Small, Pale pink to red colour colonies.

c) Blood Agar Media

Colonies of Staphylococcus aureus are frequently surrounded by zones of clear beta-hemolysis. Golden color colonies.

3.3. Coagulase test

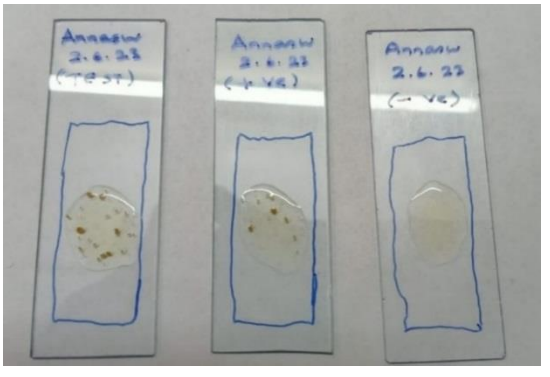

Figure 5. Slide test

Figure 6. Tube test

a) Slide coagulase test: The formation of clumps within 10-15 seconds is the positive result.

b) Tube coagulase test: Positive result is clotting. It will become a solid clot.

3.4. Plasma treatment

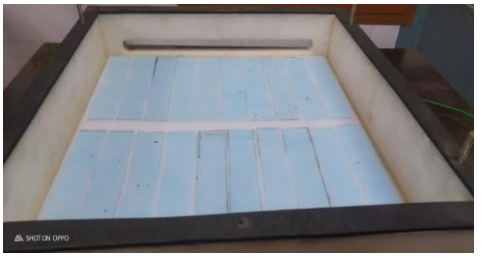

Figure 7, 8. Plasma treatment

After the plasma treatment, the fabric became rougher. The roughness of the fabric will help to the adhesion of the silver particles.

3.5. Preparation of AgNO3 and impregnation of $AgNO_3$

Figure 9. $AgNO_3$ dipped fabric

Figure 10. $AgNO_3$ dipped fabric kept for drying

3.6. Inoculation of S. aureus in Lb broth

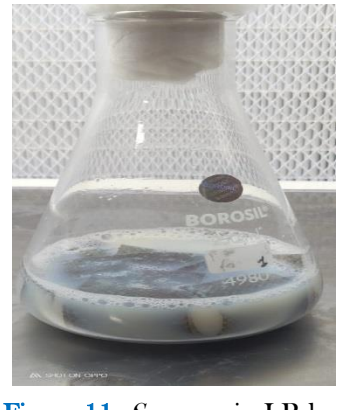

Figure 11. *S.aureus* in LB broth

3.7. Study of antimicrobial activity of Ag coated fabric

Figure 12. Ampicillin

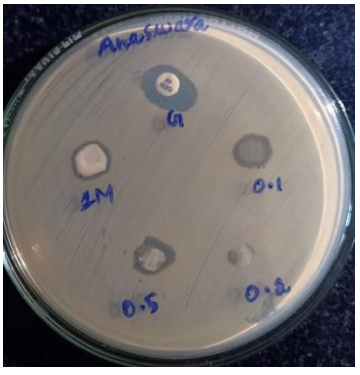

Figure 13. Gentamycin

Figure 14. Penicillin

Table 1. Standard chart

	Standard chart		
	S	I	R
Penicillin (10mg)	29	_	28
Gentamycin(10mg)	18	_	18
Ampicillin(10mg)	29	_	28

Table 2. Antimicrobial activity test

S. aureus Zone of inhibition (mm in diameter)				
	0.1 M	0.2 M	0.5 M	1.0 M
Penicillin (31)	*10*	*11*	*11*	14
Gentamycin (20)	*12*	*10*	*13*	*15*
Ampicillin(27)	*11*	*12*	*14*	*15*

