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Article

Antibacterial Activity of Superhydrophobic-SiO₂ Coatings to Inhibit the Growth of *Escherichia coli* and *Staphylococcus aureus*

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Abstract: The emergence of superhydrophobicity-based antibacterial materials represents a promising approach to upholding surface cleanliness and hygiene, effectively preventing bacterial adhesion. This research describes the synthesis of a superhydrophobic coating with antibacterial and bacteriostatic properties using silica nanoparticles (SiO₂ NPs) modified with 1H,1H,2H,2H-Perfluorodecyltriethoxysilane (PFDTES). TEM, XRD, EDS, SEM, and FTIR analyses were conducted to study its morphology and surface characterization. Additionally, the coating was applied on glass substrates using the spray coating method, varying the number of layers to evaluate antibacterial and bacteriostatic properties through turbidimetry and inhibition halo measurement techniques. The durability of the coatings was also assessed by exposing them to outdoor conditions for 35 days. This study aimed to evaluate the antibacterial capacity of the superhydrophobic coating and its weather resistance. The results demonstrate that a superhydrophobic coating with a contact angle $\geq 150^\circ$ and a sliding angle $\leq 10^\circ$ was successfully synthesized using SiO₂ NPs smaller than 10 nm modified with PFDTES. The coating exhibited the ability to inhibit bacterial growth by preventing the adhesion of bacteria such as *Escherichia coli* and *Staphylococcus aureus*; moreover, the number of layers correlated with enhanced antibacterial properties. The coating also showed good durability under outdoor conditions. These results support the potential application of superhydrophobic coatings to prevent these bacteria from adhering and growing in areas where they can be harmful.

Keywords: SiO₂ NPs; superhydrophobic coatings; SH-SiO₂ coating; PFDTES modification; antibacterial and bacteriostatic evaluation; biofilm prevention; nanotechnology; growth inhibition

1. Introduction

The research and development of bacteria-repellent coatings have emerged as a priority to enhance safety in hospital environments.[1] Microbial fouling can lead to nosocomial infections in patients when bacterial biofilms adhere to medical devices, representing a cause of death among patients [2–4]. *Escherichia coli* and *Staphylococcus aureus* are the most common microorganisms in hospital-acquired infections [5]. These bacteria pose significant challenges due to their resistance to antimicrobial treatments and ability to persist on surfaces for varying periods, increasing the risk of transmission [6–8].

Preventing bacterial adhesion is crucial to avoid infections and ensure safety in clinical settings. While biocides are designed to prevent or control the proliferation of such bacteria, there is a growing concern that their increasingly frequent use may lead to higher bacterial resistance and cross-resistance to clinically important antibiotics, resulting in significant complications [9,10]. In this context, developing antibacterial materials based on superhydrophobicity emerges as a promising

strategy for maintaining clean and hygienic surfaces and preventing bacterial adhesion [1,3,5,11,12]. Research on how *Escherichia coli* and *Staphylococcus aureus* interact with superhydrophobic surfaces is essential for developing effective strategies to prevent device-associated infections in healthcare settings [13–15].

Superhydrophobic surfaces, characterized by water contact angles (WCA) greater than 150° and sliding angles (WSA) less than 10° , repel water and facilitate a self-cleaning function by removing debris and pathogens [15,16]. Various methods and treatments can be employed to achieve this property, including the design of coatings with layers of material that provide this specific function [17]. Consequently, these superhydrophobic coatings aim to prevent bacterial adhesion, inhibit bacterial growth, and allow for the easy removal of contaminants from surfaces [1].

Superhydrophobic coatings are based on compounds with low surface energy [18] and high roughness. Superhydrophobic coatings are based on compounds with low surface energy [19]. A promising option for obtaining these coatings is the synthesis of modified SiO_2 NPs [20–22] and their application via the spray coating method [23].

SiO_2 NPs are described as a potent group of nanocarriers that have shown antimicrobial properties and drug-delivery capabilities [24]. Due to their free silanol groups, which allow for modifications, and their porous nature, which enables higher drug loading, they are positioned as a viable alternative for infection treatment. Additionally, the roughness of these nanoparticles can significantly influence their ability to inactivate bacteria, with greater roughness being associated with higher antibacterial effectiveness [25].

Moreover, the durability and stability of superhydrophobic coatings are crucial under various environmental conditions, including outdoor exposure. Studies have shown that factors such as layer thickness, particle size, and the nature of functional groups play a critical role in determining these properties [26]. Furthermore, recent research indicates that SiO_2 NPs modified with PFDTES are highly resistant to water abrasion and other environmental factors [27].

Our research focuses on developing a superhydrophobic coating with antibacterial properties for surfaces using SiO_2 NPs smaller than 10 nm modified with PFDTES. To this end, the antibacterial activity was evaluated by applying the coating on glass substrates using the spray coating method, varying the number of layers, and utilizing turbidimetry and inhibition halo measurement techniques against *Escherichia coli* and *Staphylococcus aureus* bacteria. Additionally, the durability of the coating was analyzed by exposing it to outdoor conditions for 35 days, and its morphology, composition, and hydrophobic properties were characterized. These findings confirm its effectiveness in preventing bacterial adhesion and growth on surfaces, making it a promising strategy for areas where these bacteria pose a risk.

