

Superhydrophobic Surfaces to Combat Bacterial Surface Colonization

Deepu Ashok, Samuel Cheeseman, Yi Wang, Bronte Funnell, Siu-Fung Leung, Antonio Tricoli,* and David Nisbet*

The recent COVID-19 pandemic and the accelerating rise of deaths associated with antibiotic-resistant bacterial strains have highlighted the global health and economic threats caused by the super spreading of pathogens. A major route of transmission for pathogens is via surfaces contaminated by touch or droplets generated via sneezing and coughing. Current surface disinfection strategies are having diminishing efficacy, due to the increasing number of superbugs and the short-lasting effect of disinfectants resulting in recontamination. New strategies for inhibiting surface-mediated pathogen transmission are the focus of significant multi-disciplinary efforts. Among those, the development of superhydrophobic surfaces (SHS) is increasingly regarded as a powerful alternative, or additive, to antimicrobial strategies. SHS provide a neutral/inert interface that can prevent viral and bacterial surface colonization. Here, the use of such water-repellent coatings are critically reviewed to impede the surface-mediated transmission of pathogens, addressing the challenges and future directions for their translation into real-world settings.

1. Introduction

Biofouling is a process of an undesired deposition on surfaces involving biologically active organisms including bacteria, fungi, and viruses. It has become a major problem in various environments that include the formation of biofilms and accumulation of algae, mussels, and barnacles on the hull of sea vessels, and biofilm formation in shared spaces such as hospital environments, which leads to infections, requires frequent maintenance and capital loss.^[1,2] In this review, we focus on the behavior of bacterial biofilms, biofouling in clinical and non-clinical environments, and the potential of antimicrobial surfaces with a focus on the use of superhydrophobic

D. Ashok, B. Funnell, D. Nisbet
Laboratory of Advanced Biomaterials
Research School of Chemistry
and the John Curtin School of Medical Research
The Australian National University
Canberra 2601, Australia
E-mail: david.nisbet@unimelb.edu.au

D. Ashok, S.-F. Leung, A. Tricoli
Nanotechnology Research Laboratory
Research School of Chemistry
The Australian National University
Canberra 2601, Australia
E-mail: antonio.tricoli@sydney.edu.au

D. Ashok
School of Biomedical Engineering
Faculty of Engineering
The University of Sydney
Sydney 2601, Australia

S. Cheeseman, Y. Wang, D. Nisbet
The Graeme Clark Institute
Faculty of Engineering and Information Technology
and Faculty of Medicine
Dentistry and Health Services
The University of Melbourne
Melbourne 3010, Australia

S. Cheeseman
School of Science
STEM College
RMIT University
Melbourne 3000, Australia

A. Tricoli
Nanotechnology Research Laboratory
School of Biomedical Engineering
Faculty of Engineering
The University of Sydney
Sydney 2601, Australia



The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/admi.202300324>

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DOI: 10.1002/admi.202300324

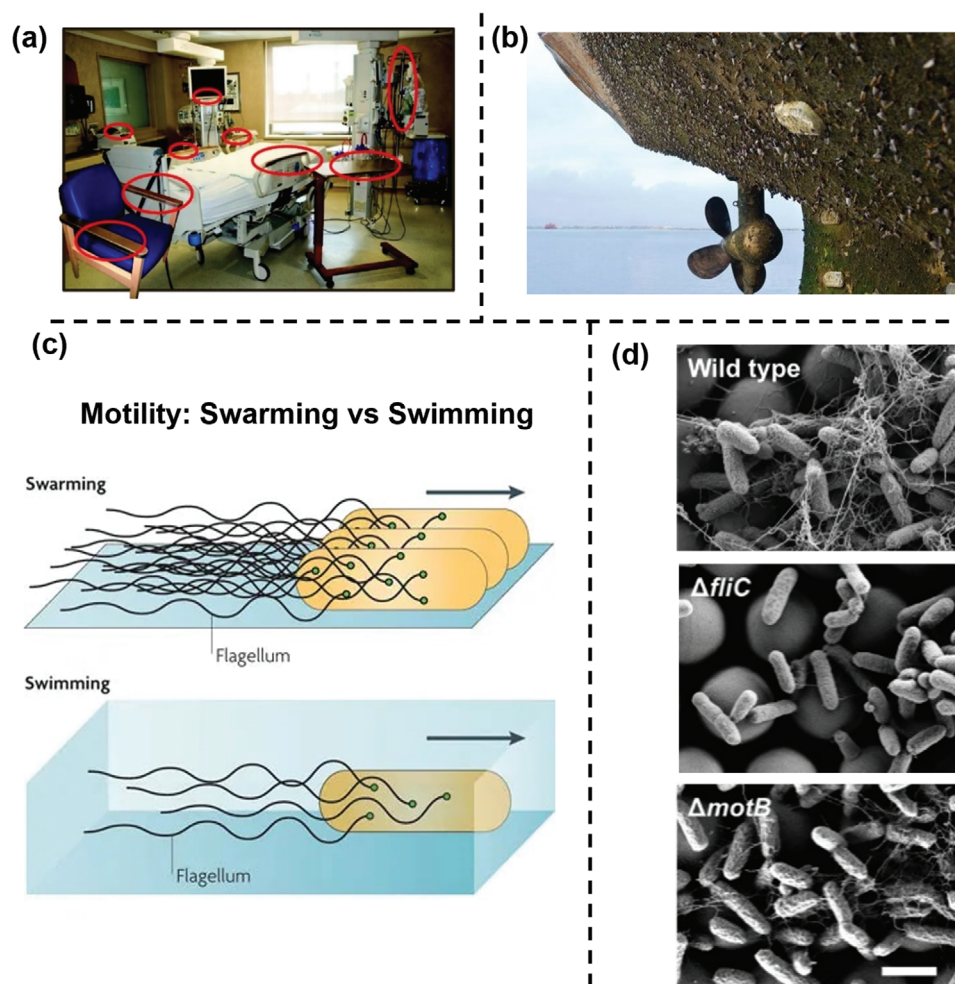


Figure 1. a) A photograph showing examples of common frequently touched sites in a health care environment, which represent areas of increased transmission of microbial pathogens.^[5] b) Photograph of a ship hull heavily fouled by marine organisms.^[32] c) Schematic showing the two major motility of bacterial cells, namely swarming on a solid surface and swimming in a liquid.^[29] d) Electron micrograph showing the biofilm formation by three different strains of *E. coli*. The biomass formed by the mutants *fliC* and *motB* was significantly less compared to the wild type. The scale bar is 2 μm .^[31]

surfaces (SHS). In one of our recent works, we engineered a robust superhydrophobic coating that can prevent the surface persistence of both bacteria and viruses. We elucidated the mechanism behind this property and investigated its failure in different conditions.^[3] The study motivated us to critically review the state of research into superhydrophobic coatings and hopefully inform a larger audience about the applicability of such surfaces to combat biofouling.

2. Biofouling

2.1. Biofouling in Healthcare Settings

Biofouling in hospitals can cause infection in patients when bacterial biofilms adhere to implanted medical devices or damaged tissue. These infections can be particularly dangerous due to the increased antibiotic resistance of biofilms.^[4] Hospital-acquired infections (HAIs) are influenced by the microbiology of the built-in health care facilities, and these infections are a leading cause

of patient deaths (**Figure 1a**).^[2,5] Commonly associated with invasive medical devices or surgical procedures, lower respiratory tract, and bloodstream infections are the most lethal, and urinary tract infections are the most common.^[6,7] The most commonly isolated microorganisms from HAIs are *Escherichia coli* and *Staphylococcus aureus*.^[8]

HAIs are thought to affect a large number of people, but unfortunately, the sparse data available from developing nations prevent conclusive statistics on the global burden to be made.^[9] A multistate point prevalence survey on 183 acute care hospitals in the United States in 2011 reported that 4.0% of inpatients had at least one HAI, yielding an estimate of 721 800 infections in the United States in 2011 and resulting in 75 000 deaths.^[10] The data drive attention to device-associated infections such as ventilator-associated pneumonia that accounted for 25.6% of all HAIs, as well as surgical-site infections that contributed 21.8% of the total infections.^[10] Allegranzi et al.^[9] found that developing nations had an average prevalence of HAIs approximately three times higher than the United States and four times higher in terms

of ICU-acquired infections. Further, the incidence of infections from medical devices in the developing world is around 16 times higher than in developed nations.^[9] In 2002, HAIs were the sixth leading cause of death in the United States.^[6,9] Estimated annual healthcare costs of the United States for HAIs are \$5 billion to \$10 billion.^[6] Estimates also say that one-third of these infections are preventable.^[11] Potential determinants of HAIs include poor environmental hygienic conditions and infrastructure, insufficient equipment, understaffing, overcrowding, a paucity of knowledge and application of basic infection control measures, prolonged and inappropriate use of invasive devices and antibiotics, and scarcity of local and national guideline policies.^[12] Among hygiene, the most important, but also one of the most neglected factors, is hand hygiene.^[13,14]

A leading cause of HAIs today is a result of biofilm colonization of health care infrastructure and implanted devices.^[15] Biofilm bacteria are significant contributors to human disease and the number of diseases associated with bacterial biofilms is notable, with colitis, vaginitis, urethritis, conjunctivitis, and otitis as common examples.^[16] Biofilm bacteria also have increased drug resistance due to factors such as protection by extracellular polymeric substances, reduced metabolic and growth rates which prevent action by antibiotics that target rapidly multiplying cells, and altered physiology of biofilm bacteria that activates specific resistance genes.^[16] Biofilm infections of antibiotic-resistant “superbugs” are difficult to eradicate, and the problem is worsened by the dry pipeline for new antimicrobials against gram-negative bacteria.^[4,7,17,18] Gram-negative strains have several mechanisms of resistance against antibiotics, often using multiple strategies against one antibiotic or a single mechanism against multiple antibiotics.^[6] Infections caused by gram-negative strains have characteristics of deleterious nature and form more than 30% of the HAIs in the United States.^[6,19] This poses a threat to the efficacy of antibacterial techniques to combat increasing drug resistance.