3.8. SEM

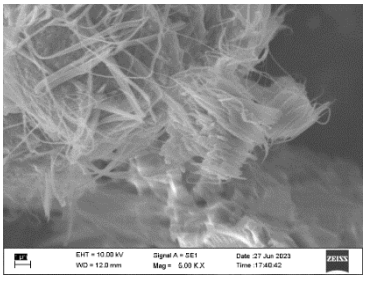

Figure 15. Control

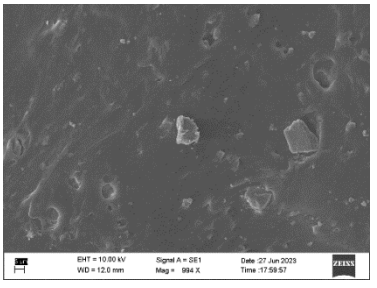

Figure 16. Plasma treated

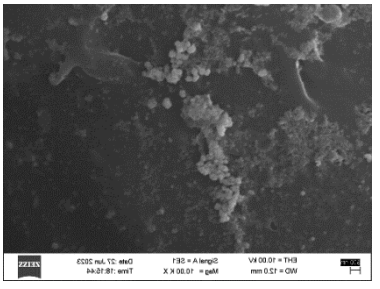

Figure 17. Silver coated+*S.aureus*

Staphylococcus aureus cells linked to the non-woven polyethylene fabric well, according to findings made under scanning electron microscopy (fig. 17). It appears to be a globular clustered structure. During adhesion, the fabric's surface shape also has a significant impact. The initial image (fig. 15) does not show any treatment. Only control exists. in order to just produce an image of the fabric's structure. Figure 16 in the second image shows a plasma-treated image. It reveals the fabric's pores. The fabric was given a plasma treatment that made it rougher than it was before the structure was altered. The fabric's rough surface made it easier for bacteria to adhere to it. That is, bacterial adhesion is promoted by rough surfaces. But the presence of silver, the bacteria were destroyed and could be used effectively to control the spread of bacteria through the fabric.

3.9. FTIR

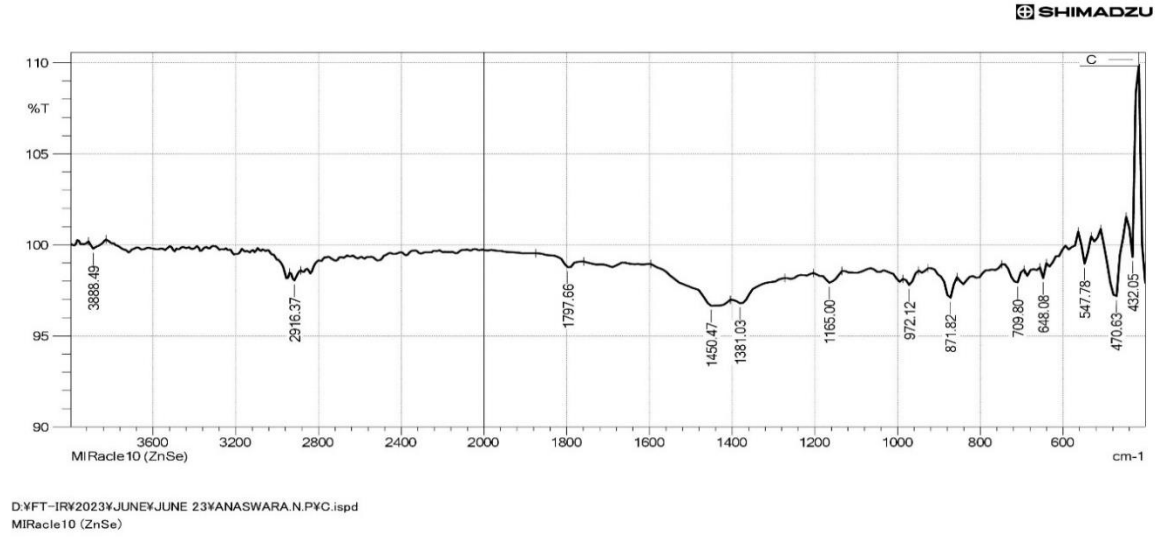

Figure 18. Control

Using FTIR spectra helps in understanding the adherence mechanism as the technique identifies the functional groups involved during the interaction between the fabric and the bacteria. The FTIR spectrum of non-woven polyethylene fabric (Fig.18) shows a broad and intense peak at 1450.47 cm^-1, indicating the presence of O-H stretching vibration. The small peaks centred at 709.80 cm^-1 correspond to the carbonyl of amide-I. This is due to the presence of an indigenous protein of the non-woven polyethylene, which might support bacterial growth for adherence. The small but sharp peak at 470.63 cm^-1 corresponds to a C-C stretch in a ring.

