Chapter 5

Superhydrophobic Surfaces Toward Prevention of Biofilm-Associated Infections

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Additional information is available at the end of the chapter

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Abstract

In this chapter, we briefly review the different strategies for surface modification as a method to fight against bacteria adhesion and biofilm formation. We focus on superhydrophobic materials and biofilm medical infections. We give some insights into common materials and preparation techniques for superhydrophobic surfaces before discussing recent bacteria interacting with superhydrophobic surfaces. These surfaces have indeed demonstrated great potential in preventing bacterial adhesion and biofilm formation due to the presence of micro- and nanostructures. Although much work has been done, further investigations are still required to improve the surface mechanical properties over time and to understand the underlying mechanism behind their antimicrobial and antifouling capability. Moreover, there is a lack of standard methodology for evaluating antibacterial properties, and biofilm prevention should be studied with longer incubation times. We strongly believe in the potential of superhydrophobic surfaces, and we encourage more research on its magnificent properties, especially for their advantages over other antimicrobial surfaces.

Keywords: superhydrophobic surfaces, biofilm-associated infections, antibacterial surface, bacterial adhesion, biofilm formation, anti-biofilm surface

1. Strategies on surface modification for antibacterial properties

The rapid proliferation of pathogenic bacteria, which is responsible for nosocomial infections, is becoming a major public health problem because of bacterial resistance to antimicrobial treatments (antibiotics and biocides) [1]. It is now well established that bacterial populations attach to solid substrates for survival, forming biofilms. Biofilms are dense microbial communities, adhering to surfaces, which secrete an extracellular matrix mainly composed of water, polysaccharides, DNA and proteins [2]. Many different strategies on surface modification have been studied over the last few years to reduce bacterial adhesion and to avoid biofilm formation.

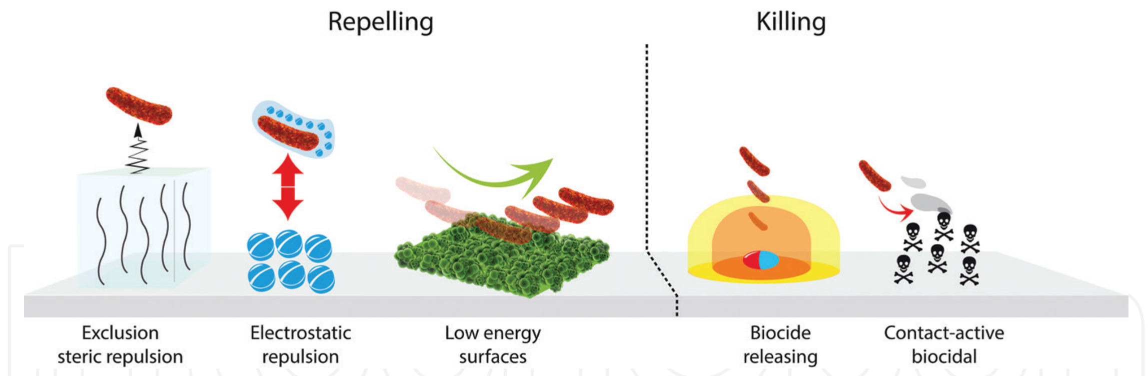

Figure 1. Antibacterial surfaces.

In general, two different global strategies can be distinguished (**Figure 1**). The first strategy relies on killing bacteria through antimicrobial compounds (either release or direct contact), such as silver ions [3] and antibiotics [4]. However, this strategy can involve tedious preparations and might represent a threat to the environment and biological systems. The second strategy relies on repelling bacterial attachment through morphological or physical-chemical interactions such as steric impediment, electrostatic interactions and low surface energy [5]. Many of the steric and electrostatic repulsion techniques proposed until now show no persistence, and surface hydrolysis may occur. Wet surfaces can provide the ideal conditions for biofilm formation; therefore, wettability properties of surfaces play a crucial role on biofilm formation. Low-surface-energy surfaces have a great potential since their antibacterial properties depend mostly on surface roughness [6]. In the present chapter, our discussion focuses on superhydrophobic surfaces. For further information regarding other techniques, useful information can be found in the review by Griesser et al. [7] and in the work by Siedenbediel and Tiller [8].