2.2. Biofouling in Non-Healthcare Settings

Marine infrastructure, once exposed to seawater, is quickly fouled by marine microorganisms and later by macro-fouling species such as mussels and barnacles.^[13,20] Biofilms create serious problems for marine industries worldwide with effects including an increase in drag force leading to greater fuel consumption, corrosion of metal, and reduction in heat transfer efficiency.^[13,21–24] Although marine biofouling is less visible in society, the problem has been staggering for the shipping and water treatment industries, where ship hulls, membrane structures, and many other surfaces can become fouled and negatively affects their performance (Figure 1b).^[22,25] For example, ships have been estimated to require ≈80% more power to maintain a cruising speed due to the drag created from heavy fouling on their hull.^[26] This is a major issue in the shipping industry where fuel is estimated to account for around 50% of running costs.^[27] This also, indirectly contributes to environmental pollution due to the increased CO₂ emissions. It is estimated that the US Navy alone costs an extra 1 billion US\$ annually due to biofouling.^[22] Marine microfouling biofilms commonly contain heterotrophic proteobacteria and cyanobacteria.^[13] Bacteria not only colonize the surface but also

act as important initiators for the successful settlement of marine invertebrate larvae.^[22] In the first stage, marine bacteria and soft fouling unicellular algae colonize and condition the surface, the growth rate of which is slower and takes hours.^[20,22] This stage is followed by fouling by complex organisms like barnacles, mussels, and tubeworms.^[22]

With a universal trend toward urbanization, human beings have been increasingly inhabiting the artificial environment.^[2] Our life revolves around our homes, workplaces, and the public environment—spaces that are intimately shared with microorganisms as well.^[2] This has facilitated increased interactions between human beings and pathogenic microorganisms, leading to an increased incidence of infectious diseases.^[28] In the following sections, we discuss the antimicrobial resistance of biofilms and methods to eradicate drug-resistant biofilms.

3. Bacterial Motility

In between the liquid media and on a solid surface, bacteria exhibit two major types of motility—swimming and swarming, which are schematically shown in Figure 1c.^[29] Swimming, as the definition suggests, is the flagella-assisted movement of planktonic bacteria in a liquid (Figure 1c).^[29] Swarming is again flagella-mediated, but is the multicellular movement of bacteria across an energy-rich, solid medium surface, powered by their rotating flagella (Figure 1c).^[29] Unlike biofilm formation where surface bacteria aggregate in a sessile fashion, swarming is often oppositely regulated.^[29] Bacteria are found to decide on either swarming motility or biofilm formation depending on favorable conditions for survival.^[30] Both processes tend to involve similar components, for example, flagella are required for reversible attachment in biofilm formation and can be crucial for biofilm structure, however, are also involved in the detachment of bacteria from biofilms through swarming motility.^[30]

Interactions of bacteria on a surface are influenced by the physical and chemical environment, and thus these conditions provide valuable cues to engineering surfaces that can prevent bacterial adhesion.^[30,31] For example, Friedlander et al.^[31] studied the adhesion of wild-type *E. coli* and its mutants on both flat and patterned polydimethylsiloxane (PDMS) surfaces to understand the role of flagella and its motility in colonization (Figure 1d).^[31] The two mutant strains *fliC* (lacking flagella) and *motB* (deletion of motor protein enabling flagellar rotation) had significantly less biofilm mass on the surfaces than the wild type, indicating a reduction in adhesion (Figure 1d).^[31] This suggests that for biofilm formation, both flagella and motility play an important role.^[31]

4. Bacterial Biofilms

Bacteria attach to solid surfaces and colonize, forming a hydrated matrix mostly composed of self-produced polysaccharides.^[18] These sessile communities of bacteria are called biofilms.^[18] Bacteria are capable of colonizing a wide range of surfaces by forming biofilms and have been found at extreme conditions such as temperatures from –12 to 110 °C and pH values between 0.5 and 13.^[21] In addition to this, bacteria in biofilms produce chemical compounds that may induce or inhibit the settlement of other fouling organisms.^[13]

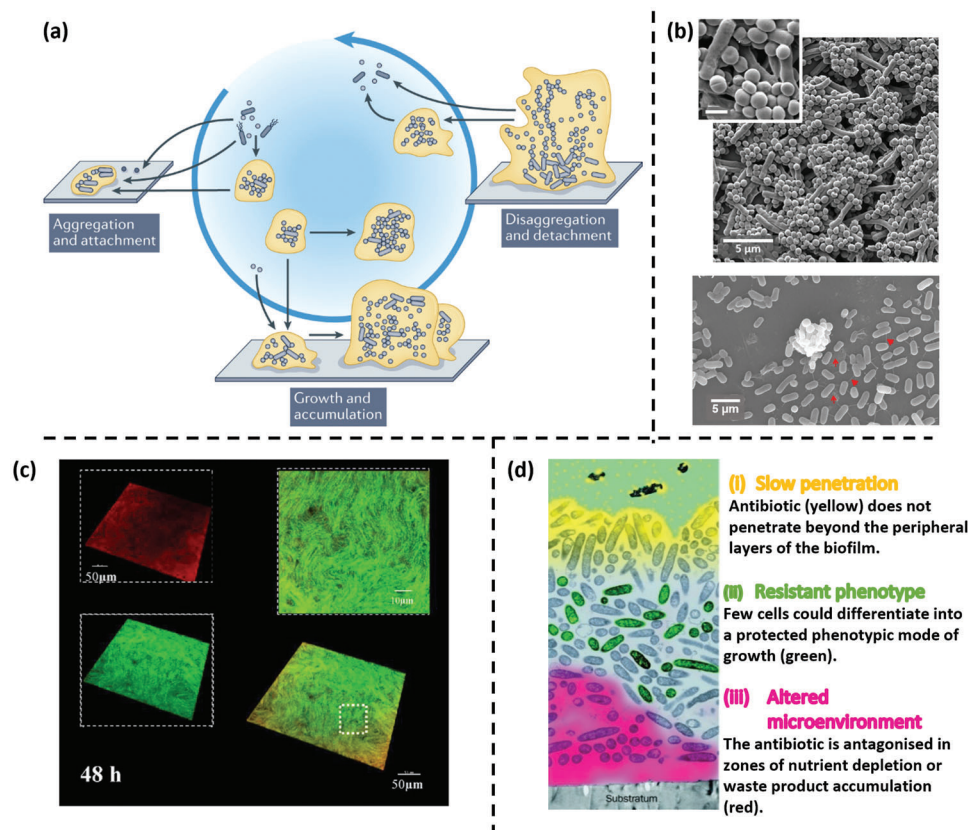


Figure 2. a) Schematic showing the lifecycle of a biofilm in 3 stages.^[36] b) Electron micrographs of mixed species biofilms. *S. aureus* and *Bacillus cereus* (top)^[39] and *S. aureus* and *Salmonella enterica* (bottom).^[41] c) Confocal laser scanning microscopy (CLSM) images of *Bacillus subtilis* following 48 h growth. The biofilms are stained with TOTO-1 for eDNA (green) and SYTO 60 for bacteria (red). The two channels are displayed separately (left) and merged (right).^[40] d) Schematic showing the 3 different hypothesised mechanisms on how biofilms develop resistance to drugs.^[4]

In natural environments, especially in aquatic ecosystems, bacteria have a propensity to colonize surfaces, which is an advantageous survival mechanism in terms of defense and finding nutrition compared to their free-ranging dwellers or planktonic form.^[18,19] **Figure 2a** shows a schematic representation of the three stages of the biofilm lifecycle. Biofilms of bacteria are ubiquitous on various surfaces in nature and are the major reason for ongoing issues, such as food contamination and spreading illnesses.^[18,33] Heterogeneous circulation of nutrients and chemical signals throughout the polymeric matrix results in different gene expressions by cells in different regions of a biofilm, resulting in phenotypically diverse cells within the biofilm.^[34,35] This allows biofilms to have a protected mode of growth in hostile conditions.^[18] Due to the pattern of gene expression that varies from the boundary to the center of a biofilm, and also the complexity of its structure and metabolism, biofilms are considered analogous to multicellular tissues of higher organisms.^[18,34] The presence of a biofilm can also act as a source of nonsessile planktonic bacteria that can disperse to other surfaces, attach and multiply, leading to the formation of new biofilms (Figure 2a).^[36]

The formation of biofilms has a deep significance in the ecosystem, proving to be both beneficial and destructive to other organisms.^[37] Due to the complexity of biofilms, including their mode of formation, an immense number of studies have taken place over the years to understand the initial attachment process

of bacteria as well as the generation of drug resistance.^[4,18,33,38] Electron micrographs of *S. aureus* (Gram-positive) and *Bacillus Cereus* (Gram-positive) (top)^[39] and *S. aureus* (Gram-positive) and *Salmonella enterica* (Gram-negative) (bottom), presented in Figure 2b, shows the dense multicellular aggregates of bacteria that are held together by diffuse extracellular polymers.^[4] Confocal laser scanning microscopy (CLSM) images of *Bacillus subtilis* are shown in Figure 2c.^[40] The following sections present an overview of the mechanisms behind biofilm formation and the generation of drug resistance. Further, a summary of the literature on antifouling strategies against biofilm formation in different environments is discussed.

4.1. Bacterial Adhesion and Biofilm Formation

The formation of biofilms begins with the adhesion of a few planktonic cells to a surface (Figure 2a).^[42] This interaction is primarily influenced by the electrostatic charge and hydrophobicity of the abiotic surface material, and also the charge of the bacterial cell wall, among other factors.^[42,43] In the case of living tissues, adhesion is dictated by specific molecular docking mechanisms (lectin, ligand, or adhesion).^[33,44] Any surface, biotic or abiotic, are potential sites for bacteria to attach and do not necessarily need to be a nutrient-rich surface. The two major phases of

bacterial adhesion are the primary docking phase and the secondary locking phase.^[33,44,45]

The primary phase is a reversible phase where the organism has to be in proximity to the surface (Figure 2a).^[33,44,45] When a cell comes into close contact with a surface (typically <1 nm), the forces present include electrostatic and hydrophobic interactions, steric hindrance, temperature, hydrodynamic forces, and van der Waals forces.^[33,43]

The secondary bacterial adhesion phase, or locking phase, involves molecularly mediated binding between the surface and the cells via specific adhesins (Figure 2a).^[33,44,45] The cells start producing exopolysaccharides that are complex with the surface aiding the cells in firmly attaching to the surface.^[33] At the end of this stage, adhesion becomes irreversible and requires significant physical or chemical treatment to remove.^[33] For example, the current treatment for biofilms formed on medical implants commonly involves long-course antibiotic therapy or revision surgery, both treatment strategies have the potential for additional health complications.^[46] Once the biofilm matures and reaches a critical mass, a dynamic equilibrium is reached where individual cells start detaching and forming colonies at different locations (Figure 2a).^[33]

The interactions between bacteria and a surface are traditionally explained using the DLVO theory. The theory suggests that the net energy of adhesion comes from the van der Waals attractive forces and the electrostatic repulsive forces. The theory does not take into consideration short-term interactions such as Brownian interactions and polar interactions (e.g., hydrophobic interactions). However, these additional forces can play an important role in cell-surface interactions, for example, it is important to consider hydrophobic/hydrophilic interactions in aqueous environments.^[33,47] Therefore an extension to the original DLVO theory, called the XDLVO theory, has been proposed to take into consideration these additional short terms interactions.^[48] In the new approach, the total free energy of interaction between two surfaces immersed in an aqueous medium is the sum of the Lifshitz–van der Waals forces, Brownian movement forces, electrostatic repulsive forces, and polar interactions. These interactions are dependent on the distance of separation between the interacting entities and their geometry. In the case of bacterial adhesion to a solid surface, a flat plate/sphere geometry is generally assumed. This is because the dimensions of the surface are usually several orders of magnitude above those of an individual cell.^[47,48]

The above-mentioned models are only reasonably good at predicting bacterial adhesion to a surface. The deviation is due to the complexity of the cell-adhesion process.^[42,49] Solid materials in various environments do not expose their bare surfaces and are usually conditioned by the adsorption of various organic and inorganic matter on them even before microorganisms adhere.^[47] The interaction between bacteria and these conditioning films significantly differs from that on a bare solid surface. The adhesion models also ignore the presence of appendages (e.g., Pili and flagella) on the bacterial cell wall. Bacteria use these appendages to pierce the energy barrier described by the DLVO theory, resulting in a deviation from the model.^[47] Notably, there is still a lot unknown about bacterial cell adhesion and this represents an important area for future fundamental research to better under-

stand the adhesion process and help design strategies to mitigate bacterial adhesion and colonization.