2. Methodology

2.1. Materials

To synthesize SiO_2 nanoparticles (NPs), tetraethyl orthosilicate (TEOS, $\text{Si}(\text{OC}_2\text{H}_5)_4$, 98%, Sigma-Aldrich) was utilized as the silica precursor. Ammonium hydroxide (NH_4OH , 28–30% NH_3 , Sigma-Aldrich) was the catalyst agent. Isopropyl alcohol ($\text{C}_3\text{H}_8\text{O}$, CEDROSA) and deionized water (18 $\text{M}\Omega\cdot\text{cm}$) were employed as solvents for the hydrolysis process. For the modification of SiO_2 NPs and the preparation of the superhydrophobic coating, 1H,1H,2H,2H-Perfluorodecyltriethoxysilane (PFDTES, $\text{C}_{16}\text{H}_{19}\text{F}_{17}\text{O}_3\text{Si}$, 97%, Matrix Scientific) was used along with hexane (C_6H_{14} , 95%, J.T. Baker) as the solvent. Glass substrates used for the deposition and analysis of the coating were obtained from Fisher Scientific.

2.2. Equipment

The synthesis and characterization of the superhydrophobic coatings were conducted using the following equipment: a laboratory stirrer (PC 410, Corning®) for mixing solutions and a water purification system (Water-Pro PS, Labconco®) for obtaining deionized water. Fourier-transform infrared spectroscopy (FTIR) analysis was performed using an infrared spectrometer (NICOLET IS50

FT-IR, Thermo Scientific). X-ray diffraction (XRD) analysis used a diffractometer (X'Pert Pro, Panalytical). Transmission electron microscopy (TEM) characterization was conducted using a transmission electron microscope (JEM-2010, JEOL) with samples deposited on carbon-coated copper grids from Ted Pella Inc. Finally, scanning electron microscopy (SEM) was performed using a scanning electron microscope (JSM-7401, JEOL).

2.3. Synthesis and Characterization

The synthesis of SiO₂ nanoparticles (NPs) was performed using the method proposed by Stöber.[28] To prepare a 100 ml solution of SiO₂ NPs, 95 ml of isopropyl alcohol was mixed at a stirring speed of 300 rpm. Subsequently, 3.65 ml of deionized water, 350 µl of NH₄OH, and 1 ml of TEOS were added. The mixture was maintained at 40°C under continuous stirring for 24 hours. Following this period, 25 ml of hexane was introduced into the solution, the temperature was increased to 60°C, and 500 µl of PFDTES was added. The solution was stirred at 300 rpm for 48 hours, yielding 125 ml of superhydrophobic coating.

For the coating application, 1, 3, 5, and 7 layers of the superhydrophobic coating were deposited onto glass substrates using the spray coating technique. This process was conducted with an air pressure of 30 psi, maintaining a distance of 15 cm between the nozzle and the substrate. The samples were then exposed to outdoor conditions for 35 days to evaluate changes in surface superhydrophobicity. The coating's water contact angle (WCA) and the water sliding angle (WSA) were characterized using custom-built laboratory equipment at room temperature. Deionized water droplets of 4 µl were used for the measurements, and the contact angle was measured five times at different positions on each sample to obtain an average value.

For FTIR and XRD characterization, the solutions were evaporated to obtain the SiO₂ NPs and the superhydrophobic coating in powder form. These powders were then deposited onto glass slides for subsequent analysis. For SEM and EDS analysis, glass substrates with varying numbers of layers were examined. For TEM analysis, the colloidal samples were deposited onto carbon-coated copper grids.

2.4. Antibacterial and Bacteriostatic Study

The methodology used to conduct the antibacterial and bacteriostatic study of the superhydrophobic coating involved depositing 1, 3, 5, and 7 layers of the coating onto glass substrates using the spray coating method.

For the antibacterial test, the turbidimetry technique was employed using 1:1 solutions (culture broth-phosphate buffer solution) with *Escherichia coli* (ATCC® 11229™) and *Staphylococcus aureus* (ATCC® 6538™). The tests were conducted by adding the bacterial solution to tubes containing the coated samples, which were then incubated for 24 hours at 37°C in a Fisher Scientific Low-Temperature Incubator. After the incubation period, the solutions were measured using UV-visible light spectroscopy (Genesys2, Thermo Spectronic) at a wavelength of 450 nm. Negative controls (bacterial solution without sample) and positive controls (buffer solution with sample) were included, and the analysis was performed in triplicate.

For the bacteriostatic characterization, all samples were sterilized using UV light within a laminar flow hood (Labconco) for 15 minutes on each side before testing. In a sterile environment, standard solutions of approximately 1.5×10^8 CFU/ml of *Escherichia coli* (ATCC® 11229™) and *Staphylococcus aureus* (ATCC® 6538™) were prepared. These solutions were then separately plated on Petri dishes containing nutrient agar (Difco 21300). The coated samples were placed on the inoculated agar, and the Petri dishes were incubated at 37°C for 24 and 48 hours to observe the inhibition halos.

3. Results

3.1. WCA

The manufactured coating exhibits water contact angles (WCA) $\geq 150^\circ$ and water sliding angles (WSA) $\leq 10^\circ$. Figure 1 presents photographs of water droplets deposited on glass substrates with

varying numbers of coating layers. Table 1 provides the contact and sliding angle values measured initially and after 35 days of exposure to outdoor conditions.

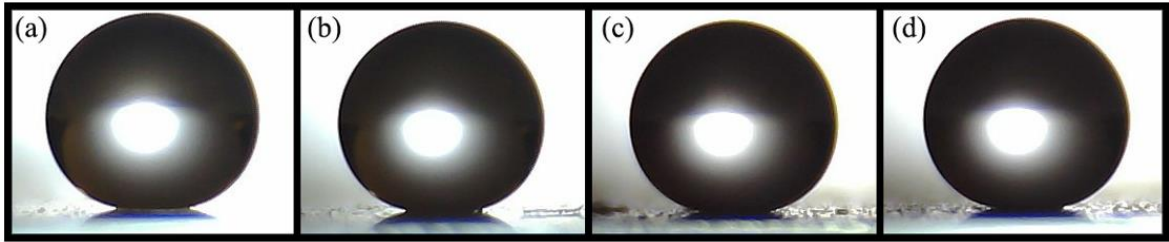


Figure 1. Images of water droplets on glass substrates with varying numbers of superhydrophobic coating layers: (a) 1 layer, (b) 3 layers, (c) 5 layers, and (d) 7 layers.