2. Superhydrophobicity

Surface wettability is an interface phenomenon reflecting the behavior of a liquid in contact with a solid surface. The control of surface wettability is present in nature and in our daily life in many applications such as waterproof coatings, cooking utensils and bathroom accessories. A material's property can be evaluated by the measurement of the contact angle (CA) between a droplet of liquid and the material surface [9].

It is well known that the interface liquid-gas (LG) area tends to be minimized due to the surface tension. When a drop is in contact with a solid surface, a balance between three-phase surface tension occurs (**Figure 2**), and it can be described by the Young's equation as follows:

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos\theta \tag{1}$$

where γ_{SG}, γ_{SL} and γ_{LG} are the solid – gas (SG), solid – liquid (SL) and liquid – gas (LG) surface tensions and θ the contact angle, also referred to as the intrinsic contact angle or smooth surface contact angle [10].

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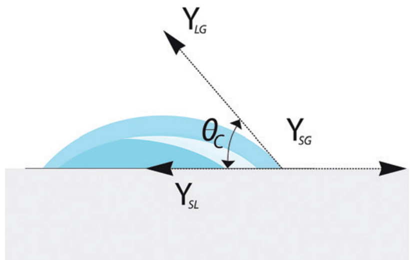

Figure 2. Liquid droplet on solid surface.

Depending on the CA value, different materials are said to be either hydrophilic or hydrophobic. For instance, if the liquid is water (or oleophilic/oleophobic when the liquid is an oil), a surface in contact with water is said to be hydrophilic if the CA is below 90°. For a CA >90°, it is hydrophobic, and for a CA >150°, it is referred to as a superhydrophobic surface. This last term refers to the repellent capability of such surfaces toward water [10].

Lotus leaves are an excellent example of superhydrophobic surfaces, as they are known to be self-cleaning. On a self-cleaning surface, particles can 'roll-off' it by their adhesion to water droplets [11]. With the technological development over the last decade, lotus leaves have been the subject of many studies. It has been found that its repellent properties are due to a hierarchical micro- and nanostructured topography. As nature demonstrates, the design of nonwettable surfaces must take parameters such as the roughness and the chemical nature of the material into consideration. The aim of this chapter is to highlight the use of superhydrophobic surfaces to prevent bacterial adhesion and biofilm formation. The main models to determine CA measurement for non-flat surfaces are described in the following section.

2.1. Wenzel and Cassie-Baxter model

As for the lotus leaf, the presence of micro- and nanostructures will contribute to the wettability of the surface, playing a crucial role on superhydrophobicity. Higher apparent CA values cannot be achieved by chemical modification, but by changing the roughness. According to Wenzel [12], the real contact area of the solid–liquid interface could be increased by changing the roughness more than changing the microscale apparent area. Wenzel's model considers that there is no gas layer between the solid–liquid interface, so the liquid fills the grooves on the surface (**Figure 3**). This angle can be referred to as the wetted contact with the surface.

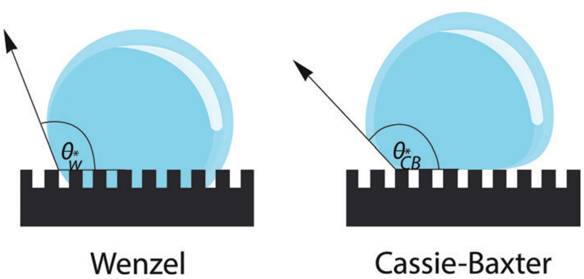

Figure 3. Wenzel and Cassie-Baxter models.

The relationship between the apparent CA θ_W and the intrinsic CA at equilibrium is:

$$\cos\theta_W = r \cos\theta \quad (2)$$

where r is the ratio of the surface area over the apparent area, defined as the Roughness r of the material. By increasing the roughness (a bigger height difference between the posts and grooves, and the density of posts), superhydrophobicity can be achieved.