4.2. Antimicrobial Resistance of Biofilms

In a clinical setting, biofilms are a hazard due to their ability to develop resistance to antibiotics.^[4] Biofilms form a protected form of growth and unlike their planktonic form, cells in a biofilm can adapt to antimicrobial agents over time.^[4,38] When it comes to implant-associated infections, it is essential to recognize the multicellular nature of biofilms that behave entirely differently from planktonic bacteria.^[4] The rise of such antimicrobial-resistant strains is a significant challenge.^[50] In some cases, very high concentrations of antibiotics are required to kill the bacteria within biofilms. For instance, Luppens et al.^[51] demonstrated that chlorine, an extremely effective killing agent, required a 600-fold increase in concentration to kill the biofilms of *S. aureus* compared to their planktonic cells.^[51] These concentrations could be inimical to mammalian cells, representing an issue for the clinical treatment of biofilm infections. Resistance to antibiotics in planktonic cells typically arises via genetic mutations and can be passed on via vertical or horizontal transmission.^[52] In addition to this genetic basis of resistance, biofilms demonstrate several other mechanisms of resistance where even cells that are susceptible in their planktonic state can demonstrate resistance.^[4,38,53] Three mechanisms of biofilm resistance to antimicrobial agents are described below and schematically shown in Figure 2d.^[4,33]

- The first hypothesis explains antibiotic resistance due to slow or incomplete penetration of the antibiotic into the biofilm (Figure 2d).^[4] The extracellular polymeric substance matrix absorbs the antibiotic, reducing the amount of antimicrobial agent reaching the cells by deactivating the antibiotic in the biofilm surface layers.^[4] Therefore, antibiotic penetration is prevented as the antibiotic is deactivated at a higher rate than it diffuses.^[4]
- The second hypothesis of antibiotic resistance is that a subpopulation of microorganisms in a biofilm differentiates and forms a unique and protected phenotypic state (Figure 2d).^[4] It is suggested that some of the bacteria enter into a spore-like state which helps them to survive when the rest of the population exposed to antibiotics are rapidly killed.^[4,54] These cells are commonly noted as persister cells.^[54,55] This mechanism is also supported by studies that show resistance in newly formed biofilms that are too thin to be resistant due to incomplete penetration as suggested in the above hypothesis.^[56]
- A third hypothesis suggests antimicrobial resistance arises due to the biofilm having an altered chemical microenvironment (Figure 2d).^[4,38] For example, local accumulation of acidic waste products could change the pH difference between the bulk fluid and biofilm interior to greater than 1, deactivating the antibiotic action.^[4]

While there have been concerted efforts to investigate different methods to eradicate already formed drug-resistant biofilms, such as via magnetic nanoparticles,^[57] it is more desirable to develop strategies that prevent their initial onto surfaces. This can

be achieved by engineering surfaces with precise chemistry, topography, and surface energy, an example being superhydrophobic surfaces (SHS).^[42] SHS have recently been investigated for their antimicrobial capability due to the nanostructured surface and low affinity for water.^[31,42,58] Developing multifunctional antibacterial surfaces could meet the requirements for preventing fouling in marine and health care environments.

5. Antibacterial Surfaces

Conventional antibacterial surfaces are based on three major mechanisms: release-based surfaces,^[59,60,61,62] contact killing,^[58,63,64] and anti-adhesion/bacteria repelling.^[63,65] The following sections briefly discuss the anti-adhesive and release-based mechanisms of antibacterial surfaces.

5.1. Bacteria-Repellent Surfaces

Anti-adhesive or anti-biofouling coatings function by preventing the earliest step of biofilm formation,^[65a] without the use of chemicals that are toxic to bacterial cells.^[65a] Since the first stage of biofilm formation is the reversible adhesion of bacterial cells, preventing adhesion is a good strategy to inhibit biofilm formation.^[33,63] Anti-adhesive properties can be generated by designing unfavorable surface topography or surface chemistry, and this mechanism can prevent biofilm formation for relatively long periods of time.^[63,65a] Surface immobilization of molecules like polyethylene glycol (PEG) and zwitterionic materials can act as an antifouling layer, forming the standard approach for the majority of such coatings.^[66] Surface modifications to alter the topography to extremely low surface energy have also been investigated as an anti-adhesion approach.^[67] These kinds of surface modifications are largely inspired by nature, hence called biomimetic surfaces.^[63]

5.1.1. Superhydrophobic Surfaces (SHS)

Biomimetic design, or bio-inspiration, is a fundamental theme of bioengineering. Nature offers tremendous opportunities for inspiration to solve many issues facing mankind. It is thought that many of the surfaces in nature have evolved over billions of years to possess bacteria-resistant properties. Natural nanostructured surfaces that inspired research into bacteria-resistant surface designs include gecko skin,^[68] lotus leaf,^[69] insect wings,^[70] and a few others.^[58,63] Superhydrophobicity is a surface property commonly found in nature where water readily forms droplets (with static water contact angles $>150^\circ$) resulting in a self-cleaning effect as the droplet slides off from the surface easily (i.e. contact angle hysteresis $<10^\circ$) carrying away any particulate contamination, resulting in a self-cleaning effect.^[71,72,73] These extreme water-repellent characteristics of a surface are attributed to a simple combination of high roughness and non-wetting surface chemistry. This combination results in the formation of a Cassie-Baxter state where a thin air film, also known as the plastron layer, forms between the solid surface and liquid, preventing the liquid from wetting the solid

(Figure 3a).^[69] Nature-inspired SHS has received huge attention in the last few decades due to its potential for numerous applications such as microdroplet manipulation,^[74] infrastructure components to reduce exposure to damaging elements,^[75,76] blood-repellent surfaces,^[77] heat transfer enhancements,^[78] reducing drag losses while transporting liquids,^[79] gas trapping and its transport,^[80] lubricating surfaces,^[81] flood control^[82] and as adjuvants for local drug delivery.^[83]

Biofilm formation starts with the adhesion of a few planktonic cells to the surface and the bacterium-surface interaction is dictated by surface charge and hydrophobicity.^[84] Superhydrophobic coatings, due to their nanopatterning and low affinity for water, resist the initial adhesion of bacteria that reside in an aqueous environment (water droplets, biological fluids, etc.). The presence of surface roughness suppresses the effect of short-range DLVO forces, especially the attractive van der Waals interactions, making superhydrophobic surfaces deviate from the traditional model.^[85] Other XDLVO models on particle interactions on superhydrophobic surfaces reported a dominance of repulsive electrostatic double-layer interactions over the net attractive interactions coming through van der Waals and Lewis acid–base interactions.^[86] Hence, particles like bacteria and protein experience an overall repulsion when approaching such a surface.^[86] This is widely observed in nature, a few of the examples being the lotus leaf, rice leaf, dragonfly wings, and duck feathers.^[87] Over the years, scientists have tried to mimic such examples from nature to develop antifouling technologies.

Studies about bacterial colonization on SHS are summarized in Table 1 and some examples are presented in Figure 3. For example, the titania nanotube functionalized with organic fluorine (Figure 3b) offers superhydrophobic repulsion to gram-negative bacteria (Figure 3c).^[88] The most comprehensive study to date was by Hizal et al.,^[90] who developed a fluorinated-nanopillar aluminum SHS.^[90] They performed bacterial adhesion studies under static and dynamic situations.^[90] The conclusion is that under a dynamic flow, the nanopillars perform over 99% of reduction in adhesion for both gram-positive and negative bacteria.^[90] This is attributed to the high aspect ratio of the nanopillars and a resulting strong dewetting nature in both static as well as dynamic conditions, irrespective of the bacterial strain (Figure 3d).^[90] The adhesion force of a bacterium to the nanopillared SHS is also calculated to be the lowest, in comparison to the flat and nanoporous surfaces (Figure 3e).^[90] Despite the promising results, superhydrophobic surfaces are metastable underwater, and the plastron has been found to disappear upon immersion in water to a certain depth.^[91] Hence, additional functionalization is needed for an SHS to be effective in wet environments such as marine applications.