Table 1. WCA and WSA values of superhydrophobic coatings with varying layers were measured initially and after 35 days of outdoor exposure.

Layers	WCA	WSA
1	156.72° ± 1.01°	2.64° ± 0.55°
3	157.01° ± 1.76°	2.01° ± 0.15°
5	157.49° ± 1.07°	2.21° ± 0.17°
7	159.92° ± 1.03°	2.13° ± 0.61°
After 35 days of outdoor exposure		
Layers	WCA	WSA
1	159.78° ± 1.07°	2.91° ± 0.10°
3	160.50° ± 1.17°	2.27° ± 0.29°
5	157.60° ± 1.05°	2.17° ± 0.94°
7	160.75° ± 1.01°	2.94° ± 0.71°

Initially, as the number of layers increases, the WCA also increases. At the same time, the WSA remains below 3°, demonstrating the necessary characteristics for superhydrophobicity. After 35 days, the coating retains contact angles $\geq 150^\circ$ and sliding angles $\leq 10^\circ$. Although slight changes are observed in both angles, the coating maintains its superhydrophobic properties.

3.2. SEM

Figures 2 and 3 show the morphology of the coatings on glass substrates with varying numbers of layers. Scanning electron microscopy observations in both figures reveal surfaces with a hierarchical structure composed of nano- and microscale particles with diameters ranging from 733.5 ± 116.9 nm to 20.046 ± 3.69 nm. This multiscale roughness is advantageous for superhydrophobic coatings, enhancing superhydrophobicity, durability, and self-cleaning properties [19].

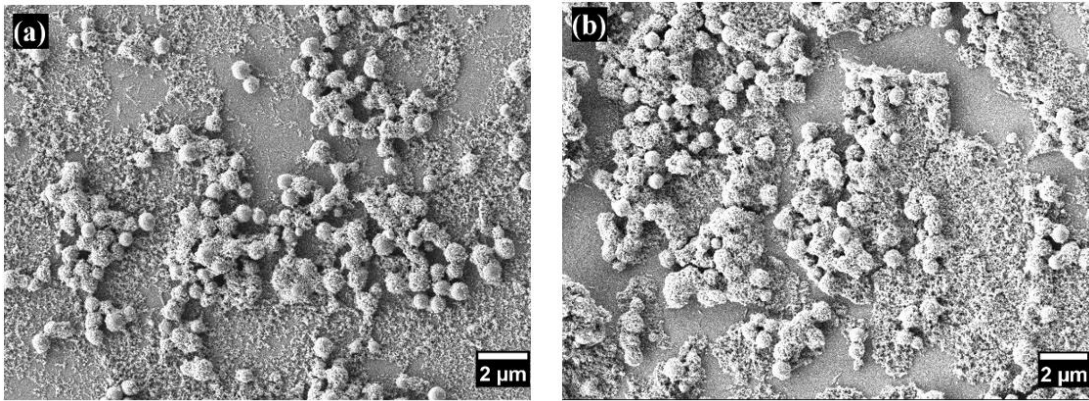


Figure 2. SEM images of superhydrophobic coatings with different numbers of layers: (a) one layer and (b) seven layers.

Figure 3 provides a detailed view of the increased porosity corresponding to the increment in coating layers. This enhanced porosity allows for more effective air trapping beneath water droplets, significantly contributing to the surface's superhydrophobic properties.

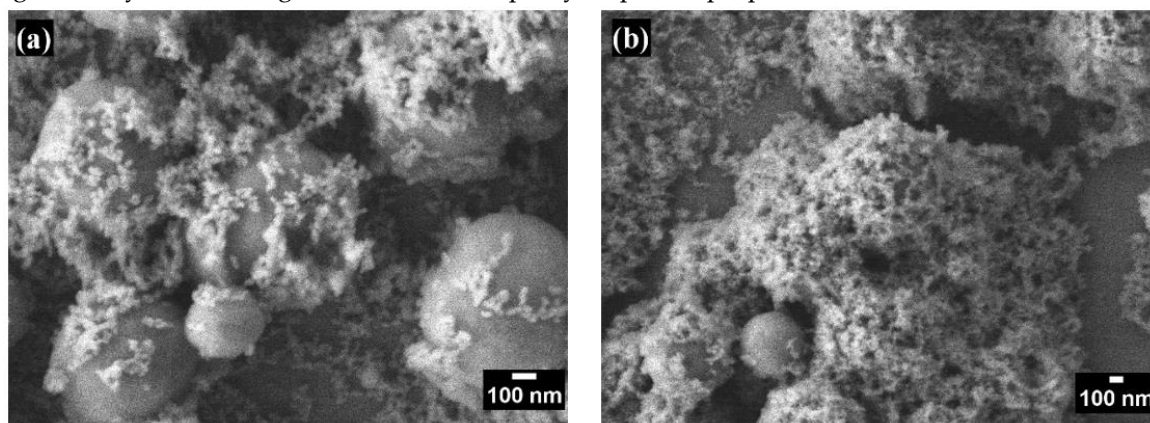


Figure 3. High-magnification SEM images of superhydrophobic coatings with different numbers of layers: (a) one layer and (b) seven layers.