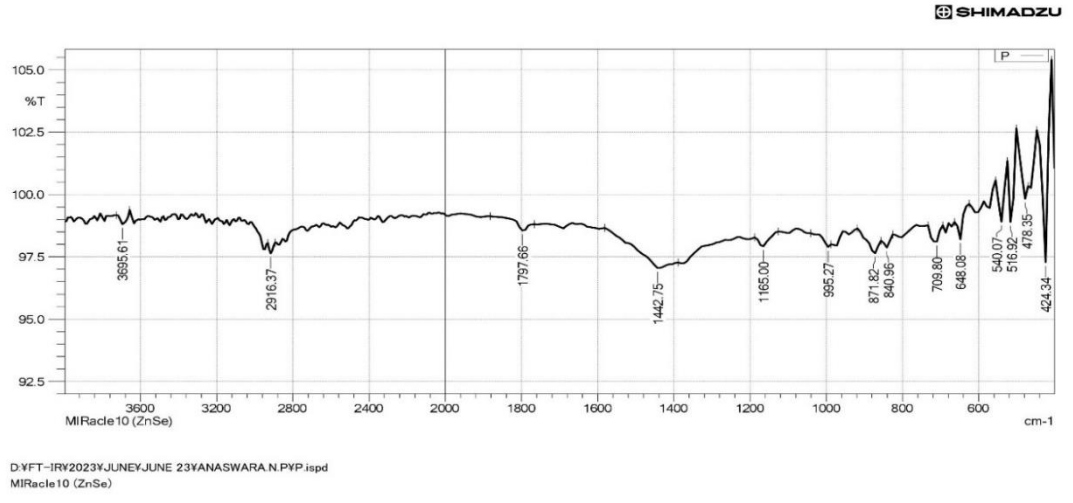

Figure 19. Plasma treated

The FTIR spectrum of this sample (Fig.19) shows the peak at 1442.75 cm^-1, indicating the presence of O-H stretching vibration. The presence of carbonyl of amide-I is at the peak of 709.80 cm^-1.