Cassie and Baxter [13] studied composite materials present in nature, finding that if the surface is hydrophobic enough, the gas phase between the solid-liquid interface will remain and the droplet will not fill the grooves on the rough surface (**Figure 3**, right). For this case, the relationship is as follows:

$$\cos\theta_{CB} = f\left(1 + \cos\theta_W\right) - 1 \quad (3)$$

The Cassie-Baxter equation adapts to any composite surface contact, which is represented as follows:

$$\cos\theta_{CB} = f_1 \cos\theta_1 + f_2 \cos\theta_2 \quad (4)$$

where θ_{CB} is the apparent CA of the composite surfaces, θ_1 and θ_2 are the intrinsic CA of two materials, and f_1 and f_2 are the fractions of two materials of the composite surfaces. f is the solid fraction of the substrate. For very rough surfaces, f will tend to zero, while θ_{CB} will tend to 180°.

Bittoun et al. [14] theoretically studied different types of surface topographies by changing roughness scales, on drop 2D systems. They studied sinusoidal, flat-top pillars and triadic Koch curves finding that Cassie-Baxter state is thermodynamically more stable, in comparison with the Wenzel state. In addition, they concluded that multi-scale roughness increases the mechanical stability of the surface and is beneficial for superhydrophobicity. Among the three topographies, round-top protrusions (sinusoidal) were shown to be the best for non-wettability, as nature has already proved.

It has been suggested that by changing the surface topography from flat to structured and by including a hierarchical organization, the water CA will be modified and superhydrophobicity may be achieved [9]. Lotus leaves have proved this theory, regardless of hydrophilic coating on their surface, the presence of hierarchical micro- and nanoscale features are responsible for their superhydrophobicity [15]. **Figure 4** shows different structures varying from flat to hierarchical.

2.2. Hysteresis

For many medical and industrial applications, the interest of a self-cleaning surface is very attractive; nevertheless, on many occasions, the CA hysteresis has been ignored in investigations. For real-life applications of repellent surfaces, the dynamic movement of the liquid drops has to be taken into consideration because it depends on the CA hysteresis. A high hydrophobicity in static CA does not imply a high hydrophobicity in dynamic CA, as proved by Oner et al. A low hysteresis CA is ideal [16].

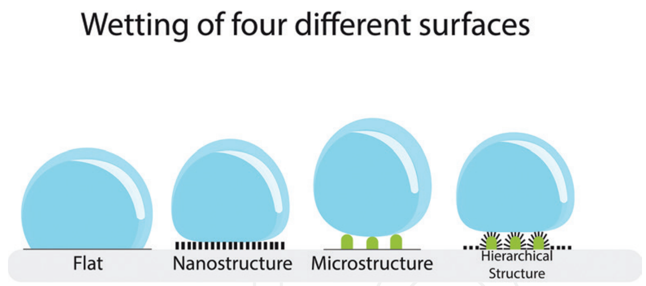

Figure 4. Different types of surface roughness and wettability.

Furthermore, CA hysteresis is important for the removal of surface contaminants when water droplets are moved along a tilted surface [9]. As we showed previously, the uniform flow of the droplet will allow or not allow the transport of the contaminant along the surface. When the flow is uniform, the material can be considered to behave as a self-cleaning surface. On the contrary, if the CA hysteresis is too high, the transport will not be very efficient and is possible that not all the contaminants will be carried by the drop (**Figure 5**) [9].