3.3. TEM

TEM images of the colloids are presented in Figure 4. Image (a) shows bare SiO₂ NPs, which are spherical, well-dispersed, and relatively uniform, with an average size of 6.309 ± 1.891 nm. In contrast, images (b) and (c) display the superhydrophobic coating at different magnifications. These images reveal a hierarchical structure of the material, confirming the presence of particles at both the micro- and nanoscale with diameters ranging from 658 ± 89.5 nm to 17.49 ± 4.83 nm.

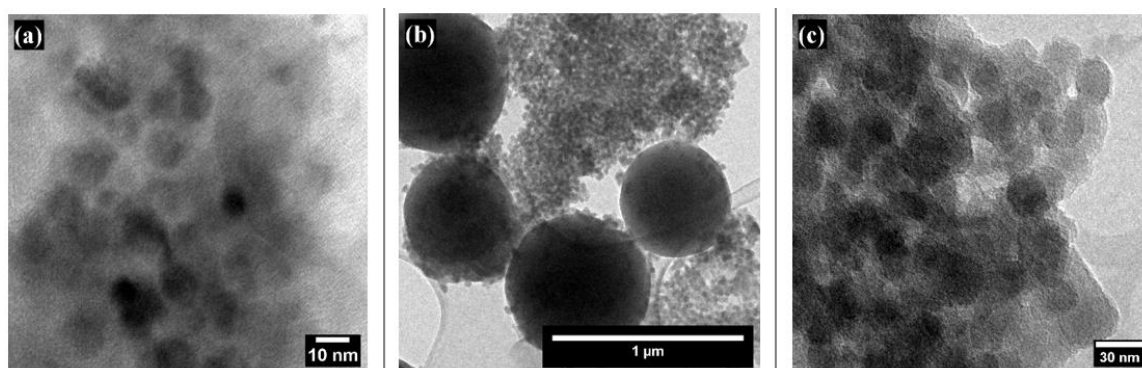


Figure 4. TEM images of SiO₂ and SiO₂ NPs modified with PFDTES at different magnifications: (a) bare SiO₂ NPs, (b) SiO₂ NPs modified with PFDTES showing both micro- and nanoparticles, and (c) SiO₂ NPs modified with PFDTES at high magnification.

3.4. EDS

The energy-dispersive X-ray spectroscopy (EDS) analysis in Figure 5 confirmed the presence of fluorine and carbon, indicating the successful modification of the nanoparticles with trifluoromethyl (CF₃) groups. These findings verify the incorporation of the desired functional groups on the surface of the silica nanoparticles, thereby supporting the functionalization and effectiveness of the superhydrophobic coating.

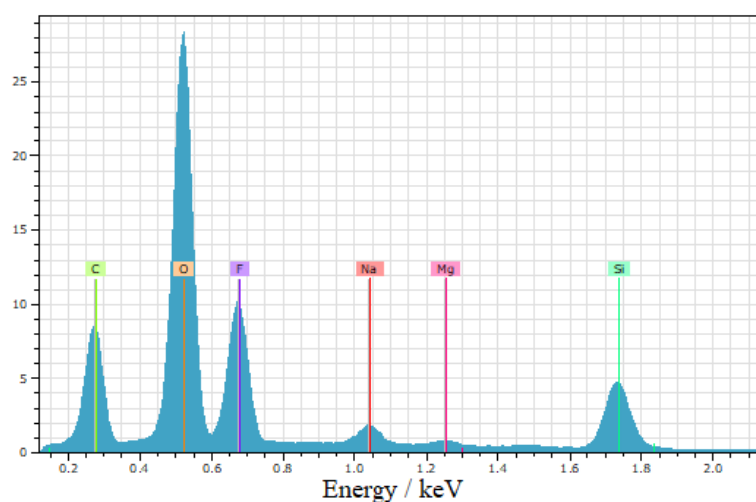


Figure 5. EDS spectra of superhydrophobic coating.

3.5. FTIR Spectroscopy

Figure 6 shows the infrared spectra of SiO₂ NPs and the superhydrophobic coating. The infrared spectrum of the SiO₂ NPs exhibits peaks at 1055 cm⁻¹, 955 cm⁻¹, and 796 cm⁻¹, corresponding to the fingerprint region of silica.[29] Both spectra also display signals at 442 cm⁻¹ and 560 cm⁻¹ attributed to the bending vibrations of O-Si-O, which are typical in amorphous silica.[30]

The FTIR spectrum of the coating reveals additional peaks, such as those at 1205 cm⁻¹ and 1147 cm⁻¹, which are likely indicative of C-F [31,32] they are stretching vibrations. The peak at 1042 cm⁻¹ confirms the presence of SiO₂, while the peaks at 905 cm⁻¹, 800 cm⁻¹, 705 cm⁻¹, and 657 cm⁻¹ are assigned to the symmetric stretching vibrations of Si-O-Si due to functionalization.[33] These functional groups are essential for ensuring the superhydrophobicity of the nanoparticles and confirming successful nanoparticle functionalization.

