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Geometric Bionics-Inspired Superhydrophobic Surfaces for Effective Antibacterial and Anti-fouling Applications—A Design Approach Leveraging Lotus-Effect Nanostructures on Polydimethylsiloxane (PDMS) Films

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***Abstract*—Bacterial adhesion and subsequent biofilm formation on surfaces pose a significant threat across various sectors, including healthcare, food safety, and marine industries. Conventional antibacterial strategies often rely on chemical agents or antibiotics, which can lead to environmental toxicity and the emergence of drug-resistant bacteria. Therefore, developing novel, environmentally friendly, and durable physical antibacterial methods is of paramount importance.Inspired by the self-cleaning properties of the lotus leaf, this study presents a facile and scalable template-assisted replication method to fabricate superhydrophobic surfaces with hierarchical micro- and nanostructures on a flexible polydimethylsiloxane (PDMS) substrate. The surface morphology and wettability were systematically characterized using Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Water Contact Angle (WCA) measurements. The antibacterial performance was evaluated against two common pathogenic bacteria, Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). Furthermore, the anti-fouling capability was assessed by quantifying the adsorption of bovine serum albumin (BSA). The biomimetic PDMS surfaces exhibited robust superhydrophobicity with a WCA exceeding 155° and a sliding angle (SA) below 5°. These surfaces demonstrated exceptional resistance to bacterial adhesion, reducing the attachment of both E. coli and S. aureus by over 95% after 24 hours of incubation compared to flat PDMS controls. This physical antibacterial effect is attributed to a stable Cassie-Baxter state, where a trapped air layer minimizes the contact area between bacteria and the material surface. Concurrently, protein adsorption was reduced by more than 95%, showcasing excellent anti-fouling properties. This research provides a novel and scalable design strategy for creating chemistry-free, long-lasting antibacterial and anti-fouling surfaces. The findings highlight the potential of leveraging geometric bionics to address the persistent challenges of surface contamination, offering a promising alternative to chemical-based approaches with broad applicability in biomedical devices, public health facilities, and marine engineering.**

Keywords*—Superhydrophobic Surface, Geometric Bionics, Antibacterial, Anti-fouling, Polydimethylsiloxane (PDMS), Lotus Effect

1. Introduction

Bacterial contamination and the subsequent formation of biofilms on material surfaces represent a pervasive and costly challenge across numerous sectors critical to human well-being and economic activity. In healthcare, biofilm-associated infections on medical devices such as catheters, implants, and surgical instruments are a leading cause of nosocomial infections, resulting in increased patient morbidity, mortality, and substantial financial burdens on healthcare systems globally [1]. The economic impact is staggering, with estimates suggesting that biofilms contribute to hundreds of billions of dollars in healthcare expenditures annually [2]. Beyond the clinical setting, the proliferation of biofilms in industrial manufacturing leads to biofouling, which can cause equipment damage, product contamination, and operational inefficiencies, with a global economic impact estimated to exceed $5 trillion annually [3][4].

The primary strategy to combat these microbial threats has historically been the application of chemical biocides and antibiotics. However, the overuse and misuse of these agents have precipitated a global public health crisis: antimicrobial resistance (AMR). The World Health Organization (WHO) has declared AMR as one of the top 10 global public health threats facing humanity [5]. In 2019 alone, bacterial AMR was directly responsible for an estimated 1.27 million deaths worldwide and contributed to nearly 5 million deaths [6]. Projections indicate that if current trends continue, AMR

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could lead to 10 million deaths annually by 2050, surpassing the mortality rate of cancer [7]. These alarming statistics underscore the urgent need for alternative antibacterial strategies that do not rely on chemical toxicity or antibiotic activity, thereby avoiding the selective pressures that drive resistance development.

Nature, through billions of years of evolution, offers elegant solutions to this problem. The lotus leaf (Nelumbo nucifera), renowned for its remarkable self-cleaning ability, provides a compelling blueprint for designing anti-fouling surfaces. This phenomenon, known as the "lotus effect," arises from a unique hierarchical micro- and nanostructure on the leaf's surface, which, combined with its waxy chemical composition, results in extreme water repellency, or superhydrophobicity [8]. On such surfaces, water droplets remain nearly spherical and roll off easily, carrying away contaminants, including bacteria. Importantly, this self-cleaning behavior is governed predominantly by surface geometry rather than chemical toxicity, making it particularly attractive for long-term and environmentally benign antibacterial applications.

Inspired by this natural design, researchers have developed a variety of biomimetic superhydrophobic surfaces with potent antibacterial and anti-fouling properties [9, 10]. These surfaces function by creating a stable air layer, commonly described as a Cassie–Baxter wetting state, between the material and the aqueous environment, which acts as a physical barrier to prevent bacterial attachment and protein adsorption [11]. According to classical wetting theory, the apparent water contact angle on such composite interfaces is governed by the fraction of solid–liquid contact area (f_s), and a sufficiently low f_s is critical for minimizing interfacial adhesion forces. As a result, bacteria suspended in liquid media experience reduced effective contact with the solid substrate, thereby hindering the initial adhesion events that precede biofilm formation. This physical repellency mechanism fundamentally differs from bactericidal approaches and is inherently less susceptible to performance degradation caused by resistance or biocide depletion.

While significant progress has been made, many existing fabrication strategies for superhydrophobic antibacterial surfaces suffer from notable limitations. Advanced lithographic techniques often require complex processing steps, high-cost equipment, and rigid substrates, restricting scalability and practical deployment. Chemical etching and sol–gel approaches, although simpler, may suffer from poor structural uniformity, limited mechanical durability, or environmental concerns. Template-based replication methods offer an attractive balance between structural fidelity and scalability; however, many reported systems remain limited to single-step replication, rigid substrates, or lack systematic evaluation of mechanical and chemical stability. These shortcomings significantly constrain their application in flexible biomedical devices and real-world environments.

Polydimethylsiloxane (PDMS) is a widely used elastomeric material in biomedical engineering, microfluidics, and wearable devices due to its flexibility, biocompatibility, and ease of processing. Nevertheless, pristine PDMS surfaces are only moderately hydrophobic and remain susceptible to bacterial adhesion and biofouling, limiting their performance in contamination-prone environments.

In this context, the present study aims to address the above-mentioned gaps by developing a facile and scalable two-step template replication approach to fabricate lotus-effect-inspired hierarchical micro/nanostructures on flexible PDMS films. The objectives of this work are threefold: (i) to fabricate and structurally characterize robust superhydrophobic PDMS surfaces with well-defined hierarchical features; (ii) to systematically evaluate their antibacterial performance against representative Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria, as well as their resistance to protein fouling; and (iii) to elucidate the underlying physical antibacterial mechanism by correlating surface geometry, wettability, and adhesion behavior within the framework of the Cassie–Baxter model. By emphasizing a purely geometry-driven, chemistry-free strategy, this work seeks to provide a sustainable and scalable design paradigm for long-lasting antibacterial and anti-fouling surfaces applicable to biomedical devices, public facilities, and marine engineering.