5.1.2. Fabricating Superhydrophobic Surfaces

Superhydrophobicity can be imparted on surfaces through unique surface topographies or surface chemistries, or a combination thereof. There are many different materials and methods that can be utilized to fabricate superhydrophobic surfaces. This section will provide a brief overview of these processes and interested readers are recommended the following comprehensive reviews.^[73,98–100]

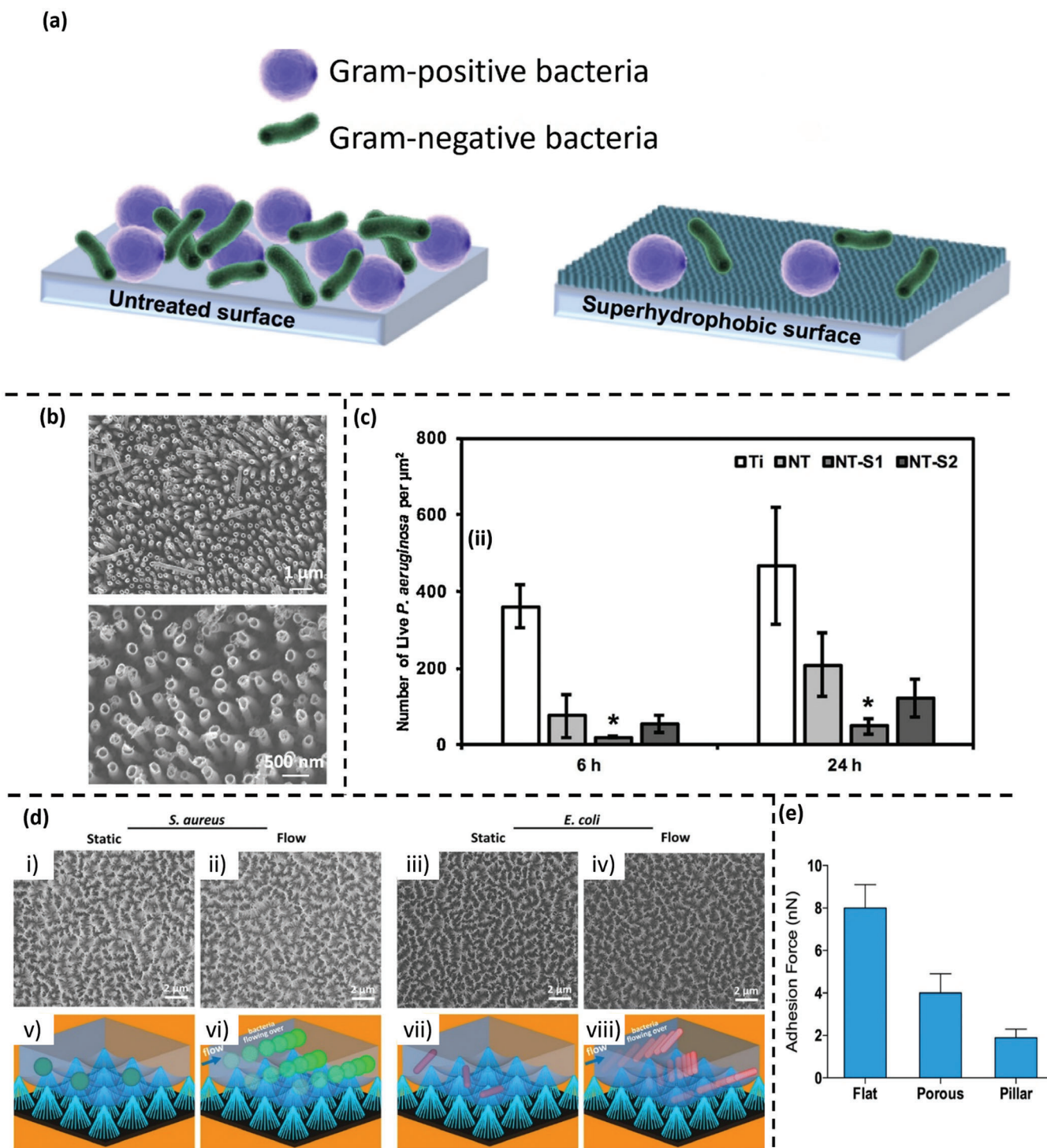


Figure 3. Monofunctional antibacterial SHS. a) Schematic showing the repellent effect of superhydrophobic coatings toward bacteria.^[68] b) Electron micrographs of an SHS made of fluorinated titania nanotubes at two different magnifications^[88] and c) the reduction in the adhesion of bacteria after 6 and 24 h on it (NT-S1).^[88] d) (i–iv) Electron micrographs of superhydrophobic nanopillars of aluminum after static and dynamic exposure to gram-positive and gram-negative bacteria, showing no attached cells. v–viii) Schematics showing the plastron effect of SHS resulting in easy removal of the cells.^[89] e) Plot showing the measured force of adhesion between bacteria and three different surfaces, with the superhydrophobic pillared surface registering the least value.^[89]

Table 1. List of the previous studies on monofunctional antibacterial SHS.

Coating characteristics	Test of antibacterial properties	Experiment duration	% Reduction in adhesion		Inference	Reference
			Gram+ve	Gram-ve		
Teflon coating applied on nanopillared and nanoporous surfaces of aluminium	– Parallel plate flow chamber – Static assay: Substrates were held inside the chamber with the bacterial suspension – Dynamic assay: The bacterial suspension flowed through the chamber at a certain rate	1 h	99.9% for dynamic assay, 98% for static assay	99.4% for dynamic assay, 99% for static assay	The high aspect ratio and the resultant roughness of the 3D pillars provided minimized contact area for bacteria, hence the reduced adhesion (Figure 3d)	[90]
A thin film of silicone elastomer of high roughness	Submersion in the bacterial suspension	1 h	80%	58%	The static assay was performed and the coated surface had a significant reduction in adhered bacteria	[92]
Xerogel coating of nanostructured fluorinated silica colloids, fluoroalkoxysilane and a silane backbone	– A parallel plate flow cell – Dynamic assay: The bacterial suspension flowed through the chamber at a certain rate	1.5 h	99%	98.2%	The highly rough fluorinated surface provided reduced contact area for the bacteria	[93]
Titanium nanotubes coated with (heptadecafluoro-1,1,2,2-tetrahydrodecyl) trichlorosilane	Substrates in a well plate were treated with 1 mL of bacterial suspension	6 h and 24 h			After 24 h of culture, there was a reduction in adhesion and no biofilm formation was observed on the superhydrophobic surfaces, whereas the uncoated surfaces had both bacteria and biofilms present	[88]
Nanorough titanium surfaces fabricated by femtosecond laser ablation (Figure 3b)	Incubation of the surface with a droplet of bacteria on it	18 h	–	–	Antiadhesion against a gram-negative strain only which could be due to the reduced surface area available for colonization (Figure 3c)	[94]
Facile multilayer deposition of poly-diallyl dimethylammonium chloride and silica particles, followed by a fluorination surface treatment	– Spraying bacteria solutions on surfaces and tilting the surface (5°), followed by incubating the surface in media – Spraying bacteria on the surface, washing with water and transferring samples to the media	– Tilting the surface 5 s after the spray, followed by 24 h of incubation. – 1 s of washing followed by incubation for 24h		93% reduction for the tilted sample and over 99% reduction for the washed sample	– After tilting increased to 90°, no bacterium remained on the surface, showing bacteria cannot contaminate an SHS under this condition – The SHS showed antiadhesion against bacteria in both assays	[95]
Picosecond laser textured hierarchical AISI 420 martensitic stainless steel	Samples were immersed in the bacterial culture for 10 minutes, taken out, dried and imaged under CLSM	Dipping cycles of 10 minutes	99%	93%	The hierarchical micro-nano structures on the steel and the robust air layer prevent bacterial adhesion	[96]
Spray coating of saturated fatty acids	Samples were incubated in the culture and live-dead staining was performed	48 h	100%	90-93%	Safe and sustainable design of organic coatings form hierarchical structures with antifouling properties	[97]

Typically surface nanoroughness is required for superhydrophobicity, which may be combined with microroughness to form a hierarchical roughness.^[98,101,102] Nanoroughness elicits superhydrophobicity by trapping small pockets of air that reduce the contact area that water can interact with. Nanoroughness can be applied to the surface through different methods.^[98,103] Most commonly, the nanostructure is created through the immobilization of nanoparticles on a surface.^[104] An intermediary layer, such as a polymer, is commonly applied between the nanoparticles and the surface to be functionalized.^[101,105] Other strategies include top-down methods, where the surface is eroded or patterned in a way to reveal nanorough features. This can be achieved through lithography, etching, and templating. Lithography relies on exposure of resists to light or electrons through a patterned mask, causing the resist to be removed (positive) or remain (negative).^[106] The non-resist coated section of the surface is then etched, creating a micro or nanopattern. This technique creates well-defined structures and is very useful for fundamental superhydrophobic studies to assess the influence of carefully tuned parameters,^[107] however large-scale manufacturing of this process can be expensive. It is also possible to etch surfaces without using lithography, which is less controllable though etching parameters can still be modulated to change the aspect ratio and resolution of surface structures.^[108] Finally, templating relies on a template to be formed which is then stamped into the material of interest. This can work well with polymer films; however, nanoscale resolutions are limited by the achievable resolution of the replica template.^[109] In contrast to the previous strategies, electrodeposition and chemical vapor deposition (CVD) are bottom-up approaches. Electrodeposition results in metallic surface coatings by exploiting the movement of ions in solution toward a charged surface to coat.^[110] Substrates are placed in an electrolyte solution and an electrical circuit is created where the substrate to be coated acts as the cathode.^[110] Controlling these parameters can result in surface superhydrophobicity.^[111] CVD involves exposure of the substrate surface to gas-phase precursors which react, resulting in the deposition of a thin film.^[112] Modifying parameters such as pressure flow rate and substrate temperature, enable controllable nanostructuring which can enable superhydrophobicity.^[103,112] Finally, superhydrophobic surfaces can also be made using hydrothermal,^[113] sol-gel,^[114] layer-by-layer deposition,^[115] and wrinkling methods.^[116]

Surface chemistry is also important, non-polar materials can be employed such as hydrocarbons that are inherently hydrophobic. As discussed earlier, low surface energy materials are also beneficial for fabricating superhydrophobic surfaces. To achieve this, materials are often functionalized with fluorine groups, which possess very low surface energy due to the high strength of the carbon-fluorine group. Combining a low surface energy functionalization (such as fluorine) with a surface nanostructure creates a synergistic effect that can also repel other liquids such as oils. For example, Huang et al.^[117] developed a superomniphobic surface coating utilizing a combination of fluorinated polydimethylsiloxane (PDMS), fluorinated silicon dioxide (FSiO₂), and a fluorinated polymer consisting of perfluoroalkyl acrylate, alkyl acrylate, and vinyl chloride.^[117] This coating was applied to substrates by a sequential spray-coating procedure and resulted in contact angles >150° with water and hexadecane

(oil). Polysiloxanes are an alternative to fluoro-functionalization, which researchers and companies are moving away from due to the emerging research into the devastating impact of “forever chemicals”.^[99] Polysiloxanes are comprised of organic groups, often an alkyl group, attached to a silicon-oxygen backbone. The most well-known example is PDMS.