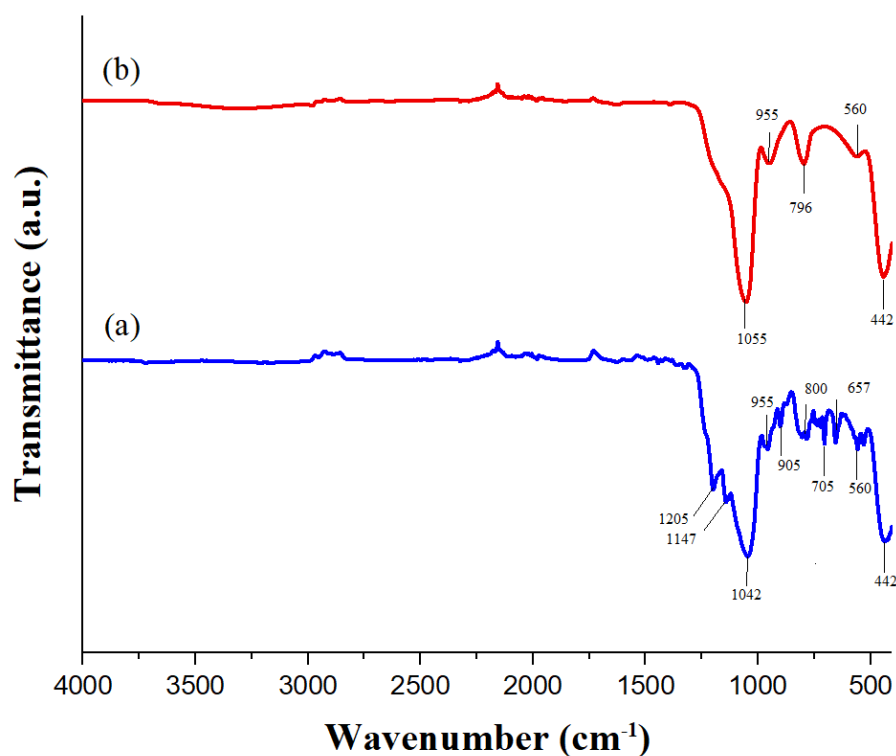


Figure 6. FT-IR spectra of (a) SiO₂ NPs and (b) superhydrophobic coating.

3.6. DRX

As shown in Figure 7, the X-ray diffraction (XRD) pattern of the SiO₂ NPs exhibits an amorphous structure consistent with the JCPDS file 00-083-2467. The broad peak observed at a Bragg angle of $2\theta = 23.37^\circ$ is characteristic of amorphous materials, including amorphous silica.[34]

Post-functionalization, the XRD pattern reveals the persistence of the amorphous nature of SiO₂ along with the emergence of three new peaks at $2\theta = 17.67^\circ$, 22.72° , and 39.44° . These new peaks suggest the formation of a new crystalline phase or a structural change due to the functionalization process. Such changes can result from compressive or expansive stresses within the material, affecting the diffraction angles. Additionally, introducing C-F groups on the surface of SiO₂ can alter the local atomic environment, leading to variations in peak intensities, the emergence of new peaks, or shifts in existing peaks.[31]

Furthermore, the hydrophobic nature of the C-F groups can reduce interactions with surrounding water molecules, potentially causing changes in the structure of the nanoparticles. This may lead to potential aggregation or changes in particle morphology.[35] Furthermore, the hydrophobic nature of the C-F groups can reduce interactions with surrounding water molecules, potentially causing changes in the structure of the nanoparticles. This may lead to potential aggregation or changes in particle morphology.

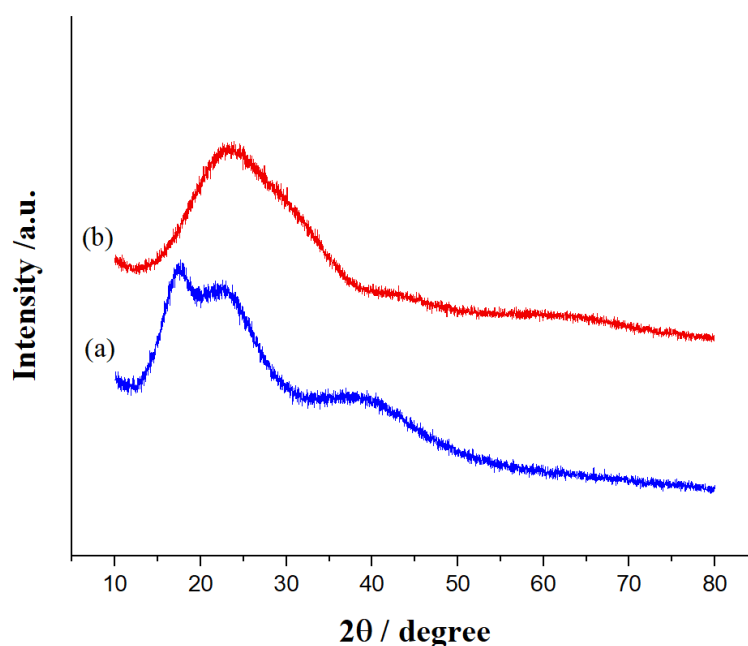


Figure 7. XRD patterns of (a) SiO₂ NPs and (b) the superhydrophobic coating.

3.7. Antimicrobial Activity

Figure 8 presents the measurement of the antibacterial activity of various samples using the turbidimetry technique. The data show that the percentage of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* increased with the number of layers, reaching 11.37% and 12.58%, respectively. *Escherichia coli*, a Gram-negative bacterium, has two membranes. In contrast, *Staphylococcus aureus*, a Gram-positive bacterium, has a thick cell wall, contributing to its resistance mechanisms against antibacterial substances. Nevertheless, the coating exhibited antibacterial activity against both types of bacteria, with the highest activity observed against Gram-positive bacteria.