2. Related Work

2.1. The Process and Impact of Biofilm Formation

Biofilm formation is a complex, multi-stage process that begins when planktonic (free-floating) microorganisms attach to a surface [12]. This initial, reversible attachment is typically mediated by weak physicochemical interactions such as van der Waals forces and hydrophobic interactions. If the microorganisms are not removed, they transition to an irreversible attachment phase, strengthening their bond to the surface through the production of specific adhesion proteins and appendages like pili and fimbriae [13]. Following this, the attached cells begin to proliferate and secrete a protective extracellular polymeric substance (EPS) matrix. This matrix, composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA), encases the microbial community, leading to the formation of microcolonies [14]. As the biofilm matures, it develops a complex, three-dimensional architecture with water channels that facilitate nutrient delivery and waste removal. In the final stage, a portion of the bacteria can detach from the biofilm, either individually or in clumps, to colonize new surfaces, thus perpetuating the cycle of contamination [15].

This resilient structure makes biofilms notoriously difficult to eradicate. The EPS matrix acts as a physical barrier, shielding the embedded bacteria from antibiotics, disinfectants, and the host immune system. Bacteria within a biofilm can be up to 1,000 times more resistant to antimicrobial agents than their planktonic counterparts [16]. This heightened resistance, combined with the close proximity of cells, facilitates horizontal gene transfer, further accelerating the spread of antibiotic resistance genes within the microbial community [17].

2.2. Conventional Antibacterial and Anti-fouling Strategies

Historically, the fight against surface contamination has been dominated by chemical-based approaches. The most common strategy involves the use of biocide-releasing coatings, which continuously leach toxic substances to kill or inhibit the growth of nearby microorganisms. These include coatings loaded with heavy metals (e.g., copper, silver), quaternary ammonium compounds, or triclosan [18]. While effective in the short term, these materials suffer from significant drawbacks. The finite reservoir of the biocide leads

to a limited effective lifespan, and the constant, low-dose release of toxic agents contributes to environmental pollution and exerts strong selective pressure for the development of resistant microbial strains [19, 20].

Another major class of materials is anti-adhesion or "fouling-release" coatings. These are typically low-surface-energy materials, such as those based on silicone or fluoropolymers, which are designed to minimize the adhesion strength of fouling organisms, allowing them to be removed by shear forces (e.g., water flow). While non-toxic, their effectiveness is often limited, especially in low-flow environments, and they can be susceptible to mechanical damage [21]. More advanced strategies include the use of hydrophilic polymer brushes (e.g., polyethylene glycol, PEG) that form a hydration layer to repel proteins and bacteria, but their long-term stability and cost can be prohibitive for large-scale applications.

2.3. Biomimetic Superhydrophobic Surfaces

In recent years, biomimicry—learning from and emulating nature's strategies—has emerged as a powerful paradigm for materials design. Superhydrophobic surfaces, inspired by the lotus leaf, represent a particularly promising platform for combating bacterial adhesion through a purely physical mechanism. The water-repellent nature of these surfaces is governed by two key factors: low surface energy chemistry and, more importantly, a specific hierarchical surface topography.

The wetting behavior of a liquid on a rough surface is typically described by either the Wenzel model or the Cassie-Baxter model. In the Wenzel state, the liquid fully penetrates the surface asperities, leading to a large contact area and strong adhesion. In contrast, the Cassie-Baxter state occurs when the liquid is suspended on top of the surface roughness, trapping pockets of air within the texture. This composite interface minimizes the solid-liquid contact area, leading to a high water contact angle (>150°) and a low sliding angle (<10°), which are the defining characteristics of superhydrophobicity [8].

Various techniques have been developed to fabricate these intricate topographies, including lithography, chemical etching, sol-gel processes, electrochemical deposition, and template replication, each with its own advantages and limitations [22, 23]. Among these, template replication offers a compelling combination of simplicity, cost-effectiveness, and scalability. It allows for the precise transfer of micro- and nanostructures from a master template to a polymer, making it an ideal method for creating biomimetic surfaces on flexible substrates like PDMS [24].

2.4. Physical Antibacterial Mechanism of Superhydrophobic Surfaces

The antibacterial properties of superhydrophobic surfaces stem directly from the Cassie-Baxter state. The entrapped air layer acts as a physical barrier that prevents bacteria from making direct contact with the solid surface [11]. This non-contact state drastically reduces the adhesion forces—governed by factors like surface charge, motility, and hydrodynamic conditions—between the bacteria and the material, making it difficult for them to attach and initiate the biofilm formation process. Any bacteria that do manage to land on the surface are poorly adhered and can be easily removed by even gentle water flow, leading to a "self-cleaning" effect.

3. METHODOLOGY

3.1. Research Strategy

The overall research strategy followed a logical progression of biomimetic design, fabrication, multi-faceted characterization, and mechanistic analysis. The workflow, depicted in Figure 1, began with the preparation of a suitable template, followed by the replication of its structure onto a PDMS substrate. The resulting surfaces were then thoroughly characterized for their morphology and wettability. Finally, their performance was systematically evaluated through a series of antibacterial, anti-fouling, and stability tests to validate the design and understand the underlying principles.

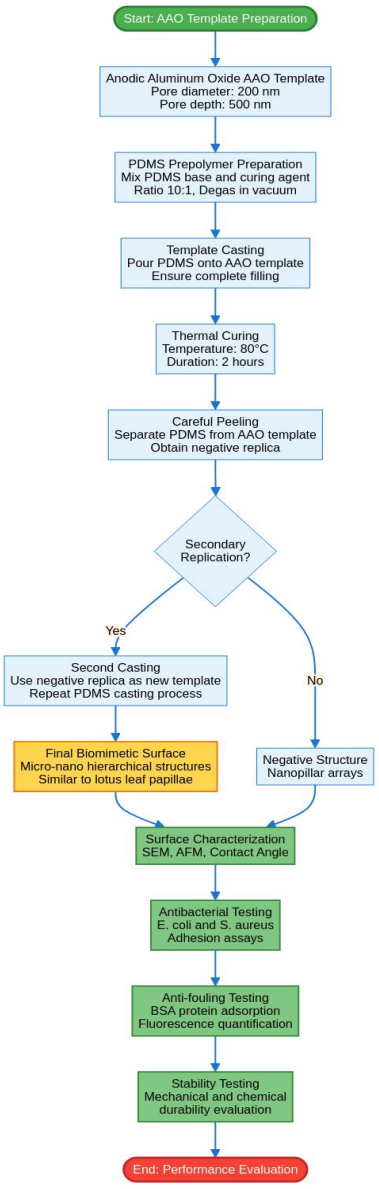

Figure 1. Schematic illustration of the experimental workflow, from template preparation to surface fabrication, characterization, and performance evaluation.