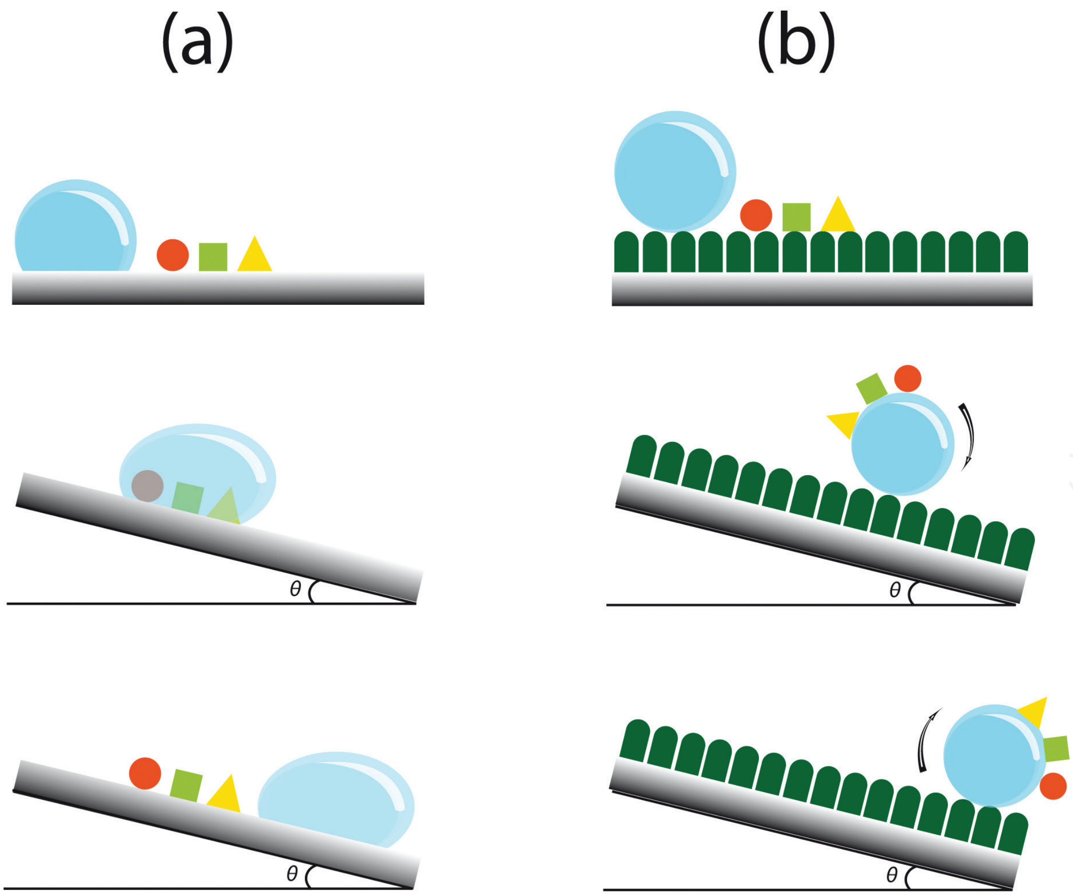

Figure 5. Dirty flow on surfaces with high (a) and low (b) CA hysteresis. While the drop is moving on a high CA hysteresis surface, it will not be efficient enough to transport the contaminants on the surface.

2.3. Superhydrophobic surfaces toward biofilm prevention

Biofilms represent a high risk for nosocomial infections in which different pathogenic bacteria are involved in biofilm-associated infections (BAI) [1]. Remarkable efforts have been made toward superhydrophobic surfaces, as they are considered self-cleaning. As we have previously mentioned, superhydrophobicity is usually achieved by a combination of surface structure, often at a micro/nanoscale, with low-surface-energy compounds. Numerous studies have been published over the last decade, regarding the use of superhydrophobic surfaces or coatings to reduce bacterial adhesion and thus to prevent biofilm formation.

Figure 6 shows how the interest in the development and study of antibacterial superhydrophobic surfaces has increased dramatically over the last decade. Despite all the efforts made until now, there is still a lack of standard methodology to assess the effectiveness of materials against biofilm formation. Incubation times and bacterial strains differ dramatically from publication to publication. In the following sections, we try to review the most relevant publications, first discussing the main preparation techniques and then describing some of the results obtained according to the bacterial strains studied and the incubation times. The aim of this review is to give the reader general tools to be able to compare and understand the key conclusions from different studies, since the conditions change from one to another.