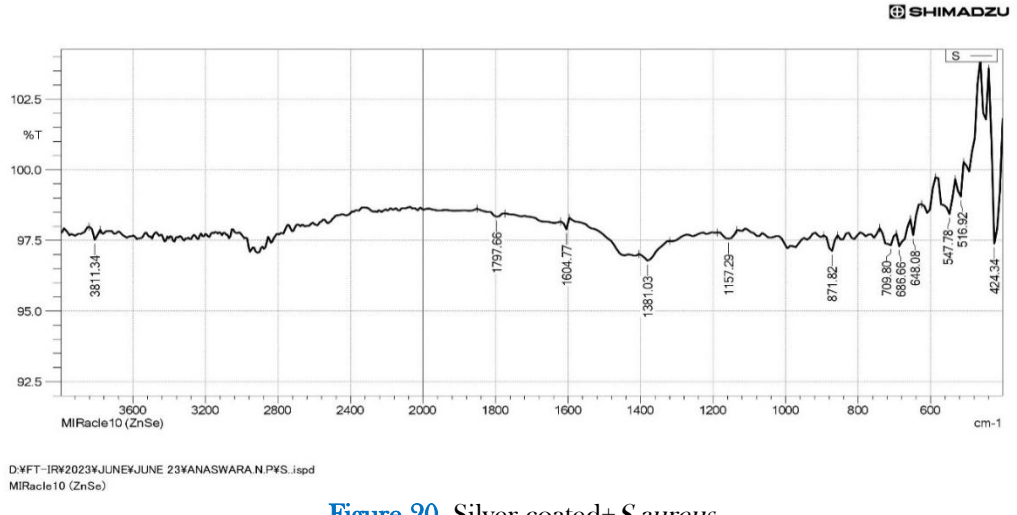

Figure 20. Silver coated+*S.aureus*

In this (Fig.20) shows the peak at 1381.03 cm^-1, indicates the presence of O-H group. The small peaks centered at 1157.29 cm^-1 corresponds to carbonyl of amide-I. This indicates the presence of indigenous protein of the non-woven polyethylene fabric, which might support bacterial growth of attachment. The small and sharp peak at 871.82 cm^-1 corresponds to C-C stretch in a ring.

3.10. Contact Angle

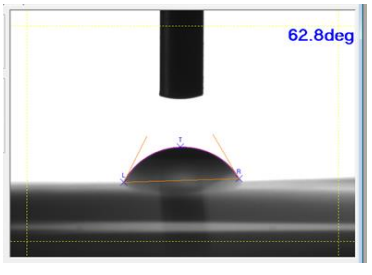

Figure 21. Control

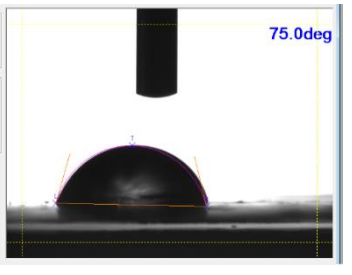

Figure 22. Plasma treated

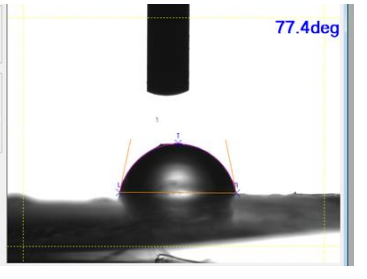

Figure 23. Silver coated+*S.aureus*

When the contact angle is 0°, the droplet spreads completely on the solid surface and this is referred to as complete wetting. When the contact angle is 180 ° the droplet stands on the surface and it is named as non-wetting. When water is used as the liquid, the surface is hydrophilic when the contact angle is less than 90° Superhydrophilic surfaces are those with contact angles for water less than 5° On the other hand, a surface is hydrophobic when the contact angle for water is above 90° and superhydrophobic when it is more than 150°.

In the images (Fig.19,20,21), the contact angle is less than 90° So that the fabric surface is hydrophilic. So that, it will help in the fast adhesion of *silver particles.*

4. Summary and Conclusion

Using different fabric treatments, we quantified the bacterial adhesion on non-woven polyethylene fabric in this study. the verification of Staphylococcus aureus adhesion to the fabric using SEM pictures and FTIR analyses. SEM pictures showed that adhesion was reliant on the surface shape of the fabric and that adhering bacterial cells did not evenly cover the entire fabric surface. Studies using FTIR further supported the finding that fabric hydrophilicity/hydrophobicity also plays a significant effect in the adhesion of silver, in turn, will kill the bacteria. The silver coating of one molar concentration is effective in destroying the bacteria. The fabric's improved hydrophilicity was revealed by the contact angle test. It is important to keep in mind that this research has discovered a straightforward method for calculating the amount of bacterial adhesion to materials. This method may be applied to various

bacterial strains and types of fabric to quantify the adhesion of bacterial cells. It would be very helpful in the development and evaluation of different antimicrobial finished textile products.

References

[1] F.H. Paul, Talking rot and mildew, Textiles, North West fungus Group, (1999) 46-50.