3.2. Materials and Reagents

Polydimethylsiloxane (PDMS) prepolymer and curing agent (Sylgard 184) were purchased from Dow Corning (USA). High-purity, ordered Anodic Aluminum Oxide (AAO)

templates (pore diameter: ~200 nm, thickness: ~100 μm) were obtained from Whatman (UK). Tryptic Soy Broth (TSB), Luria-Bertani (LB) broth, and agar were sourced from Oxoid Ltd. (UK). Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 29213) were obtained from the American Type Culture Collection (USA). For anti-fouling tests, Bovine Serum Albumin (BSA, Fraction V, ≥98%) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (USA). The LIVE/DEAD™ BacLight™ Bacterial Viability Kit was sourced from Thermo Fisher Scientific (USA). All other chemical reagents were of analytical grade and used as received without further purification.

3.3. Fabrication of Biomimetic Superhydrophobic PDMS Surfaces

The antibacterial performance of the fabricated PDMS surfaces was systematically evaluated using representative Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacterial strains. Prior to adhesion experiments, both bacterial species were cultured under standard conditions to the logarithmic growth phase, harvested by centrifugation, and resuspended in sterile phosphate-buffered saline (PBS) to obtain a uniform bacterial suspension with a concentration of 1×10^7 CFU/mL. This preparation ensured consistent physiological states of bacteria during subsequent adhesion tests.

For bacterial adhesion assays, the biomimetic and flat PDMS samples (1 cm × 1 cm) were first sterilized by ultraviolet irradiation and then placed individually in a 24-well culture plate. Each sample was incubated with 1 mL of the bacterial suspension at 37 °C for predetermined time intervals (2, 6, and 24 h) under static conditions to allow bacterial attachment. After incubation, the samples were gently rinsed with PBS to remove non-adherent bacteria, thereby isolating the firmly attached bacterial population for further analysis.

Qualitative assessment of bacterial adhesion was carried out using both scanning electron microscopy (SEM) and fluorescence-based live/dead staining. For SEM observation, the samples were chemically fixed with glutaraldehyde, dehydrated through a graded ethanol series, and dried prior to imaging. In parallel, bacterial viability on the surfaces was examined using a LIVE/DEAD™ BacLight™ staining kit and visualized by confocal laser scanning microscopy (CLSM), enabling differentiation between viable and non-viable bacterial cells. This dual-imaging approach provided complementary morphological and viability information regarding bacterial–surface interactions.

Quantitative evaluation of bacterial adhesion was performed by detaching the adherent bacteria from the PDMS surfaces through vigorous vortexing in sterile PBS, followed by serial dilution and plate counting to determine colony-forming units (CFU) per unit area. The antibacterial rate was calculated relative to the flat PDMS control, allowing direct comparison of bacterial adhesion resistance between surfaces. All antibacterial experiments were conducted using independent samples and repeated measurements to ensure data reliability, and the results were expressed as mean ± standard deviation..

3.4. Surface Characterization

The antibacterial performance of the biomimetic superhydrophobic PDMS surfaces was systematically investigated and compared with that of flat PDMS controls using both qualitative and quantitative approaches. After 24 h of incubation with Escherichia coli and Staphylococcus aureus, scanning electron microscopy (SEM) images revealed pronounced differences in bacterial adhesion behavior between the two surfaces. As shown in the representative micrographs, the flat PDMS surfaces were extensively colonized by bacteria, with dense bacterial clusters and early-stage microcolonies clearly observable, indicating favorable conditions for bacterial attachment and proliferation. In contrast, the biomimetic PDMS surfaces exhibited remarkably sparse bacterial coverage, with only a limited number of isolated bacterial cells distributed across the surface and no evidence of aggregation or biofilm formation.

Fluorescence-based LIVE/DEAD staining further corroborated the SEM observations and provided insight into the viability of adherent bacteria. On flat PDMS surfaces, a high density of bacteria emitting green fluorescence was observed, indicating that the majority of adhered cells remained viable after incubation. Conversely, the biomimetic superhydrophobic surfaces displayed a drastic reduction in the number of attached bacteria, and the few adherent cells were predominantly viable, suggesting that the antibacterial effect of the surface arises from inhibition of bacterial adhesion rather than bactericidal activity. This observation confirms that the surface operates through a physical repellency mechanism rather than chemical toxicity.

Quantitative analysis of bacterial adhesion supported the qualitative findings. Over the entire incubation period, the number of colony-forming units (CFU) recovered from the biomimetic surfaces remained consistently and significantly lower than that from the flat PDMS controls. After 24 h, the biomimetic PDMS surfaces achieved a reduction in bacterial adhesion exceeding 95% for both E. coli and S. aureus, demonstrating robust and sustained antibacterial performance. The time-dependent adhesion profiles further indicated that the superhydrophobic surface effectively suppressed bacterial accumulation at both early and later stages of incubation, thereby impeding the initial events required for biofilm development.

3.5. Antibacterial Performance Evaluation

In addition to bacterial adhesion, the resistance of the PDMS surfaces to nonspecific protein adsorption was evaluated, as protein fouling is widely recognized as a critical precursor to subsequent bacterial colonization. Bovine serum albumin (BSA) was selected as a model protein, and its adsorption behavior on the flat and biomimetic PDMS surfaces was examined using fluorescence microscopy following incubation with fluorescein-labeled BSA.

The flat PDMS surfaces exhibited strong and uniform fluorescence signals, indicating substantial protein adsorption across the surface. This result is consistent with the moderate hydrophobicity of pristine PDMS, which facilitates protein–surface interactions. In contrast, the biomimetic superhydrophobic PDMS surfaces displayed only weak background fluorescence, suggesting minimal protein attachment. The pronounced difference in fluorescence intensity between the two surfaces highlights the effectiveness

of the hierarchical surface topography in suppressing protein fouling.

Quantitative analysis of fluorescence intensity revealed that protein adsorption on the biomimetic PDMS surfaces was reduced by approximately 95.7% compared to the flat PDMS controls. This significant reduction can be attributed to the formation of a stable air layer at the solid–liquid interface, which minimizes the effective contact area between the protein molecules and the surface. By preventing the formation of an initial protein conditioning layer, the superhydrophobic surface disrupts a key step in the biofouling cascade, thereby contributing to its overall antibacterial and anti-fouling functionality.

3.6. Anti-fouling Performance Test

BSA was labeled with FITC according to established protocols. The PDMS samples were incubated in a 1 mg/mL solution of FITC-BSA in PBS at 37°C for 3 hours. After incubation, the samples were rinsed thoroughly with PBS to remove non-adsorbed protein. The fluorescence intensity of the adsorbed protein on the surfaces was observed and quantified using the CLSM. The average fluorescence intensity from at least five randomly selected areas per sample was calculated.

3.7. Stability Tests

- Mechanical Stability: The robustness of the superhydrophobic surface was evaluated using a tape-peeling test. A piece of 3M Scotch tape was firmly applied to the surface and then rapidly peeled off at a 90° angle. This process was repeated up to 200 times on the same spot. The WCA was measured after a set number of cycles.
- Chemical Stability: The chemical stability was assessed by immersing the samples in aqueous solutions with a wide range of pH values (from 2 to 12) for 24 hours. After immersion, the samples were rinsed with DI water, dried, and their WCA was measured.