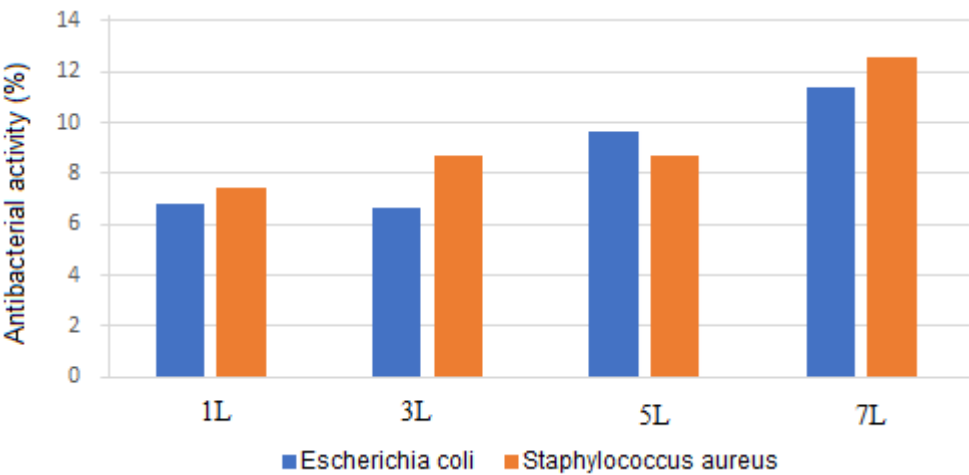


Figure 8. Graphical representation of the antibacterial activity of samples with different numbers of superhydrophobic coating layers: 1L (one layer), 3L (three layers), 5L (five layers), and 7L (seven layers) after 24 hours of exposure to *Escherichia coli* and *Staphylococcus aureus*.

Table 2 presents the results of antibacterial activity measured by the inhibition halo. No inhibition halos were formed in any of the samples. However, the samples exhibited bacteriostatic activity, successfully inhibiting bacterial growth on the coating's surface. This effect is attributed to the coating's ability to interfere with bacterial DNA replication and cellular metabolism [36]. Notably, samples with seven layers (7L) and five layers (5L) demonstrated higher bacteriostatic activity, indicating that the concentration of the coating layers influences bacteriostatic efficacy.

Table 2. Antibacterial activity of samples with varying numbers of layers (1L to 7L) after 24 and 48 hours of exposure to *Escherichia coli* and *Staphylococcus aureus*.

	Escherichia coli	Staphylococcus aureus	Escherichia coli	Staphylococcus aureus
	24 h	24 h	48 h	48 h
7L and 5L				
3L and 1L				

4. Discussion

The water contact angle (WTC) results presented values from 156 to 159° when the coating was not exposed outdoors. The value increased when the number of layers was bigger. Different research presented results from 125° to 160° when the coating was based on silicones. The values obtained in this research are inside of specs. The increase of WCA when exposed after 35 days is due to the hydrophobic membrane generating a possible chemical polymerization of coating [16,21,22,24,28,33].

The hierarchical structure in our coating helps keep the water droplets in place [37]. One important point in developing a good hydrophobic coating is to generate hierarchical structures and modify the rough surface. The 35 days of exposure modified the coating roughness, generating a more hydrophobic layer [38].

Authors have used 1H, 1H, 2H, and 2H-Perfluorodecyltriethoxysilane (PFDTES) with a nano hierarchical structure to suppress the bacteria addition [38]. The presence of a nano hierarchical structure made it easier to avoid the proliferation of bacteria according to the size of the structure, which was 6.309 ± 1.891 nm and also 658 ± 89.5 nm to 17.49 ± 4.83 nm according to the TEM results [8,10,11,22,24,33,34,39–41].

5. Conclusions

The developed superhydrophobic coating has demonstrated significant antibacterial activity, particularly against Gram-positive bacteria. Additionally, all samples exhibited bacteriostatic activity, indicating the coating's effectiveness in inhibiting bacterial growth on the treated surface. The antibacterial activity of the coating was shown to be influenced by the number of layers, with samples containing a higher concentration of layers displaying greater effectiveness.

Morphological and structural characterization revealed a hierarchical structure in the coating with nano- and microscale particles contributing to its roughness and superhydrophobicity, evidenced by contact angles $\geq 150^\circ$ and sliding angles $\leq 10^\circ$. The successful functionalization of silica nanoparticles with trifluoromethyl (CF₃) groups in the coating confirms the efficacy of the functionalization and the coating's ability to repel water and resist biofilm formation.

These findings suggest that the superhydrophobic coating holds significant promise for applications requiring clean surfaces, resistance to biofilm formation, and effective antibacterial properties.

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References

- Privett, B.J.; Youn, J.; Hong, S.A.; Lee, J.; Han, J.; Shin, J.H.; Schoenfisch, M.H. Antibacterial Fluorinated Silica Colloid Superhydrophobic Surfaces. *Langmuir* **2011**, *27*, 9597–9601, doi:10.1021/la201801e.
- Schmidt, M.G.; Attaway, H.H.; Sharpe, P.A.; John, J.J.; Sepkowitz, K.A.; Morgan, A.; Fairey, S.E.; Singh, S.; Steed, L.L.; Cantey, J.R.; et al. Sustained Reduction of Microbial Burden on Common Hospital Surfaces through Introduction of Copper. *J. Clin. Microbiol.* **2012**, *50*, 2217–2223, doi:10.1128/JCM.01032-12.
- Ashok, D.; Taheri, M.; Garg, P.; Webb, D.; Parajuli, P.; Wang, Y.; Funnell, B.; Taylor, B.; Tschärke, D.C.; Tsuzuki, T.; et al. Shielding Surfaces from Viruses and Bacteria with a Multiscale Coating. *Adv. Sci.* **2022**, *9*, 1–15, doi:10.1002/advs.202201415.
- Zarb, P.; Coignard, B.; Griskeviciene, J.; Muller, A.; Vankerckhoven, V.; Weist, K.; Goossens, M.M.; Vaerenberg, S.; Hopkins, S.; Catry, B.; et al. The European Centre for Disease Prevention and Control (ECDC) Pilot Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use. *Eurosurveillance* **2012**, *17*, 1–16, doi:10.2807/ese.17.46.20316-en.