5.2. Bactericidal Surfaces

Bactericidal surfaces work by releasing bactericidal active materials that kill both adhered and adjacent planktonic bacteria in the vicinity of the coating.^[65a] The release of the loaded bactericidal agents is achieved by diffusion into the aqueous medium, hydrolysis of covalent bonds, or erosion/degradation.^[118] This approach releases a high concentration of killing agents locally, controlling release kinetics so that the concentration does not surpass systemic or ecological toxicity levels.^[65a] Thus, an antibacterial effect is created, where it is needed, hence minimizing the development of resistance and harmful systemic repercussions.^[65a] However, since coatings that operate through the release-based mechanism only have a limited reserve of antibacterial agents, the surfaces become exhausted of the required molecules and hence their antibacterial action is only temporary.^[65a] The main challenges, and therefore opportunities for improvement, of release-based surfaces are identified as controlled release (the ability for antibacterial agents to only be released when in contact with bacteria), multifunctionality (additional mechanisms such as multiple release mechanisms or antimicrobial agents) and the long-term stability (such as improved physical and chemical resistance).^[65a]

Over the past decade, there has been a significant development in a broad range of biocide-releasing coatings.^[65a] Common fabrication methods include simple impregnation, coating an antimicrobial compound, or soaking a porous material.^[65a] Fast release is caused by a lack of a particular bonding mechanism within the coating.^[65a] Widely used biocide agents include metal and metal oxide nanoparticles (NPs) like silver,^[119] copper, zinc, and their oxides,^[118,120,121,122,123] antibiotics like vancomycin,^[124] nitrogen dioxide,^[125] metal-organic frameworks,^[126] metallic glasses^[62] and graphene.^[61] The frequently used carrier materials for antibacterial agents include poly(methacrylic acid) (PMMA), polyacrylic acid (PAA), poly(lactic-co-glycolic acid) (PLGA), and polyurethane.^[118] The NPs could themselves disrupt the cell membrane depending on its size, or release ions or reactive oxygen species that compromise the integrity of the cell wall, enter the cytoplasm, and disrupts the genetic material in it.^[120,123,127] For instance, functionalized silver nanoparticles were adhered to ϵ -polylysine-g-butyl, thereby releasing silver ions. This nanocomposite significantly inhibited both *Pseudomonas aeruginosa* and *S. aureus*.^[122] The readers are advised to go through detailed and excellent reviews on released-based antibacterial surfaces.^[60,65a] In the next section, we discuss the research on bifunctional SHS with antibacterial properties.

6. Bifunctional Antibacterial Superhydrophobic Surfaces

Bifunctional SHS are superhydrophobic surfaces with an additional functionalization, for example, incorporating biocides with

a release-based mechanism into the coating.^[128] This is advantageous to applications involving long exposure to a wet environment, as the release of biocides kills fouling cells that colonize damaged areas of the coating.^[128] For example, metallic silver nanoparticles have been widely investigated for their antibacterial properties, as the nanoparticles release silver ions that disturb the permeability and respiration functions of cell membranes, as well as target the process of DNA replication in the cell, causing cytoplasm leakage and even cell death.^[128,129] **Figure 4a** schematically demonstrates the mechanism behind a bifunctional superhydrophobic surface. Short-term protection from bacterial colonization is created by bacterial solution roll-off, due to the low liquid sliding angle of superhydrophobic surfaces (**Figure 4a(i)**). Long-term protection is provided by biocidal agents embedded in the micro-nano texture that is released when the plastron collapses, to attack bacteria in the vicinity of the coating (**Figure 4a(ii)**).^[65a,128,130]

Table 2 summarizes investigations into bifunctional superhydrophobic surfaces. For example, Ren et al.^[131] fabricated a transparent fluoro-silica-based superhydrophobic coating that incorporates copper oxide nanoparticles. Antibacterial characterizations both in dry (**Figure 4b**), as well as in wet environments (**Figure 4c**), are carried out (detailed in **Table 2**). A reduction in 1500 colonies cm⁻¹ was observed on the coated surface compared to a non-coated one.^[131] In the wet analysis, nearly all the cells are either unable to adhere to the surface or killed by the NPs in the test sample compared to the non-coated controls.^[131] It is interesting to note that several studies on bifunctional superhydrophobic coatings are catered to fabrics. All of these studies stand on a common trend of creating multilayers on cotton fabrics, where the middle layer mostly contains a biocidal NP, and the topmost layer would be always, if not all, a fluorinated or silanized superhydrophobic coat.^[132–134,135,136] For example, a self-healing and antibacterial coating on cotton fabrics is developed by the sequential deposition of branched poly(ethylenimine) (PEI), silver NPs and fluorinated decyl polyhedral oligomeric silsesquioxane (F-POSS) (**Figure 4d**).^[132] The coated fabric accommodates an abundance of F-POSS, which restores any damaged superhydrophobicity by migrating to the surface, resulting in a self-healing property.^[132] This self-healing also retains the antibacterial property of the silver NPs.^[132] Surface plasmon resonance of the silver NPs endows the fabric's adjustable colors (**Figure 4d(ii–iv)**).^[132] Long incubations in bacterial culture promoted the release of silver ions which killed the cells.^[132] No increase in optical density was found in the case of the culture incubated with the coated cotton fabric test sample, indicating that bacteria growth was inhibited (**Figure 4e**). Since it is on fabrics, the reliability of these coatings to combat biofouling in aquatic environments is not investigated.

7. Critical Perspective

The following section critically reviews the research on both monofunctional and bifunctional SHS, providing a discussion on the significance that SHS could hold for future antibacterial applications, the challenges that it faces in reaching our everyday life, and opportunities for future investigation.

7.1. Standardization of Antibacterial Testing Protocols

As evident in **Tables 1** and **2**, there are multiple approaches to testing the antimicrobial properties of monofunctional and bifunctional superhydrophobic coatings. Often, studies focus on testing them either in dry or wet conditions, however in reality the conditions that a coating is subject to are dependent on the specific application. For example, experimental procedures that involve immersing the coated surface in a culture are not relevant for determining if that coating is suitable for high-touch surfaces in a clinical setting, which is dry. Studies should aim to assess the antibacterial nature of superhydrophobic coatings in both wet and dry conditions so that the results are relevant to a wider range of applications.

Methodologies for assessing antibacterial activity vary greatly between research laboratories. The two most common methods include plate count enumeration, or some variation of plate counting, and confocal laser scanning microscopy (CLSM) with the aid of fluorescent dyes that differentiate live and dead cells,^[146] and often cell counting software.^[147] The advantage of CLSM is that it is ideal for gaining information from only the surface and so free-floating planktonic bacteria do not affect the results.^[148] However, there are well-noted issues with this technique including the variability in results due to inexperience, batch-to-batch differences in dye quality, non-uniformity of staining procedures, and the susceptibility to manipulating results through artificially high or low gain or laser power.^[149] CLSM can also provide information on cell adhesion, colonization, and cell counting, however for live/dead experiments only a percentage can be gained, not a total microbial reduction which is often more relevant for applications.

Plate counting is a traditional method within microbiology that provides a total enumeration of live cells through the use of a dilution series that are spread onto agar plates until single bacterial colonies, known as colony-forming units (CFU) are formed.^[150] Enumeration of the CFUs and recalculation of the dilution series can then lead to an understanding of the initial concentration or total number of bacteria present and with a suitable control, provides information about the reduction of cell adhesion and survival on a surface.^[151] One of the biggest challenges in plate counts on surfaces is accurately assessing the cells on the surface without killing them or missing cells that remain surface-adhered and hence introduce research artifacts. A common methodology is to swab a test surface with a cotton tip, or similar, which then serves as the inoculum for the dilution series. However, differences in pressure and time of the swab can lead to high variability between samples.^[152]

Finally, a general consideration of antibacterial testing is that there is a lack of standardization between studies in terms of exposure time, exposure concentration, physiological condition of the bacteria, medium (e.g., nutrient broth vs buffer), and other experimental parameters, making it difficult to comprehensively compare different antibacterial surfaces reported in the literature.^[153,154] On some occasions, researchers follow protocols from standards bodies such as ISO 22196 from the International Organization for Standardization.^[153–155] However, differences in efficacy have been shown between standards.^[153] It

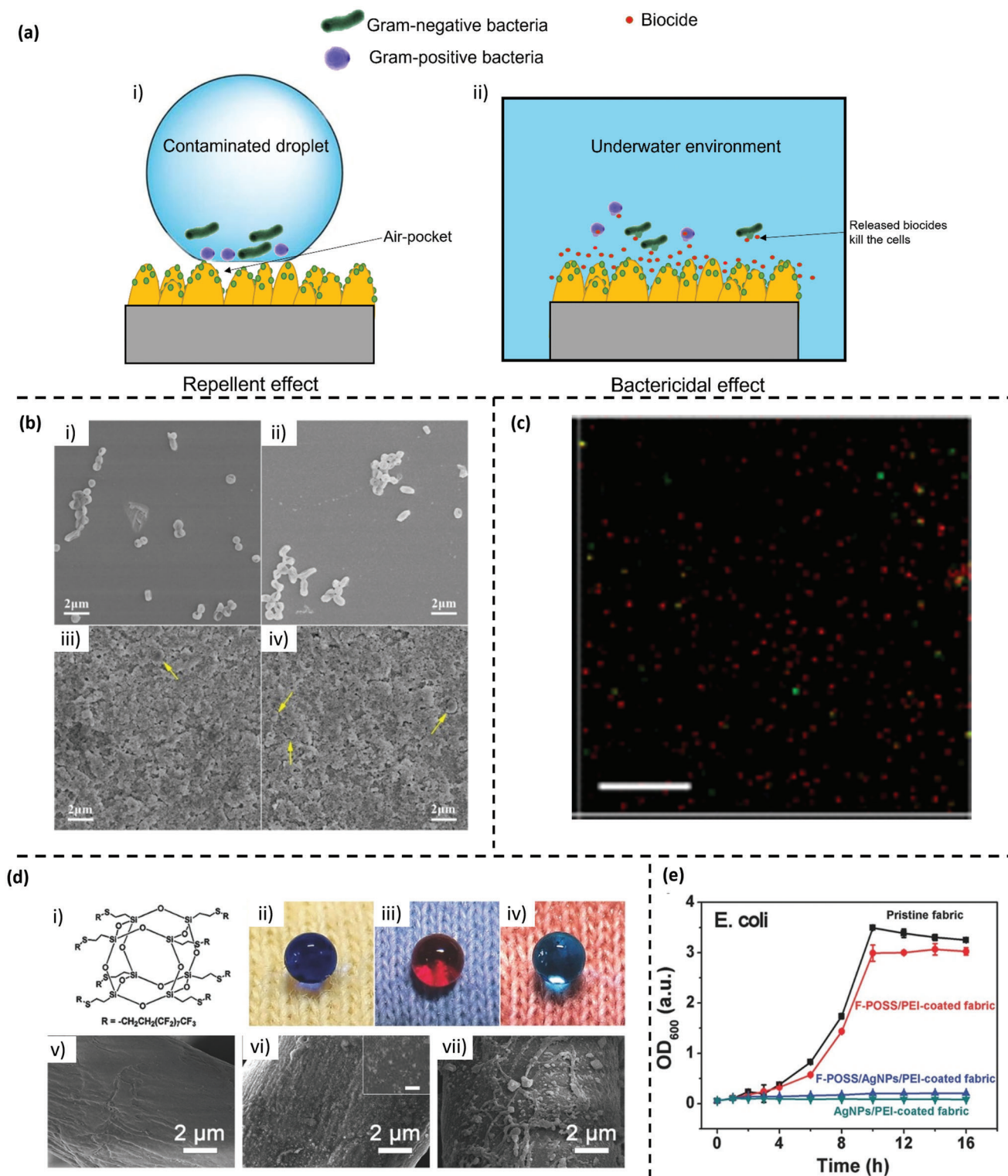


Figure 4. a) Schematic showing the antibacterial mechanism of a bifunctional superhydrophobic surface. b) Electron micrographs showing the attachment of bacteria on i) bare glass, ii) copper oxide-coated glass, iii) fluorosilica-coated glass, and iv) fluorosilica and copper oxide-coated glass.^[131] c) Fluorescence images of fluorosilica-copper oxide-coated glass after exposure to bacteria in the solution for 24 h. Green and red refer to live and dead bacteria, respectively.^[131] d) i–vii) A superhydrophobic antibacterial coating on cotton fabric.^[132] i) Chemical structure of F-POSS (ii–iv) water droplets on the coated fabric of different colors, electron micrographs of v) pristine fabric, vi) silver NPs/PEI-coated fabric, inset scale bar is 200 nm (vii) silver NPs/PEI/F-POSS coated fabric. d) Plot showing the growth curve against culture time for non-coated and coated fabrics.^[132]

must also be acknowledged that these standards can become unavailable to researchers with less funding. Challenges such as resource limitations between institutions and proposed end-use applications of the research are a consideration that makes a set of rigid standardized experimental protocols difficult to design.