3.8. Statistical Analysis

All quantitative experiments were performed in triplicate, and the data are presented as mean ± standard deviation (SD). Statistical significance between groups was determined using a two-tailed Student's t-test or one-way analysis of variance (ANOVA) where appropriate. A p-value of less than 0.05 was considered statistically significant.

4. RESULTS

4.1. Surface Morphology and Wettability

The surface morphology of the fabricated PDMS samples was first investigated using SEM and AFM. As shown in Figure 2a and Figure 3a, the control PDMS sample exhibited a smooth and featureless surface, with a low root-mean-square (Rq) roughness of approximately 12.3 ± 0.8 nm. In stark contrast, the biomimetic PDMS surface, fabricated through the two-step replication process, displayed a distinct hierarchical topography. Figure 2b reveals a dense and uniform distribution of micro-scale papillae, reminiscent of the lotus leaf structure. Higher magnification AFM imaging (Figure 3b) further resolved that these micro-papillae were covered with nano-scale textures, resulting in a significantly increased surface roughness with an Rq value of 388.4 ± 6.5 nm.

The profound difference in surface topography led to a dramatic shift in wettability, as summarized in Table 1 and visualized in Figure 4. The flat PDMS surface was moderately hydrophobic, with an average WCA of 108.2° ± 1.5°(Figure 4a). A water droplet placed on this surface was pinned and would not slide off even when the surface was tilted to 90°. Conversely, the biomimetic PDMS surface demonstrated excellent water repellency. A water droplet on this surface formed a nearly perfect sphere, exhibiting a high WCA of 156.8° ± 1.2° (Figure 4b). Furthermore, the droplet was extremely mobile, rolling off the surface at a very low sliding angle (SA) of less than 5°, confirming its superhydrophobic and low-adhesion nature.

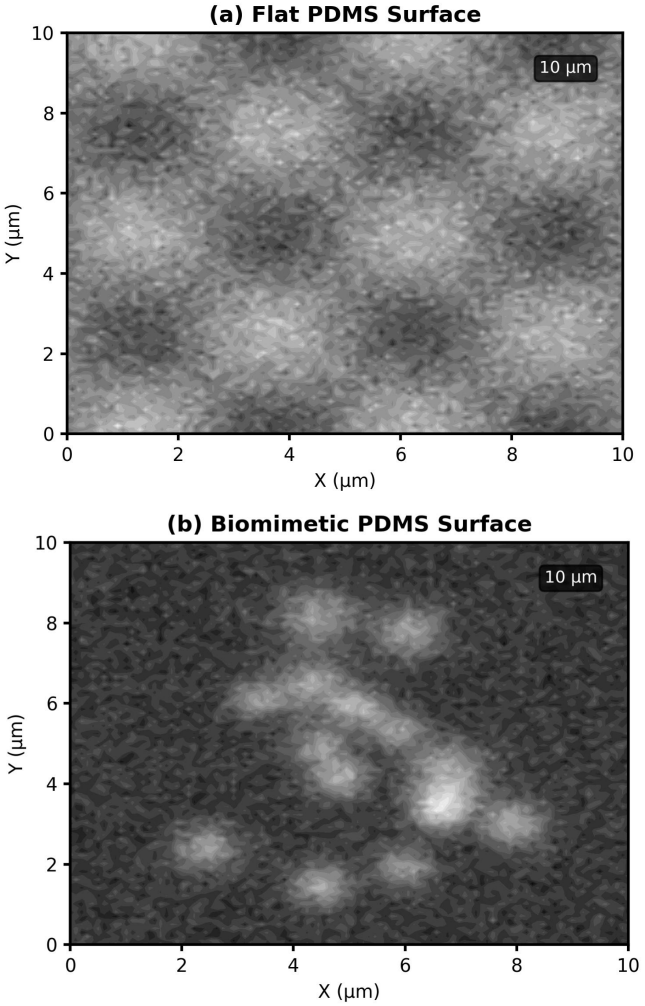

Figure 2. Top-down SEM-style images comparing (a) the relatively featureless surface of flat PDMS with (b) the hierarchical micro-structured topography of the biomimetic PDMS surface.

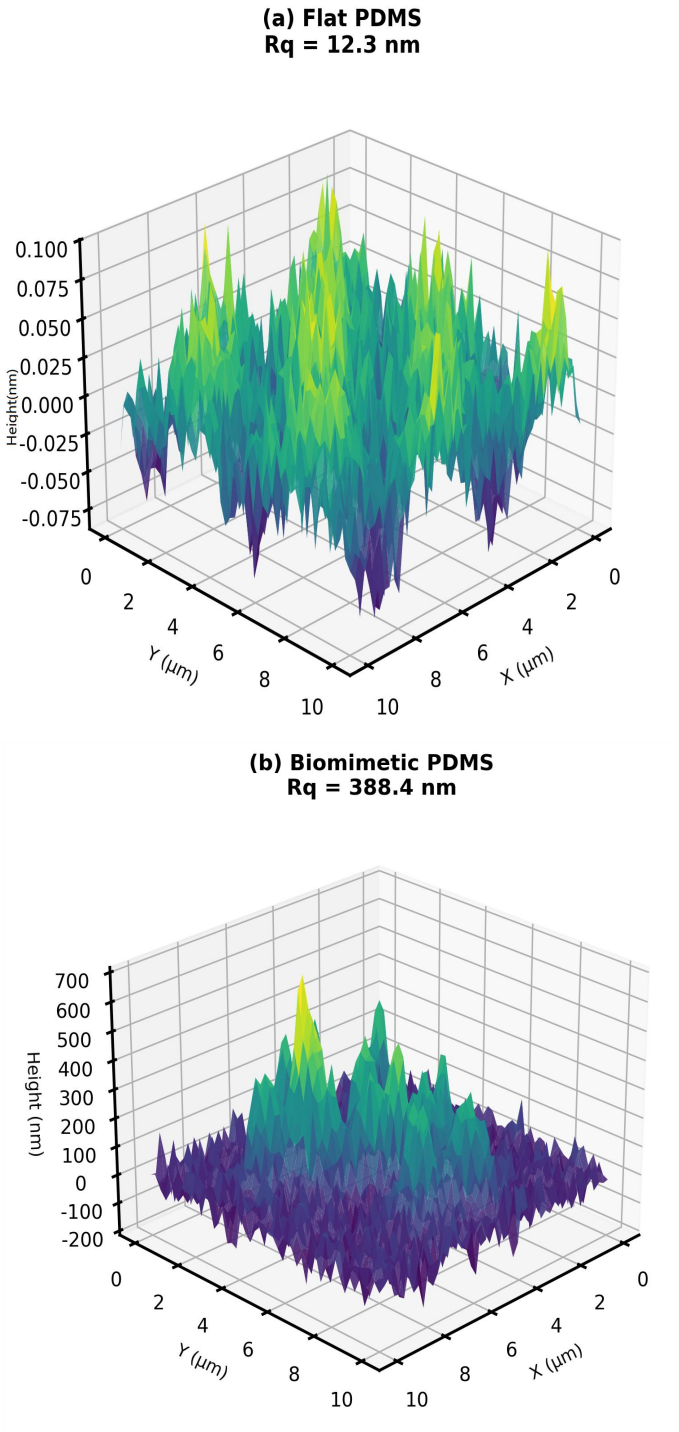

Figure 3. 3D AFM surface profiles illustrating (a) the low surface roughness of the flat PDMS control (Rq ≈ 12.3 nm) and (b) the significantly rougher, hierarchical topography of the biomimetic PDMS (Rq ≈ 388.4 nm)