5. Ashok, D.; Cheeseman, S.; Wang, Y.; Funnell, B.; Leung, S.F.; Tricoli, A.; Nisbet, D. Superhydrophobic Surfaces to Combat Bacterial Surface Colonization. *Adv. Mater. Interfaces* **2023**, *10*, doi:10.1002/admi.202300324.
6. Donlan, R.M. Biofilms: Microbial Life on Surfaces. *Emerg. Infect. Dis.* **2002**, *8*, 881–890, doi:10.3201/eid0809.020063.
7. Kokare, C.R.; Chakraborty, S.; Khopade, A.N.; Mahadik, K.R. Biofilm: Importance and Applications. *Indian J. Biotechnol.* **2009**, *8*, 159–168.
8. Stewart, P.S.; Costerton, J.W. Antibiotic Resistance of Bacteria in Biofilms. *Lancet* **2001**, *358*, 135–138, doi:10.1016/S0140-6736(01)05321-1.
9. Poole, K. Mechanisms of Bacterial Biocide and Antibiotic Resistance. *J. Appl. Microbiol.* **2002**, *92 Suppl*, 55S–64S.
10. Zhang, X.; Wang, L.; Levänen, E. Superhydrophobic Surfaces for the Reduction of Bacterial Adhesion. *RSC Adv.* **2013**, *3*, 12003–12020, doi:10.1039/c3ra40497h.
11. Falde, E.J.; Yohe, S.T.; Colson, Y.L.; Grinstaff, M.W. Superhydrophobic Materials for Biomedical Applications. *Biomaterials* **2016**, *104*, 87–103, doi:10.1016/j.biomaterials.2016.06.050.
12. Berendjchi, A.; Khajavi, R.; Yazdanshenas, M.E. Fabrication of Superhydrophobic and Antibacterial Surface on Cotton Fabric by Doped Silica-Based Sols with Nanoparticles of Copper. *Nanoscale Res. Lett.* **2011**, *6*, 1–8, doi:10.1186/1556-276X-6-594.
13. Crick, C.R.; Ismail, S.; Pratten, J.; Parkin, I.P. An Investigation into Bacterial Attachment to an Elastomeric Superhydrophobic Surface Prepared via Aerosol Assisted Deposition. *Thin Solid Films* **2011**, *519*, 3722–3727, doi:10.1016/j.tsf.2011.01.282.
14. Marmur, A. Superhydrophobicity Fundamentals: Implications to Biofouling Prevention. *Biofouling* **2006**, *22*, 107–115, doi:10.1080/08927010600562328.
15. Ciasca, G.; Papi, M.; Businaro, L.; Campi, G.; Ortolani, M.; Palmieri, V.; Cedola, A.; De Ninno, A.; Gerardino, A.; Maulucci, G.; et al. Recent Advances in Superhydrophobic Surfaces and Their Relevance to Biology and Medicine. *Bioinspiration and Biomimetics* **2016**, *11*, 11001, doi:10.1088/1748-3190/11/1/011001.
16. Erbil, H.Y. Practical Applications of Superhydrophobic Materials and Coatings: Problems and Perspectives. *Langmuir* **2020**, *36*, 2493–2509, doi:10.1021/acs.langmuir.9b03908.
17. Sreekantan, S.; Hassan, M.; Murthe, S.S.; Seeni, A. Biocompatibility and Cytotoxicity Study of Polydimethylsiloxane (Pdms) and Palm Oil Fuel Ash (Pofa) Sustainable Super-Hydrophobic Coating for Biomedical Applications. *Polymers (Basel)*. **2020**, *12*, 1–13, doi:10.3390/polym12123034.
18. Mahadik, S.A.; Pedraza, F.; Mahadik, S.S.; Relekar, B.P.; Thorat, S.S. Biocompatible Superhydrophobic Coating Material for Biomedical Applications. *J. Sol-Gel Sci. Technol.* **2017**, *81*, 791–796, doi:10.1007/s10971-016-4244-4.
19. Feng, B.L.; Li, S.; Li, Y.; Li, H.; Zhang, L.; Zhai, J.; Song, Y.; Liu, B.; Jiang, L. Superhydrophobic Surfaces: From Natural to Artificial. *Adv. Mater.* **2002**, 1857–1860.
20. Liang, Z.; Geng, M.; Dong, B.; Zhao, L.; Wang, S. Transparent and Robust SiO₂/PDMS Composite Coatings with Self-Cleaning. *Surf. Eng.* **2020**, *36*, 643–650, doi:10.1080/02670844.2019.1691367.
21. Zhao, Y.; Huo, M.; Huo, J.; Zhan, P.; Shao, X.; Zhang, X. Preparation of Silica-Epoxy Superhydrophobic Coating with Mechanical Stability and Multifunctional Performance via One-Step Approach. *Colloids Surfaces A Physicochem. Eng. Asp.* **2022**, *653*, 129957, doi:10.1016/j.colsurfa.2022.129957.
22. Zhang, Z.; Xu, C.; Liu, W.; Wang, K.; Rao, Y.; Jiang, C.; Li, D.; Zhang, Y.; Jiang, X.; Chen, X.; et al. Ultrasonic Assisted Rapid Preparation of Superhydrophobic Stainless Steel Surface and Its Application in Oil/Water Separation. *Ultrason. Sonochem.* **2021**, *81*, 105848, doi:10.1016/j.ultsonch.2021.105848.
23. Ye, H.; Zhu, L.; Li, W.; Liu, H.; Chen, H. Constructing Fluorine-Free and Cost-Effective Superhydrophobic Surface with Normal-Alcohol-Modified Hydrophobic SiO₂(2) Nanoparticles. *ACS Appl. Mater. Interfaces* **2017**, *9*, 858–867, doi:10.1021/acsami.6b12820.
24. Alavi, M.; Thomas, S.; Sreedharan, M. Micro Nano Bio Aspects Modification of Silica Nanoparticles for Antibacterial Activities: Mechanism of Action. *Micro Nano Bio Asp.* **2022**, *2022*, 49–58.
25. Wang, F.; Pi, J.; Song, F.; Feng, R.; Xu, C.; Wang, X.-L.; Wang, Y.-Z. A Superhydrophobic Coating to Create Multi-Functional Materials with Mechanical/Chemical/Physical Robustness. *Chem. Eng. J.* **2019**, *381*, 122539, doi:10.1016/j.cej.2019.122539.
26. Xue, F.; Shi, X.; Bai, W.; Li, J.; Li, Y.; Zhu, S.; Liu, Y.; Feng, L. Enhanced Durability and Versatile Superhydrophobic Coatings via Facile One-Step Spraying Technique. *Colloids Surfaces A Physicochem. Eng. Asp.* **2022**, *640*, 128411, doi:https://doi.org/10.1016/j.colsurfa.2022.128411.
27. Liu, X.; Zhou, Z.; Chen, M.; Liu, Z.; Jiang, S.; Wang, L. Preparation of Durable Superhydrophobic Coatings Based on Discrete Adhesives. *Coatings* **2024**, *14*, 463, doi:10.3390/coatings14040463.
28. Stöber, W.; Fink, A.; Bohn, E. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *J. Colloid Interface Sci.* **1968**, *26*, 62–69, doi:https://doi.org/10.1016/0021-9797(68)90272-5.
29. Stuart, B.H. Infrared Spectroscopy: Fundamentals and Applications. *Infrared Spectrosc. Fundam. Appl.* **2005**, *8*, 1–224, doi:10.1002/0470011149.