7.2. Understanding the Superhydrophobic Mechanism

Studies on superhydrophobic coatings for antibacterial surfaces, such as those discussed in Tables 1 and 2, hypothesize and schematically demonstrate that the mechanism of antibacterial

Table 2. Previous studies on bifunctional antibacterial SHS.

Antibacterial Coating		Testing protocol	Results	Reference
Superhydrophobic (anti-adhesive)		Release based		
Hierarchical micro-nano structures of polyelectrolyte coated with fluoro-silane	Ions from silver NPs embedded in the hierarchical structures	Incubating the samples in culture for 1 day and 7 days followed by imaging	Superhydrophobicity prevented adhesion for up to 7 days, after which the superhydrophobic coating decreased and silver ions were released, which damaged the bacterial cells and reduced adhesion	[137]
Hierarchical polydimethylsiloxane (PDMS) surface	Copper ions from copper hydroxide nanowires	Incubating the samples in the culture for 0, 3, 18 and 24 h	After 18 h, no cells were found on the test surface against nearly $5 \log_{10}$ observed in the control – The sample containing PDMS with copper ions had the highest antimicrobial efficiency compared to the control and PDMS surfaces, attributed to the bifunctional nature of the coating	[138]
Hierarchical bimetallic composite surface with metal oxide coating on porous copper foam substrate	Silver and copper ions	– Incubation in the culture followed by optical density measurement. – Zone of inhibition analysis	– For the test sample, the optical density reached a maximum of 0.073, compared to the control with 1.614 – Inhibition zone diameter for the test sample is 10mm, compared to 5mm for the nanoscale roughness alone and 0.7mm for the copper foam sample – Combination of bimetallic composition, presence of metal oxides and large surface area endows an enhanced antibacterial effect in wet and dry conditions	[102]
Octyltriethoxysilane	Silver NPs	Zone of inhibition assessment	The test samples had a zone of inhibition, showing antibacterial activity toward gram-positive and gram-negative bacteria	[133]
Colloidal silica nanoparticles treated with hexadecyltrimethoxysilane	Copper NPs	Contact assessment method by putting bacteria on the samples for 0 h and 24 h, then washing with phosphate-buffered saline (PBS), incubating the resulting solution on nutrient agar, and counting colony units	There were more than 70% and 90% bacteria growth reduction in gram-negative and gram-positive bacteria respectively	[135]
γ -Alumina layer modified by heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane	Silver NPs	Incubating surfaces in the culture, followed by washing with water, ultrasonicated the samples to remove the bacteria, and then plate dilution	The test surface with silver NPs had an 88% reduction in the number of viable bacteria, whereas the superhydrophobic sample alone had no reduction, indicating that the ions create the antibacterial effect	[139]
Thin and porous PVC films on thermoplastic polyurethane	Silver phosphate NPs	Samples in bacteria were incubated for 24 h, then taken out and ultrasonicated to remove bacteria, and this solution was then vortexed and diluted for plate counting	There was a higher than 99% reduction in bacterial adhesion for the test sample in comparison to the neat PVC film, for both gram-positive and gram-negative bacteria	[140]

(Continued)

Table 2. (Continued).

Antibacterial Coating		Testing protocol	Results	Reference
Superhydrophobic (anti-adhesive)	Release based			
Silica NPs functionalised by fluoro-silanes	Copper oxide NPs	– Anti adhesion assay: Samples incubated in bacterial culture for 4 h, followed by slight rinsing with PBS to remove unadhered bacteria. Electron microscopy of samples followed – Bactericidal assay: Similar to above with 24 h incubation, followed by washing with PBS, live-dead staining and confocal microscopy	– There was a reduction in adherent bacteria by up to 3.2 log cells/cm ² for the test sample compared to the control – In solution, the majority of the bacterial cells were unable to adhere to the coated surface, and nearly all that did were killed by the copper ions	[131]
Sequential deposition of branched PEI, silver NPs and F-POSS on fabrics	Silver NPs	The coated fabrics were immersed in a bacterial culture, followed by optical density measurements for up to 16 h	The test samples with silver NPs recorded almost no increase in optical density over time, suggesting the ions can bind with bacteria and annihilate them	[132]
Fluorination of poly(vinylidene) fluoride (PVDF) nano-sized fiber, micro-sized polytetrafluoroethylene (PTFE) particles	Photoactive Chlorine (e6)	The samples were incubated in bacterial culture for 2 h, and then rinsed in PBS to remove the adherent cells. The rinsed solution was plate diluted. Bactericidal assessment was done using the zone of inhibition method.	Only 3.6% and 4.3% of gram positive and gram negative bacterial adhered. Under light illumination, 100% of the cells were killed as well.	[141]
Fluorosilanes	Zinc oxide and copper NPs	Samples were incubated in a continuous dip flow reactor	99.9% of reduction in adhesion of gram-positive bacteria	[142]
Thin film based on silica NPs and organofluorosilane	Lysozyme enzyme		Test samples reduced Gram-positive and Gram-negative bacteria by 99.999%	[143]
Electrodeposition of copper oxide nanopetal architectures	Copper ions/rupture of cell walls due to nano-roughness	Specimen were incubated in bacterial culture and optical density was measured over time	Gram-negative: optical density of 0.476 for the test sample against 4.47 for the control. Gram-positive: optical density of 1.1 compared to 5.58 for the control. Culture duration was 4 h	[144]
Dip-coating of graphene and titanium dioxide NPs on poly(lactic acid) films	Photoactive Titanium dioxide NPs	Antibiofilm assay was performed by inoculating the samples in bacteria, followed by rinsing and crystal violet staining of the surface-adhered bacteria. Antibacterial assay involved incubating the samples in bacteria for 20 h and plate-diluting the culture.	Antibiofilm assay showed a twofold reduction in optical density for the test samples was observed. Antibacterial assay showed 2 log scale growth reduction for the test samples.	[105]
Fluorinated nanopillars on polycarbonate substrate	Photoactive Chlorine (e6)	Samples were incubated in bacteria, washed and the supernatant was plate diluted.	≥ 99% bactericidal efficiency	[145]

surfaces is due to the Cassie-Baxter state (or plastron) that prevents microbial adhesion (**Figure 5a**). However, very few studies experimentally show how bacteria would float on the plastron.^[3] Microscopy imaging of the interaction between bacteria and superhydrophobic surfaces would aid in scientists' understanding of the mechanism. A notable investigation was performed by Poetes et al.,^[91] where they mechanistically studied the rupture of the plastron and demonstrated it through confocal microscopy.^[91] The presence of plastron changes the cell-surface interactions.^[156] As discussed previously, on an abiotic surface, bacterial cells choose between swarming or biofilm formation.^[29,31] Demonstrating how the presence of a plastron

affects these interactions and studying the consequences is important. In addition, it would also help to demonstrate why additional functionalization such as release-based antibacterial mechanisms is required. An improvement for future studies would be to further visualize the interactions between bacteria and superhydrophobic coatings, to increase the understanding of the mechanism of antibacterial surfaces, where they fail, and demonstrate the conditions where an incorporated biocidal agent is necessary.

One of the drawbacks of the SHS is its metastability of hydrophobicity underwater.^[91] When an SHS is immersed in a liquid, the Cassie-Baxter state, characterized by a thin layer of air