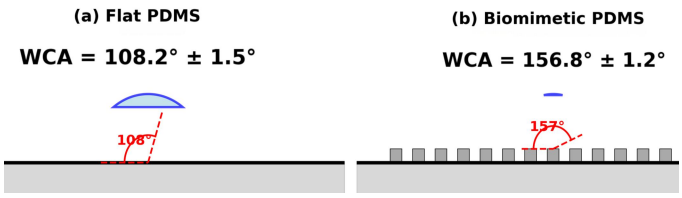

Figure 4. Water contact angle measurements showing (a) a WCA of ~108° on the flat PDMS surface and (b) a superhydrophobic WCA of ~157° on the biomimetic PDMS surface, indicating extreme water repellency.

TABLE I. SURFACE ROUGHNESS AND WETTABILITY PROPERTIES OF FLAT AND BIOMIMETIC PDMS SURFACES.

Surface Type	Roughness (Rq, nm)	Water Contact Angle (WCA, °)	Sliding Angle (SA, °)
Flat PDMS	12.3 ± 0.8	108.2 ± 1.5	> 90
Biomimetic PDMS	388.4 ± 6.5	156.8 ± 1.2	< 5

4.2. Antibacterial Performance

The ability of the superhydrophobic surface to resist bacterial colonization was evaluated against both Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. After 24 hours of incubation, SEM imaging revealed extensive bacterial adhesion on the flat PDMS control surface. As seen in Figure 5, large areas were covered by dense layers of bacteria, with evidence of microcolony and early biofilm formation. In sharp contrast, the biomimetic PDMS surface remained remarkably clean (Figure 6). Only a few, isolated bacteria were sparsely distributed across the surface, with no signs of aggregation or biofilm development.

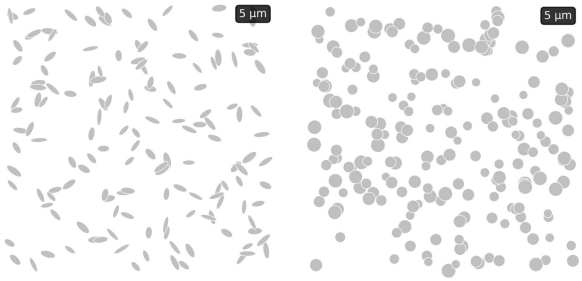

Figure 5. SEM-style micrographs after 24h incubation showing extensive adhesion and microcolony formation of (a) E. coli and (b) S. aureus on the flat PDMS control surface.

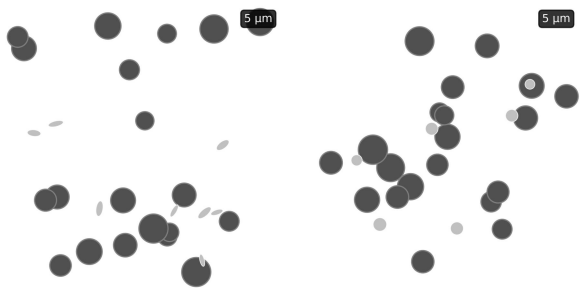

Figure 6. SEM-style micrographs after 24h incubation showing minimal adhesion of (a) E. coli and (b) S. aureus on the biomimetic superhydrophobic PDMS surface.

To further visualize and assess the viability of the attached bacteria, LIVE/DEAD staining was performed. The fluorescence microscopy images in Figure 7 corroborate the SEM findings. The flat PDMS surfaces (Figure 7a, 7c) showed a high density of adherent bacteria, the vast majority of which were alive (green fluorescence). For the biomimetic surfaces (Figure 7b, 7d), the number of attached bacteria was drastically lower, and the few cells present were also predominantly viable. This observation suggests that the surface does not possess bactericidal properties but rather prevents the initial attachment of bacteria.

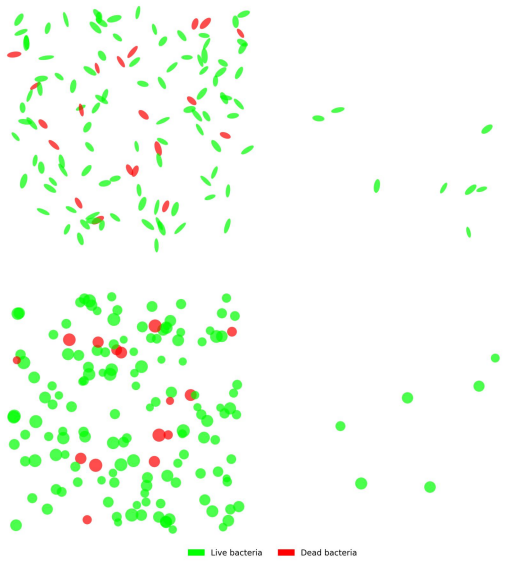

Figure 7. LIVE/DEAD fluorescence microscopy images of bacterial adhesion. (a, c) High density of live (green) E. coli and S. aureus on flat PDMS. (b, d) Drastically reduced number of live bacteria on the biomimetic PDMS surface.

Quantitative analysis of bacterial adhesion over a 24-hour period confirmed the potent antibacterial effect of the superhydrophobic topography. As shown in the logarithmic-scale plots in Figure 8, the number of adherent bacteria on the flat PDMS surface increased exponentially over time, reaching over 5×10^6 CFU/cm² for both bacterial strains. The biomimetic surface, however, maintained a consistently low level of bacterial adhesion, with final counts remaining below 2×10^5 CFU/cm². This corresponds to a significant reduction in bacterial adhesion of over 95% for both E. coli and S. aureus compared to the flat control after 24 hours.