30. Zhuravlev, L.T. The Surface Chemistry of Amorphous Silica. Zhuravlev Model. *Colloids Surfaces A Physicochem. Eng. Asp.* **2000**, *173*, 1–38, doi:10.1016/S0927-7757(00)00556-2.
31. Lee, H.; Dellatore, S.M.; Miller, W.M.; Messersmith, P.B. Mussel-Inspired Surface Chemistry for Multifunctional Coatings. *Science (80-.)*. **2007**, *318*, 426–430, doi:10.1126/science.1147241.
32. Zhu, Y.; Murali, S.; Cai, W.; Li, X.; Suk, J.W.; Potts, J.R.; Ruoff, R.S. Graphene and Graphene Oxide: Synthesis, Properties, and Applications. *Adv. Mater.* **2010**, *22*, 3906–3924, doi:10.1002/adma.201001068.
33. Coates, J. Interpretation of Infrared Spectra, A Practical Approach. *Encycl. Anal. Chem.* **2000**, 1–23, doi:10.1002/9780470027318.a5606.
34. Musić, S.; Filipović-Vinceković, N.; Sekovanić, L. Precipitation of Amorphous SiO₂ Particles and Their Properties. *Brazilian J. Chem. Eng.* **2011**, *28*, 89–94, doi:10.1590/S0104-66322011000100011.B.D. & Stock, S.R.C. *Elements of X-Ray Diffraction*; 2001;
35. Das, P.; Ganguly, S.; Bose, M.; Mondal, S.; Choudhary, S.; Gangopadhyay, S.; Das, A.K.; Banerjee, S.; Das, N.C. Zinc and Nitrogen Ornamented Bluish White Luminescent Carbon Dots for Engrossing Bacteriostatic Activity and Fenton Based Bio-Sensor. *Mater. Sci. Eng. C* **2018**, *88*, 115–129, doi:10.1016/j.msec.2018.03.010.
36. Jin, M.; Xing, Q.; Chen, Z.; Jin, M.; Xing, Q.; Chen, Z. A Review: Natural Superhydrophobic Surfaces and Applications. *J. Biomater. Nanobiotechnol.* **2020**, *11*, 110–149, doi:10.4236/JBNB.2020.112008.
37. Yu, M.; Cui, Z.; Ge, F.; Man, C.; Lei, L.; Wang, X. Fabrication of Durable and Roughness-Regeneration Superhydrophobic Composite Materials by Hot Pressing. *Compos. Part B Eng.* **2019**, *179*, 107431, doi:10.1016/J.COMPOSITESB.2019.107431.
38. Sarkar, D.K.; Farzaneh, M.; Paynter, R.W. Superhydrophobic Properties of Ultrathin Rf-Sputtered Teflon Films Coated Etched Aluminum Surfaces. *Mater. Lett.* **2008**, *62*, 1226–1229, doi:10.1016/J.MATLET.2007.08.051.
39. Jia, W.; Kharraz, J.A.; Choi, P.J.; Guo, J.; Deka, B.J.; An, A.K. Superhydrophobic Membrane by Hierarchically Structured PDMS-POSS Electrospray Coating with Cauliflower-Shaped Beads for Enhanced MD Performance. *J. Memb. Sci.* **2020**, *597*, 117638, doi:10.1016/J.MEMSCI.2019.117638.
40. Xu, P.; Li, X. Fabrication of TiO₂/SiO₂ Superhydrophobic Coating for Efficient Oil/Water Separation. *J. Environ. Chem. Eng.* **2021**, *9*, 105538, doi:10.1016/J.JECE.2021.105538.

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