2.4. Common materials and preparation techniques for superhydrophobic surfaces

As previously mentioned, superhydrophobic surfaces can be obtained by a wise combination of factors, among which, surface roughness and low surface energy are of particular importance. These features can be achieved using a wide variety of materials, involving organic and inorganic substrates. Among the most common techniques to modify the surface of the substrate, we find laser ablation, vapor deposition, electrochemical polymerization, lithography, sol-gel processing and layer-by-layer deposition (**Figure 7**). As a result, a vast range of surfaces are available. Unfortunately, only a few of these surfaces could be used for practical applications due to the high cost of production and feasibility.

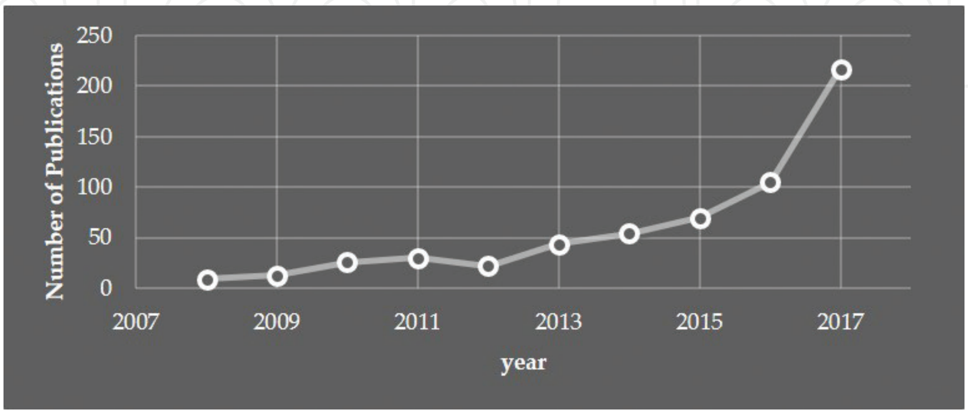

Figure 6. Number of publications per year based on the keywords: Superhydrophobic and antibacterial, from science direct database.

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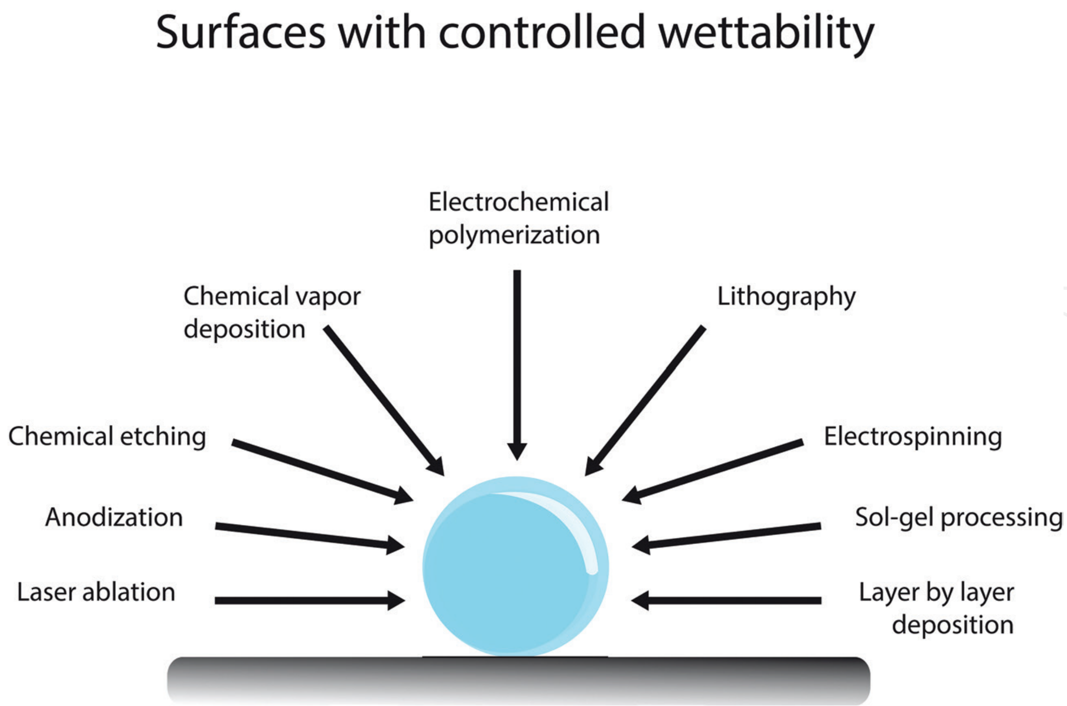

Figure 7. Most used techniques to obtain superhydrophobic surfaces and coatings.