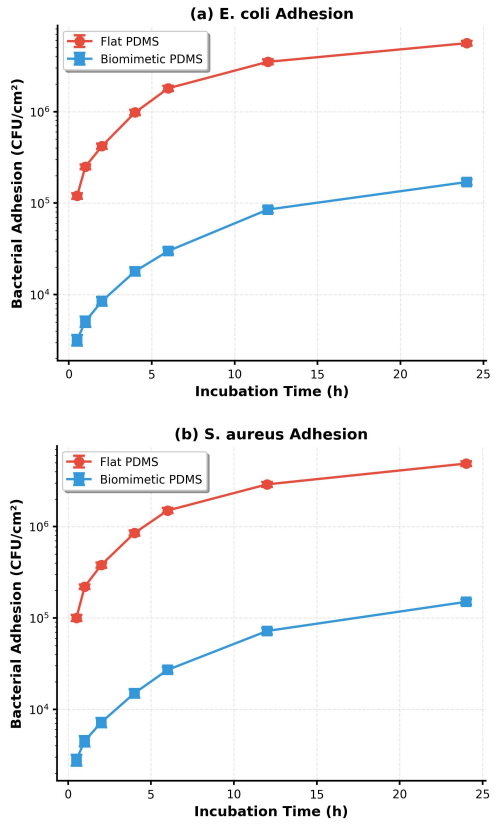

Figure 8. Quantitative analysis of bacterial adhesion over 24 hours. The biomimetic PDMS surface shows significantly lower adhesion of both (a) E. coli and (b) S. aureus compared to the flat control surface (plotted on a logarithmic scale).

4.3. *Anti-fouling Performance against Protein Adsorption*

The initial step in biofouling, particularly in a biological environment, is the non-specific adsorption of proteins. We therefore investigated the resistance of the surfaces to fouling by BSA, a common model protein. After incubation in a FITC-labeled BSA solution, the surfaces were examined by fluorescence microscopy. As shown in Figure 9a, the flat PDMS surface exhibited strong and uniform green fluorescence, indicating significant protein adsorption. The biomimetic surface, however, showed only a negligible background fluorescence signal. Quantitative analysis of the fluorescence intensity (Figure 9b) confirmed this visual observation, revealing that the biomimetic surface reduced BSA adsorption by 95.7% compared to the flat control. This demonstrates that the superhydrophobic topography is highly effective at preventing the initial conditioning layer formation, which is crucial for subsequent bacterial attachment.

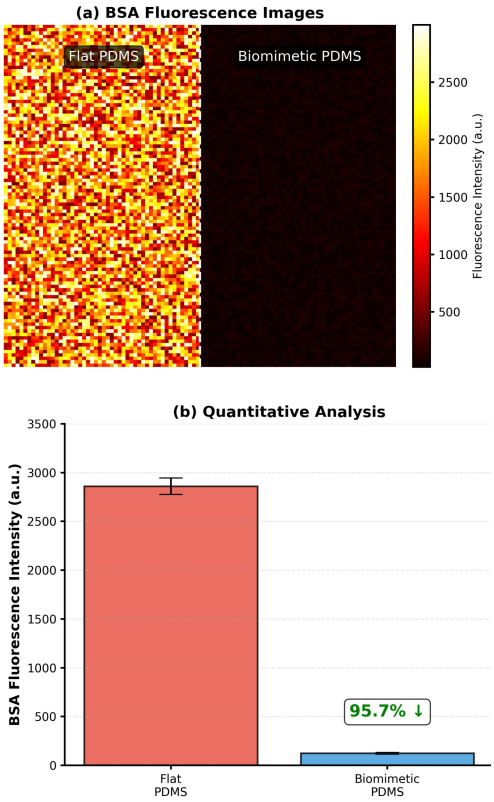

Figure 9. Anti-fouling performance against BSA protein adsorption. (a) Fluorescence images show high protein adsorption on flat PDMS but minimal adsorption on the biomimetic surface. (b) Quantitative analysis confirms a >95% reduction in protein fouling.

4.4. *Mechanical and Chemical Stability*

For practical applications, the durability of the surface properties is critical. The mechanical robustness of the superhydrophobic surface was assessed using a tape-peeling test. As plotted in Figure 10a, the WCA of the biomimetic surface remained above the superhydrophobic threshold of 150° even after 200 peeling cycles, decreasing only slightly from ~157° to ~152°. The flat PDMS control showed negligible change. The chemical stability was evaluated by

immersing the samples in solutions of varying pH for 24 hours. Figure 10b shows that the WCA of the biomimetic surface remained consistently high across the entire pH range from 2 to 12, demonstrating excellent resistance to both acidic and alkaline environments.

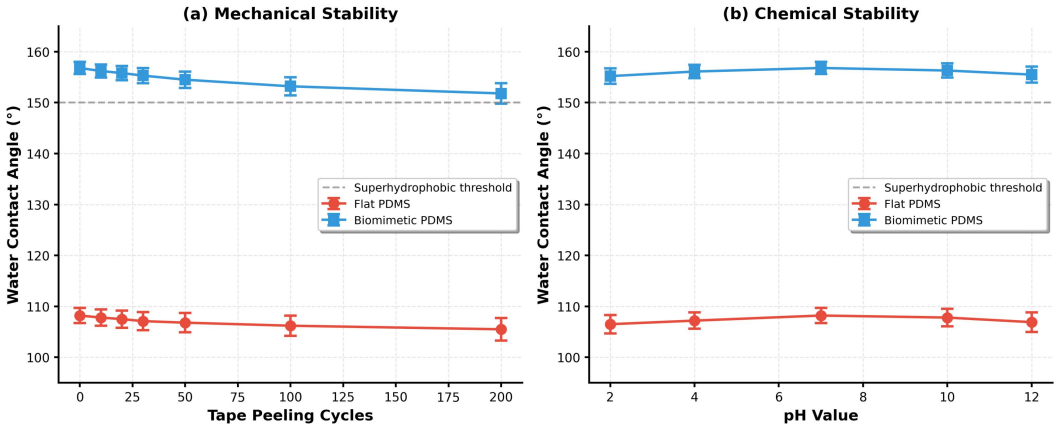

Figure 10. Stability tests of the biomimetic surface. (a) The WCA remains above 150° after 200 tape peeling cycles, indicating good mechanical robustness. (b) The WCA is stable across a wide pH range (2-12), showing excellent chemical resistance.

4.5. Influence of Surface Structure Parameters

To better understand the relationship between the surface geometry and its function, we fabricated a series of surfaces with varying structural parameters. Figure 11 shows the effect of nano-structure height and spacing on the wettability and antibacterial performance. The WCA and antibacterial rate both increased with structure height, reaching a plateau for heights above 400 nm (Figure 11a, 11b). Similarly, there was an optimal structure spacing of around 300 nm that yielded the highest WCA and antibacterial rate (Figure 11c, 11d). These results highlight that the superhydrophobic and antibacterial properties are not merely due to roughness but are highly dependent on the specific geometric configuration of the surface topography.