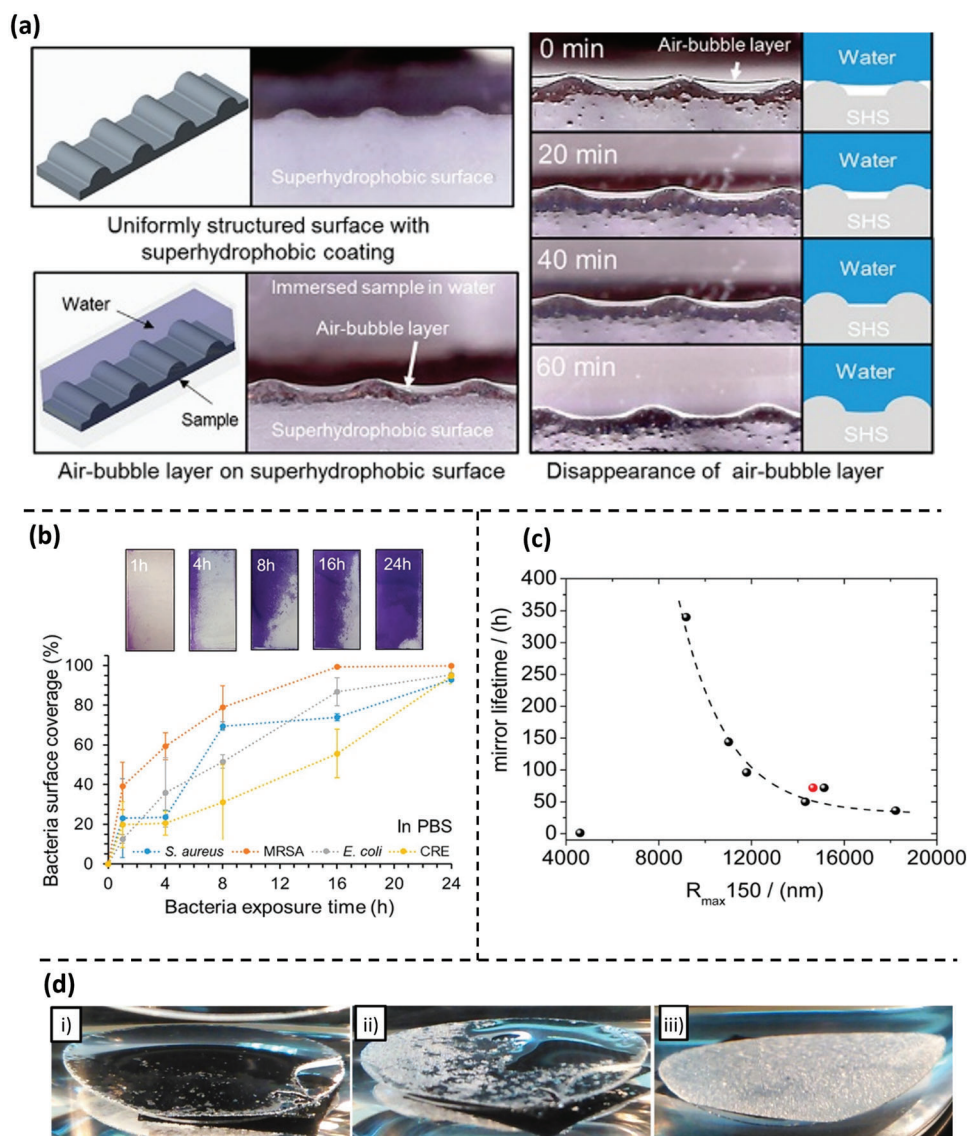


Figure 5. a) Microscopy images showing the disappearance of the air-layer or plastron between the water and the superhydrophobic surface.^[3] b) i–iii) Stability study of plastron underwater based on the disappearance of the mirror effect going from left to right.^[158] c) Plot showing the dependence of topography on plastron lifetime.^[158] d) Total surface coverage by different strains of bacteria on an immersed superhydrophobic surface in PBS after 1, 4, 8, 16, and 24 h.^[159]

(or plastron) starts to break down. The liquid pressure on top of the plastron layer causes the rapid dissolution of the air from the pores of the coating after an onset time, which is found to be dependent on the depth of immersion.^[91] The number of dissolved gases in the liquid medium of testing and the physical and chemical properties of this medium could alter the stability of the plastron.^[130] The underwater metastability and plastron lifetime are also found to be affected by vibrational pressure, the geometry of the coating, and temperature, but this has not been thoroughly investigated.^[157,158] These factors were often overlooked in the studies discussed in Tables 1 and 2, which could also be the reason why for some of the studies, the adhesion increased as the duration of the experiments increased.^[92,137,159] Temperature affects the solubility of gases dissolved in liquid, and hence,

the temperature of the medium used in the antibacterial studies performed should be taken into account.^[158]

When a superhydrophobic surface is immersed in a liquid, the reflection of light on the plastron at a certain glancing angle gives it a mirror effect as shown in Figure 5b(i,ii).^[158] Stability studies can be carried out by observing the disappearance of this mirror effect over time (Figure 5b(iii)). Plastron stability is also found to be dependent on surface topography.^[158] Surfaces with the smoothest topography registered the highest plastron lifetime (Figure 5c).^[158] Hence, theoretically, it was proved that when considering only the topographical factors, the infinite life of a plastron is possible if the roughness of the surface extends to infinity, and the depth of immersion is shallow.^[160] Realistically, this stability cannot be achieved unless energetically assisted.^[161]

The presence of bacteria in the medium could also affect plastron stability as bacterial motion could locally change the pressure and temperature inside the medium.

The formation of defects on superhydrophobic surfaces can lead to a localized loss of the underlying plastron layer and ultimately failure of the coating. These local defects can act as nucleation-sites for bacterial adhesion and eventual attachment of secondary cells on top of the surface-immobilized bacteria. The formation of these defects detrimentally affects the performance of a superhydrophobic surface, however, the robustness of superhydrophobic surfaces is commonly overlooked in many studies. For antibacterial applications, the presence of a secondary release mechanism has been demonstrated to prevent the adhesion of bacteria on defect sites.^[3] Additional self-healing strategies have been investigated, including stimuli-responsive microspheres consisting of antimicrobial ZnO.^[162] Future research should focus on developing surface coatings that are highly durable and can self-repair or possess high function following localized damage.

The rupture of the plastron exposes the solid surface underneath, offering bacteria sites to attach to (Figure 5a).^[163] It has even been suggested that due to the exposed nanostructured high surface area of the coatings, the disappearance of the plastron may lead to enhanced attachment of bacteria over the long-term, as the surface acts as a bacterial reservoir (Figure 5d).^[3,163] Therefore, testing the impact of factors on plastron collapse becomes important if the intended application is one where conditions are not the standard laboratory conditions, for example, the prevention of marine biofouling on ship hulls for drag reduction and corrosion resistance. The lack of stability of the plastron underwater results in a limited scope for commercial applications, as it is not currently viable for SHS to be used in permanently submerged conditions.^[91] Comprehensively studying the effect of factors that lead to the collapse of the plastron may lead to a strategy where the plastron can be stabilized for a longer duration, providing an alternative to additional antibacterial functionalization. This, in turn, would improve the potential for underwater applications of superhydrophobic surfaces which would have a significant impact globally, for example in the marine industry.

7.3. Understanding the Antibacterial Agents

As evident from Table 2, the antibacterial agents are usually metal NPs or their compounds. Numerous mechanisms have been proposed for the killing action of these NPs, schematically shown in Figure 6a(i).^[126] The biocidal mechanism of silver NPs includes the release of silver ions and increased membrane permeability to it, loss of the proton motive force, de-energization of cells, leakage of cellular content, and prevention of DNA replication.^[164] Copper NPs release ions that adhere to the bacteria because of opposite charges, resulting in a copper reduction at the cell wall.^[165] In a different study, soluble species of copper have been found to cause cytolysis of cells and leak cellular content.^[166] Metal oxide NPs like zinc oxide and copper oxide are photoactive and form reactive oxygen species (ROS) under photo-activation. The ROS are highly reactive and penetrate the cell wall, causing damage to the genetic material of the bacteria.^[127,120] Electron micrographs of *E. coli* cells untreated and treated with copper oxide NPs are

shown in Figure 6a(ii,iii), featuring the significant cell damage in the latter.^[120] The metal oxide NPs are also known to release ions that kill the cells.^[167] Understanding the mechanisms is crucial since these pathways could be toxic to other aquatic organisms. Also, most of the superhydrophobic surfaces are made of fluorinated compounds, which could be environmentally toxic. Hence, environment-friendly alternatives should be engineered.^[99,168]

7.3.1. Status of the Released Agent

The physicochemical properties of the media significantly affect the antibacterial effect of released agents.^[169,170] Metal and metal oxide NPs are the most investigated killing agents and the assumption that they are stable is refuted by numerous studies. Once they are released into the surrounding medium, they degrade depending on the chemistry of the medium.^[169,170] This implies that the availability of the killing agent is dictated by how it degrades or reacts to products, the effects that are unique to the environmental exposure. Johnston et al.^[169] find that the activity of silver NPs is specific for each exposure environment.^[169] The media and its components (for example tryptone and yeast extract in an LB medium) dictate the number of silver ions released, and also the effect of silver NPs and their ions on bacterial growth.^[169] In another study, the toxicity of zinc oxide NPs to bacteria at the same concentration is found to decrease in different media in the order: ultrapure water > 0.85% NaCl > minimal Davis (MD) > Luria–Bertani (LB) > phosphate-buffered saline (PBS).^[167] The concentration of zinc ions released from the NPs is decreased by the formation of precipitates ($\text{Zn}_3(\text{PO}_4)_2$ in PBS and zinc complexes in MD and LB), hence explaining the lower toxicity in these media.^[167] Similar observations were found for NPs of zeolitic imidazolate-8 (ZIF-8) (Figure 6b(i)).^[171,172] Solution chemistry affects the degradation kinetics and speciation of an NP, giving different observations on toxicity and dosimetry in different biological environments (Figure 6b(ii)).^[173] The significance of these studies was even more accentuated when Leareng et al.^[170] studied the toxicity of zinc oxide and iron oxide to *B. subtilis* in two natural river water samples, namely Elands River (ER) and Bloubank river (BR).^[170] The cell viability, cell membrane integrity, and ATP production are diminished in the ER compared to BR (Figure 6b(iii)).^[170]

For a hospital environment, the durability of the surface, along with antifungal, antiviral, and antiserum properties are of great importance.^[77] A coating that exhibits all of these properties would be a significant development. Bifunctional antimicrobial surfaces combining superhydrophobicity and release-based mechanisms could be a viable approach to prevent biofouling in both dry and wet environments.

7.3.2. Controlling the Release

The release-based mechanism of a bifunctional surface also has challenges, such as controlling the release of the antimicrobial agent, being resistant to damage, and remaining biocidal in a range of different conditions.

One of the recent successful approaches to control the release rate of antimicrobial agents from coatings is to use polyelectrolyte multilayers (PEMs), which are nanostructured polymeric