A brief summary of different substrate materials and processing techniques is given below (**Table 1**) on superhydrophobic surfaces where bacterial interaction was tested (nonexhaustive list).

Among these techniques, we can distinguish two categories: the first category concerns the intrinsic superhydrophobic surfaces, in particular, where the surface structuration is applied directly on the substrate. In the second category, we can find the films and surface coatings, such as polymeric or oleic coatings deposited on a substrate. We focus here on biomedical infections and biomedical applications. Medical implants require a very homogenous and stable surface. The main materials used for medical implants are titanium, stainless steel, cobalt-chromium and some polymers. For this reason, many of the superhydrophobic studies made until today concern titanium surfaces [28]. In most of the cases, titanium surfaces belong to the first category, since their main application is in medical implants. Infections due to medical implants represent about 60–70% of nosocomial infections. BAI have been found in almost 100% of medical implants, such as prosthesis (heart valves, orthopedic, vascular, ocular), urinary catheters, contact lenses and intrauterine contraceptive devices [29]. Biofilms are found to be 100–1000 times more resistant to antibiotics [30]. BAI can cause a number of health complications such as chronic inflammation, antibiotic resistance, chronic and recurrent infections and, in the worst cases, sepsis [29]. Nowadays, BAI are related not only to medical implants and intensive care units but also to non-intensive care hospital areas and many other healthcare settings [29]. Thus, the implementation of self-cleaning surfaces, which facilitate the removal of microorganism on any common surfaces such as desks, tables, walls and clothes, has become a key step in preventing BAI in nosocomial settings.

Transfer from the laboratory to real-life applications, from an applied material point of view, requires testing the mechanical stability over time, in both dry and wet conditions. Even if a

Substrate material	Material processing and coating	Ref.
Titanium	Electrochemical anodization. TiO_2 nanoscale tubes and after silanization deposition.	Tang et al. [17]
	Laser ablation self-organized micro- and nanostructures.	Fadeeva et al. [18]
	Laser ablation self-organized micro- and nanostructures.	Truong et al. [19]
Polyurethane	Soft lithography micro and nano pillar-structured surface.	Xu and Siedlecki [20]
Silicon	Plasma etching and Teflon/oil coating micro- and nanostructured porous surface.	Epstein et al. [21]
Silica	Based-catalyzed hydrolysis and condensation. Nanostructured silica fluorinated colloids with xerogel coating.	Privett et al. [22]
Aluminum	Anodization, post-etching process and Teflon coating. Al_2O_3 nanoporous and nanopillared surface obtained.	Hizal et al. [23]
Steel, glass, polystyrene	Thermal deposition of n-paraffin and fluorinated waxes.	Pechook et al. [24]
Stainless steel	Multilayer depositions of polydopamine (PDA) and silver (Ag) nanoparticles followed by post-modification with 1H, 1H, 2H, 2H–perfluorodecanethiol.	Qian et al. [25]
Aluminum wafer	Electrochemical deposition of silver coating.	Che et al. [26]
Poly-dimethylsiloxane (PDMS)	Aerosol assisted chemical vapor deposition of copper nanoparticles.	Oskan et al. [27]

Table 1. Example of most common substrates and techniques used to obtain superhydrophobic surfaces.

surface is considered self-cleaning, mechanical stress will be applied under normal conditions through disinfection and wear. Besides mechanical stability, thermal and chemical resistance would be required due to the oxidation in the environmental conditions of almost any application. There are many other fields that would benefit from self-cleaning surfaces, such as naval, the food industry, the energy industry and also for fuel storage. **Figure 8** shows some examples of medical devices that could implement superhydrophobic surfaces.