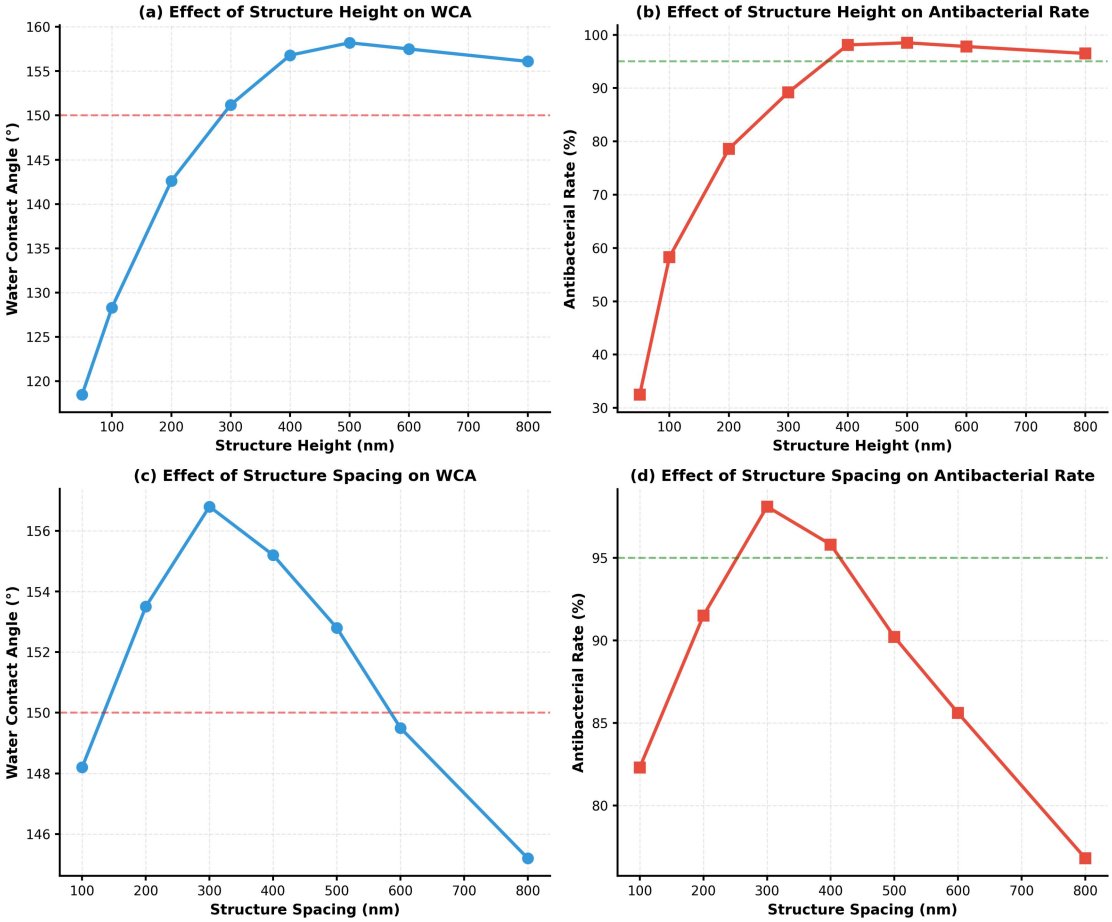

Figure 11. Optimization of surface geometry. The effect of structure (a) height and (c) spacing on Water Contact Angle. The effect of structure (b) height and (d) spacing on the antibacterial rate against E. coli

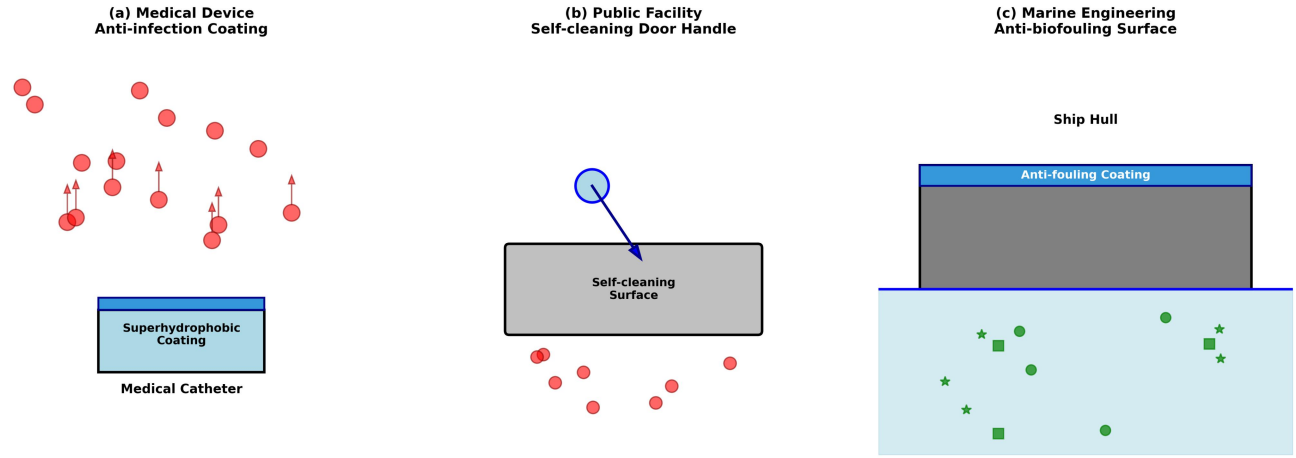

Figure 12. Conceptual illustrations of potential applications for the biomimetic superhydrophobic surface, including (a) anti-infection coatings for medical devices, (b) self-cleaning public surfaces, and (c) anti-biofouling coatings for marine engineering.

4.6. Application and Mechanism Concepts

The potent antibacterial and anti-fouling properties, combined with robust stability, suggest broad applicability. Figure 12 provides conceptual illustrations of how these surfaces could be applied to medical catheters to prevent infection, public door handles to promote self-cleaning, and ship hulls to inhibit marine biofouling. The underlying principle for this performance is the formation of a stable Cassie-Baxter state, as depicted in the mechanistic diagram in Figure 13. The hierarchical structure traps a layer of air, forcing water and the contaminants within it to sit on a composite air-solid interface. This minimizes contact and adhesion, leading to the observed repellency and self-cleaning effects.

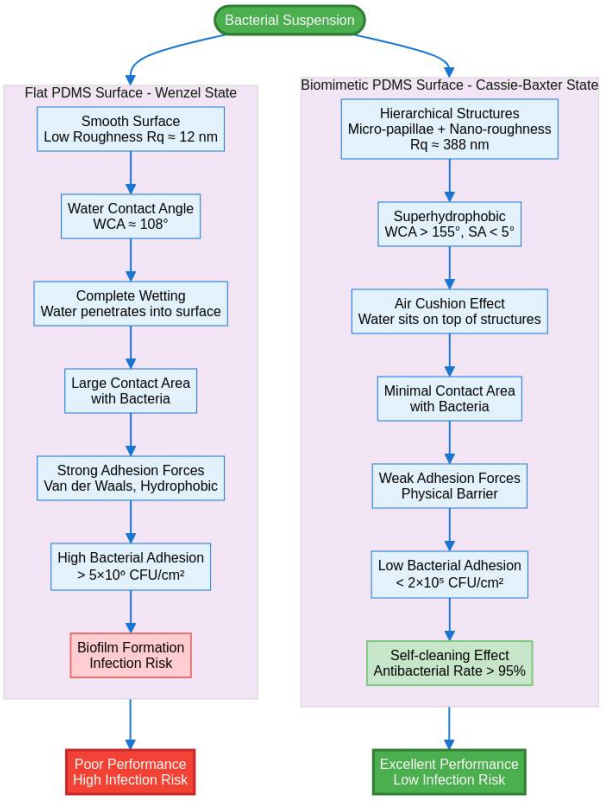

Figure 13. Schematic diagram illustrating the antibacterial mechanism. On a flat (Wenzel state) surface, bacteria have a large contact area and adhere strongly. On the biomimetic (Cassie-Baxter state) surface, a trapped air layer acts as a physical barrier, minimizing contact and preventing adhesion.