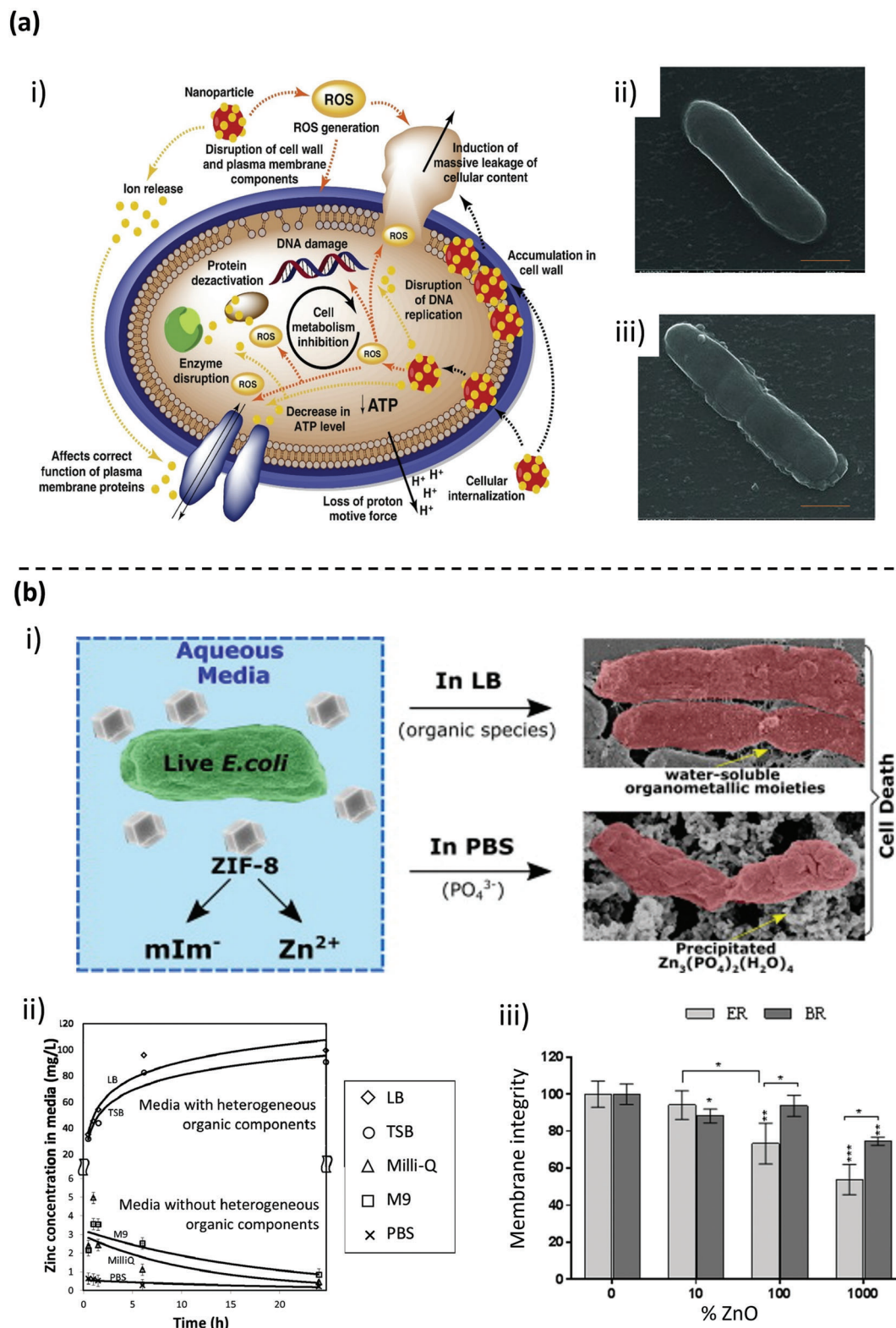


Figure 6. Understanding the toxicity and chemistry of killing agents in different biological environments. a) (i) An illustration showing different pathways of bacteria-killing action by NPs.^[126] a) (ii,iii) Electron micrographs of gram-negative bacteria without and with exposure to copper oxide NPs respectively, depicting the difference in their morphology. The scale bar is 500 nm.^[120] b) (i) An illustration along with electron micrographs of bacteria treated with ZIF-8 showing the formation of different species in different media.^[172] (ii) Plot showing the concentration of zinc in the supernatant over time for media with and without complex organic components.^[173] (c) Plot showing the differential bacterial cell-membrane integrity in two different natural water samples.^[170]

systems^[65a] that can be formed through layer-by-layer (LBL) deposition, which consists of growing alternate polymer layers of contrasting charges.^[65a] The antimicrobial agent is generally trapped in between layers.^[174] This approach is simple, versatile, and economical.^[174] The release kinetics and timeframe of antibacterial agent release are highly dependent on the application.^[65a] It usually follows first- or second-order kinetics, with an initial burst release followed by a slow release usually lasting between a few hours and a few days.^[65a] This might be desirable for coatings on implanted devices since a high-dose, short-term release of an antibacterial agent would provide important protection from pathogens in the postoperative period, a critical stage with high infection risk.^[65a] However, there are applications where long-term release is desirable, for example, in marine applications and high-touch surfaces in healthcare settings.^[65a] Also, for preventing HAIs and marine biofouling, a steady release lasting over a long time is preferred.^[23,175] The release kinetics of an antibacterial agent can be tuned by engineering strategies such as altering the concentration, distribution, size, and charge of the antimicrobial agents, the porosity, roughness, and functional groups of the carrier matrix, and tuning the overall micro/nanostructure of the coating.^[65a]

Another approach to controlling the release is by adding a thin polymeric top layer that limits the rate by acting as a barrier.^[65a] The release kinetics are influenced by the thickness, degree of crosslinking, and hydrophobicity of the thin layer.^[176] This strategy leads to near zero-order release kinetics and prevents the initial burst release.^[65a] Plasma posttreatments have also been found to be a viable technique to induce crosslinking on the top surface of polymeric carrier coatings to control the initial burst release.^[177]

7.4. Improving Coating Durability

Despite the advancements in fabrication techniques, the highly rough micro/nanoscale structures of SHS are inherently fragile to mechanical damage. The low abrasion resistance of SHS limits their translation for real-world applications.^[72,178] The durability of SHS is critical, especially in the case of antibacterial coating designs, as the industry demands low-cost, simple, and robust designs.^[179] Recently, there have been several studies^[75,81,101,180,181] that report the fabrication of highly robust SHS that can retain hydrophobicity under abrasion. Strategies to enhance surface durability include the use of hierarchical micro- and nanoscale structures, the inclusion of self-healing or self-restoration materials, utilizing strongly adherent and tough polymers.^[98,100,178,182] Hierarchical roughness utilizes a combination of micro and nanoscale features to improve wear resistance through the protection of the nanoscale superhydrophobic component, improved adhesion to the substrate (compared to the nanocoating in isolation) and the ability to form newly hierarchical structures and superhydrophobic properties as the top surface is abraded with large-scale features.^[183] In a notable example, Wang et al.^[180] utilized a microstructure consisting of micropockets filled with highly hydrophobic, but mechanically fragile nanoparticles.^[180] The pockets are generated by interconnected surface frames and prevent damage to the housed nanoparticles within from abrasants larger than the frame size.^[180] Conversely,

researchers have explored coating chemistries to increase adhesion to the surface through covalent bonding and biomimetic chemistries.^[182,184] Coating toughness^[101] and elasticity^[185] are two additional considerations to designing durable superhydrophobic coatings.

Despite the development of durable SHS, there is a lack of assessing bacterial colonization on these surfaces following abrasion. For example, there are several studies that report a superhydrophobic and antimicrobial coating, while also testing the coating response to abrasion, however they do not test the antimicrobial properties following abrasion.^[136,186]

Beyond abrasion and mechanical damage, coatings also need to be resistant to other factors such as harsh chemicals,^[101,187–189] electromagnetic radiation,^[101] cleaning cycles,^[188] and temperature.^[187,188] Similar to abrasion testing, these studies typically involve exposing the coating to chemicals, light, cleaning cycles, or high temperatures and then measuring the water contact angle before and after. However, such studies rarely investigate the antimicrobial properties before and after exposure to these harsh environmental conditions. This is important as the maintenance of superhydrophobicity and antimicrobial activity of SHS contributes significantly to its success in real-world applications and hence future studies should place great importance on assessing water repellence and antimicrobial performance before and after exposure to harsh conditions.

7.5. Self-Healing Antimicrobial Coatings

Another important consideration for designing robust antimicrobial coatings is to incorporate self-healing capacity. Self-healing coatings can have an increased lifetime, reducing the overall cost of the coating. Researchers have designed unique ways to design in-built repair mechanisms to restore both superhydrophobic and antimicrobial properties. For example, Wu et al.^[132] report a self-healing superhydrophobic coating that restores itself through the incorporation of a fluorinated silicon–oxygen organic framework that autonomically migrates to the surface following damage.^[132] Pan et al.^[190] employed a different strategy, utilizing a micropillar array that could restore its shape following mechanical crushing. Additionally, this micropillar array was decorated with fluoroalkylsilane encapsulated in pH-responsive capsules, which enabled the restoration of low-surface energy chemistry when stimulated by acidic solutions.^[190] Ni et al.^[162] utilize stimuli-responsive microspheres consisting of ZnO, mesoporous polydopamine, amino-modified silicon oil, and hydrophobic SiO₂ nanoparticles.^[162] Interestingly, this coating was able to repair both superhydrophobic and antimicrobial functions following physical and chemical damage.^[162] Finally, Li et al.^[191] demonstrated a superhydrophobic and antibacterial fabric coating that could be thermally healed via increased temperature or exposure to light, resulting in photothermal conversion by the metal nanoparticles within.^[191] For large industrial applications, the fabrication process of such coatings must be optimized to be simple, reproducible, economical, and scalable.^[192] Overall, self-healing antimicrobial coatings is an exciting and rapidly emerging research area within the field, that despite several

challenges, has good potential to improve and broaden the applications of superhydrophobic antimicrobial coatings.^[193]

8. Conclusions and Prospects

To conclude, this review provides a summary issue and formation of biofilms, followed by a discussion and critical appraisal of the research surrounding antibacterial surfaces, including anti-adhesion, bactericidal, and bifunctional superhydrophobic surfaces. Both monofunctional and bifunctional SHS are examples of anti-adhesive surfaces, with bifunctional surfaces also utilizing release-based mechanisms to prevent biofilm formation. We have also critically reviewed the advancement in the field of antibacterial SHS and have presented the challenges faced by the current research, as well as opportunities for future investigation.

Despite the advancement in nanotechnology and fabrication techniques that has resulted in many antibacterial surfaces, only a few of them have made their way to real environments such as healthcare settings, non-healthcare high-touch surfaces, or maritime infrastructure. The challenge in translating the research to real environments is largely due to its complexity and different environments require surface designs to meet different requirements depending on the applications. For example, a coating for ship hulls requires surfaces that exhibit enhanced corrosion resistance and robustness in addition to preventing fouling by diverse organisms, while a clinical environment requires a coating resistant to a broad range of pathogens not exclusively bacteria. Industries further demand low-cost, simple, and scalable techniques that are sustainable. Hence, objectively evaluating the surfaces consistent with the relevant applications should be given due importance. Further, consistency in in-vitro methodologies of testing the surfaces in the laboratory environment and thereafter must be maintained. A collaborative effort from scientists in different disciplines could tackle the challenges, working toward the realistic potential for the use of superhydrophobic antibacterial surfaces. Given the technological advancements, it has become quite easy to develop a superhydrophobic surface. In this scenario, the question to be asked is: what novelty exists in engineering another superhydrophobic coating if we are overlooking the existing research gaps? Perhaps research should be more inclined toward understanding how the limit of current superhydrophobic surfaces, such as the limited stability of the plastron layer, can be overcome to achieve a suitable technological solution for practical environments and applications.

Acknowledgements

This work was partially funded by an Australian Research Council Linkage Project (LP170101157) to A.T. and D.N. and a National Health and Medicine Research Council Project grant to D.N. D.R.N. was supported by an NHMRC Research Leadership Fellowship GNT1135657 and A.T. by the Australian Research Council for a Future Fellowship FT200100939. Access to the facilities of the Centre for Advanced Microscopy (CAM) with funding through the Australian Microscopy and Microanalysis Research Facility (AMMRF) is gratefully acknowledged.

Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

antibiotic resistance, antimicrobial, biocides, biofilms, coatings nanoparticles, superhydrophobic

Received: June 6, 2023
Published online: July 20, 2023

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