[2] V. Bajpai, A. Dey, S. Ghosh, S. Bajpai, M.K. Jha, Quantification of bacterial adherence on different textile fabrics, International Biodeterioration & Biodegradation, 65(8), (2011) 1169-1174. https://doi.org/10.1016/j.ibiod.2011.04.012

[3] A. Banu, M. Anand, N. Nagi, White coats as a vehicle for bacterial dissemination, Journal of Clinical and Diagnostic Research, 6(2012) 1381-1384. https://doi.org/10.7860/jcdr/2012/4286.2364

[4] P. Gupta, N. Bairagi, R. Priyadarshini, A. Singh, D. Chauhan, D. Gupta, Bacterial Contamination of nurses` white coats made from polyester and blend fabric, Journal of Hospital Infection, 94(1), (2016) 92-94. https://doi.org/10.1016/j.jhin.2016.05.016

[5] J.K. Oh, Y. Yegin, F. Yang, M. Zhang, J. Li, S. Huang, S.V. Verkhoturov, E.A. Schweikert, K. Perez-Lewis, E.A. Scholar, T.M.Taylor, A. Castillo, L. Cisneros-Zevallos, Y. Min, M. Akbulut, The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion, Scientific Reports, 8 (2018) 17247. https://doi.org/10.1038/s41598-018-35343-1

[6] D. Lebeaux, J.M. Ghigo, C. Beloin Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics, Microbiology and Molecular Biology Reviews, 78(3), (2014) 510-543. https://doi.org/10.1128/mmbr.00013-14

[7] A.M. Grancari`c, N. Ristic`, A. Tarbuk, I. Ristic`, Electrokinetic phenomena of Cationised cotton and its dyeability with reactive dyes, FIBRES & TEXTILES in Eastern Europe, 6(102) (2013) 106-110.

[8] J. Wu, H. Liu, P. Wang, D. Zhang, Y. Sun, E. Li, Oxygen reduction reaction affected by sulfate – reducing bacteria: different roles of bacterial cells and metabolites, Indian Journal of Microbiology, 57(2017) 344-350. https://doi.org/10.1007/s12088-017-0667-z

[9] Z.U. Iyigundogdu, O. Demir, A.B. Asutay, F. Sahin, Developing novel antimicrobial and antiviral textile products, Applied Biochemistry and Biotechnology, 181(3), (2017) 1155-1166. https://doi.org/10.1007/s12010-016-2275-5

[10] W.K. Son, J.H. Youk, T.S. Lee, W.H. Park, Preparation of antimicrobial ultrafine cellulose acetate fibers with silver nanoparticles, Macromolecular Rapid Communications, 25(18), (2004) 163-167. https://doi.org/10.1002/marc.200400323

[11] S. Anita, T. Ramachandran, R. Rajendran, C.V. Koushik, M. Mahalakshmi, Study of the antimicrobial property of encapsulated copper oxide nanoparticles on cotton fabric, Textile Research Journal, 81(10), (2011) 1081-1088. https://doi.org/10.1177/0040517510397577

[12] R. Nayak, R. Padhye, Antimicrobial finishes for textiles. In: Paul R, editor. Functional finishes for textiles: improving comfort, performance and protection, Amsterdam Woodhead publishing Ltd. (2014) 361-85.

[13] I. Cerkez, H.B. Kocer, S.D. Worley, R.M. Broughton, T.S. Huang, N-Halamine biocidal coatings via a layer-by-layer assembly technique, Langmuir, 27(7), (2011) 4091-4097. https://doi.org/10.1021/la104923x

[14] Y. Xing, X. Yang, J. Dai, Antimicrobial finishing of cotton textile based on water glass by Sol-gel method, Journal of Sol-Gel Science and Technology, 43(2), (2007) 187-192. https://doi.org/10.1007/s10971-007-1575-1

[15] C.M. Chan, T.-M. Ko, H. Hiraoka, Polymer Surface Modification by Plasmas and Photons, Surface and Interface Analysis, 24(1-2) (1996) 1-54. https://doi.org/10.1016/0167-5729(96)80003-3

Acknowledgements

The Authors like to acknowledge The Members of the Management and Dr. K. Navaneetha Pandiyaraj, Assistant Professor, Department of Physics, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Sri Ramakrishna Vidyalaya Post, Periyanaickenpalayam, Coimbatore for providing the support through Plasma Treatment Facility for this study.

Conflict of interest: The Authors has no conflicts of interest to declare that they are relevant to the content of this article.