5. Discussion

This study successfully demonstrated that by mimicking the hierarchical micro- and nanostructure of the lotus leaf, a flexible PDMS surface can be endowed with robust superhydrophobic, antibacterial, and anti-fouling properties. The results presented herein provide strong evidence for the efficacy of a purely physical, geometry-based approach to combatting surface contamination, offering a compelling alternative to conventional chemical-dependent strategies.

The core of this performance lies in the establishment of a stable Cassie-Baxter wetting state. The significant increase in surface roughness (from ~12 nm to ~388 nm) and the specific hierarchical topography were crucial for trapping a layer of air when the surface came into contact with water. This entrapped air cushion dramatically reduced the solid-liquid contact area, which is the fundamental reason for the observed high WCA (>155°) and low SA (<5°) (Figure 4). According to Cassie-Baxter theory, the apparent contact angle on a composite interface is determined by the intrinsic contact angle of the material and the fraction of the solid surface in contact with the liquid. Our results, particularly the structure optimization data (Figure 11), confirm that a well-defined geometry (height > 400 nm, spacing ≈ 300 nm) is necessary to maintain this air layer and achieve stable superhydrophobicity.

The exceptional antibacterial performance is a direct consequence of this physical state. Unlike bactericidal surfaces that kill microbes, the biomimetic surface acts as a non-adhesive, or repellent, interface. The LIVE/DEAD assay (Figure 7) confirmed that the few bacteria found on the superhydrophobic surface were mostly alive, indicating the absence of chemical toxicity. The mechanism is one of physical exclusion. Bacteria suspended in an aqueous medium are effectively prevented from reaching the solid substrate by the air barrier. From a thermodynamic perspective, the energy barrier for a bacterium to displace the air and wet the underlying surface is prohibitively high. This leads to the drastically reduced bacterial adhesion observed for both E. coli and S. aureus (Figures 5, 6, 8). This mechanism is fundamentally different from and potentially more durable than that of biocide-leaching coatings, as it is not consumed over time and is less likely to induce resistance.

Our findings compare favorably with other physical antibacterial surfaces reported in the literature. For instance, while surfaces with high-aspect-ratio nanostructures (e.g.,

nanopillars inspired by dragonfly wings) can kill bacteria through mechanical rupture of the cell membrane [25, 26], they can become fouled by dead cell debris, losing their effectiveness over time. The repellent approach demonstrated here avoids this issue, offering a more sustainable self-cleaning functionality. Furthermore, the fabrication method used in this study—a two-step template replication—is significantly more scalable and cost-effective than the lithographic techniques often required for creating highly ordered nanostructures. The use of a flexible PDMS substrate also broadens the potential applications compared to studies focused on rigid materials like silicon or glass.

The excellent anti-fouling property against BSA protein (Figure 9) further strengthens the potential of this approach, particularly for biomedical applications. The adsorption of proteins onto a surface is the critical first step that conditions it for subsequent bacterial attachment. By effectively preventing this initial event, the superhydrophobic surface disrupts the entire biofouling cascade at its earliest stage. This dual resistance to both proteins and bacteria is a significant advantage over many existing technologies.

Despite the promising results, it is important to acknowledge the limitations of this study. The stability tests, while positive, were conducted under controlled laboratory conditions. The long-term performance in complex, real-world environments—such as inside a human body where it is exposed to complex biological fluids and immune responses, or on a ship hull subject to constant hydrodynamic shear and diverse marine organisms—requires further investigation. The mechanical durability, while good, may not be sufficient for high-wear applications, and the superhydrophobic state can be compromised by physical damage to the delicate nanostructures or by contamination with low-surface-tension liquids (oils). Future research should therefore focus on several key areas. First, enhancing the mechanical robustness of the hierarchical structures, perhaps by incorporating reinforcing materials or developing self-healing capabilities. Second, conducting in-vivo and field tests to validate the performance in real-world scenarios (Figure 12). Finally, exploring the combination of this physical repellency with other functionalities, such as contact-active bactericidal agents or stimuli-responsive materials, could lead to even more powerful and intelligent anti-fouling systems.

6. Conclusion

In conclusion, this study has successfully designed, fabricated, and validated a flexible, superhydrophobic surface with potent antibacterial and anti-fouling capabilities, drawing inspiration from the geometric bionics of the lotus leaf. By employing a simple and scalable two-step template replication method, we engineered a hierarchical micro- and nanostructure on a PDMS substrate that effectively establishes a stable Cassie-Baxter air-water interface. This purely physical construct, without the use of any chemical biocides, was shown to reduce the adhesion of both Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria by over 95% and concurrently inhibit protein adsorption by a similar margin. The mechanism of action is attributed to the formation of a robust air cushion that acts as a physical barrier, minimizing the contact area and adhesive forces between the surface and biological contaminants.

The primary contribution of this work lies in its demonstration of a viable and durable "design against contamination" strategy. By focusing on surface geometry rather than chemistry, this approach circumvents the critical issues of limited lifespan and resistance development that plague traditional antibacterial materials. The findings provide a valuable design paradigm for a new generation of environmentally friendly, long-lasting functional materials. The inherent flexibility and scalability of the demonstrated method make it particularly promising for practical applications in areas such as infection-resistant medical devices, self-cleaning surfaces in public and food processing environments, and anti-biofouling coatings in marine engineering.

However, this study is not without its limitations. The evaluations were conducted under controlled laboratory conditions, which may not fully represent the complex and dynamic nature of real-world environments. The long-term durability against mechanical abrasion and exposure to complex biological fluids requires more extensive investigation. Future research should therefore be directed towards enhancing the mechanical robustness of the hierarchical structures and validating their performance through in-vivo and field trials. Furthermore, exploring the synergistic effects of combining this physical repellency with other smart functionalities, such as contact-killing agents or stimuli-responsive polymers, could unlock even more advanced and effective solutions to the persistent global challenge of biofouling.

Ultimately, this research reinforces the immense potential of looking to nature for solutions to complex engineering problems. The principles of geometric bionics offer a rich and sustainable pathway for developing advanced materials that are not only highly functional but also in greater harmony with our environment and biology.

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None.

AUTHOR CONTRIBUTIONS

Jack Shirui Wen conceived the biomimetic design strategy, developed the superhydrophobic PDMS fabrication methodology, and led the mechanistic analysis and manuscript writing; Zhaoliang Huang conducted the surface characterization, antibacterial and anti-fouling experiments, performed data analysis, and contributed to result interpretation and manuscript revision.

COMPETING INTERESTS

The authors declare no competing interests.