Figure 8. Example of medical devices which could implement superhydrophobic surfaces.

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2.5. Bacterial adhesion and biofilm formation

We can find in the literature tests on several bacterial strains, among which we can highlight *Staphylococcus aureus* and *Staphylococcus epidermidis* (Gram positive, coccus), and *Pseudomonas aeruginosa* and *Escherichia coli* (Gram negative, rod shape), as well as all facultative anaerobic bacteria. *S. aureus* and *P. aeruginosa* recently have been cataloged by the World Health Organization (WHO) as priority pathogens to be considered as threat because of their resistance to antibiotics [31]. *S. epidermidis* is among the leading causes of nosocomial sepsis [29], and *E. coli* can be found on almost any surface, besides being the most prevalent microbe identified from positive blood cultures [32].

As it is known, biofilm formation can be described in five different stages as follows (**Figure 9**) [33]:

Stage I: Reversible planktonic cell landing on a surface and initial attachment.

Stages II and III: Bacterial growth and microcolony formation, irreversible attachment.

Stage IV: biofilm maturation.

Stage V: dispersion of planktonic cells capable of forming new colonies.

It is important to highlight the fact that the formation of a mature biofilm will be dependent on the bacterial strain and incubation conditions. For discussion, as an example, we can consider *P. aeruginosa* biofilm as a representative. Rasamiravaka et al. [33] studied the development of *P. aeruginosa* biofilm over time by using fluorescence microscopy. After 2 h of incubation, the bacteria culture could be cataloged at Stage I; after 8 h, bacteria attachment was considered irreversible. Microcolony formation was observed after 14 h and biofilm formation and maturation from 1 to 4 days. Stage V was observed after 5 days. Although much research has been made so far, many studies ignored the biofilm formation, focusing only on a few

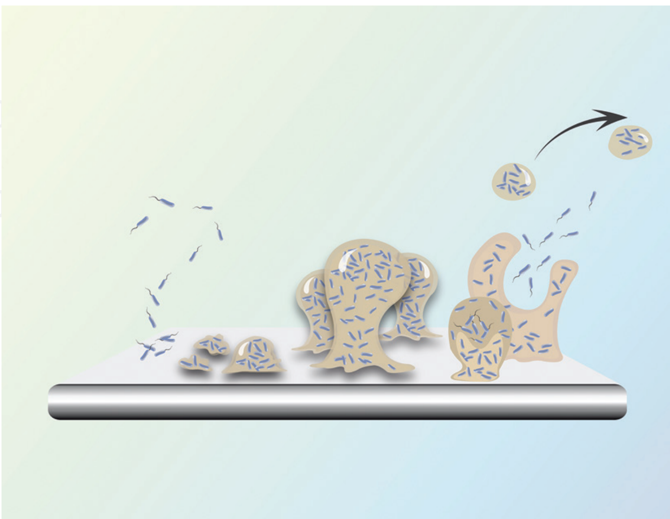

Figure 9. Dirty flow on surfaces with high (left) and low (right) CA hysteresis. While the drop is moving on a high CA hysteresis surface, it will not be efficient enough to transport the contaminants on the surface.

hours of incubation (<24 h). In such cases, we must say that only bacterial adhesion has been studied, without any knowledge as to whether the attachment is either reversible or irreversible. Nevertheless, to study biofilm prevention properties of surfaces, longer incubation times should be considered and for real-life application even longer times where all the phases should be observed. In **Table 2**, we present recent examples of relevant studies of bacterial interactions on superhydrophobic surfaces.

Although **Table 2** does not summarize all the studies to date on antibacterial superhydrophobic surfaces, we can observe that most of the studies have focused only on bacterial adhesion rather than the prevention of biofilm formation. Qian et al. [25] recently published a remarkable work on superhydrophobic multilayer film deposition on stainless steel with antibacterial properties,

Ref.	Bacterial strain	Incubation time	Mode	Biofilm prevention
Tang et al. [17]	*S. aureus*	2, 4 h	Stationary	Not tested
Fadeeva et al. [18]	*S. aureus*	18 h	Stationary	No for *S. aureus*
	P. aeruginosa			Yes for *P. aeruginosa*
Truong et al. [19]	*S. aureus*	1 h	Stationary	Not tested
	S. epidermidis			
	P. maritimus			
Xu and Siedlecki [20]	*S. epidermidis*	1, 2 h	Flow	Not tested
Epstein et al. [21]	*P. aeruginosa*	24, 48 h and 7 days	Flow	Yes
	S. aureus			
	E. coli			
Privett et al. [22]	*S. aureus*	1.5 h	Flow	Not tested
	P. aeruginosa			
Hizal et al. [23]	*S. aureus*	1 h	Both	Not tested
	E. coli			
Pechook et al. [24]	*B. cereus*	24 h and 7 days	Stationary	Yes
	P. aeruginosa			
Qian et al. [25]	*S. aureus*	1, 3 days	Stationary	Yes
	E. coli			
Che et al. [26]	*E. coli*	12 h	Stationary	Reduced
Oskan et al. [27]	*S. aureus*	1 h	Stationary	Not tested
	E. coli			

Table 2. Recently published studies on antibacterial properties of superhydrophobic surfaces, summarizing the bacterial strains, incubation mode and times and more importantly, if biofilm formation prevention was evaluated or not (nonexhaustive list).

evaluating the incubation with *E. coli* and *S. aureus* at 1 or 3 days. The surfaces were prepared with hierarchical micro/nanostructures using polydopamine (PDA) and silver (Ag) nanoparticles. These were compared against non-structured surfaces. For both strains, no cells were observed after day 1, and until day 3, cell quantity was by far less than the other surfaces studied. They proved that the biofilm formation could be prevented using these hierarchical micro/nanostructured superhydrophobic surfaces. They studied two different bacterial strains, gram positive and negative, and also long incubation times to thoroughly assess if biofilm formation is prevented or not.

Fadeeva et al. [18] prepared microstructured superhydrophobic titanium surfaces by femtosecond laser ablation. They incubated samples with *S. aureus* or *P. aeruginosa* for 18 h, finding significant reduction for *P. aeruginosa*, but not for *S. aureus*. The aim of the study was to investigate further the behavior of two different shaped pathogens on their surfaces in order to evaluate antifouling properties; nevertheless, they were not able to determine the mechanism by which this was achieved. They suggested that the ability of *S. aureus* to colonize the superhydrophobic surfaces could be dependent on their shape. However, this assumption cannot be generalized as other studies [21, 22] have shown that some surfaces are effective independent of the shape of bacteria. It is important to insist on the need of testing superhydrophobic surfaces with different bacteria (shape, gram positive and negative). Moreover, it would be even more interesting to test a mixture of bacteria to see if the antibacterial capability of the surface is independent of these multiple factors.

2.6. Insights into the mechanisms below prevention of biofilm formation by superhydrophobic surfaces

It has been proved that the Lotus leaf superhydrophobicity originates from the hierarchical micro/nanostructures on their surface even though a hydrophilic layer covers these structures [15]. Micro-/nanostructuration allows the surface to encapsulate air between, creating an air cushion which reduces the water adhesion onto the surface, thus preventing the adhesion of microorganisms and other fouling molecules.

Studying bacterial distribution on the surfaces by imaging techniques has provided important insights on the mechanisms used by the bacteria to attach to the surfaces. With SEM images, Truong et al. [19] observed the distribution of different bacterial strains on superhydrophobic titanium surfaces, finding that the upper regions of the surfaces were not covered by bacteria as much as the crevices between the upper regions. This suggested that the capability to reduce bacterial adhesion of superhydrophobic surfaces can be reduced over time after the trapped air is completely excluded under complete submersion conditions. However, Ma et al. [34] compared the adhesion of bacteria (*P. aeruginosa*) and non-biological adhesion under partially and completely submersion conditions on superhydrophobic *Colocasia esculenta* leaves. They found that the adhesion was dependent on the nanostructure density on the surface rather than the air-cushions trapped on micro/nanostructures. These results demonstrate the efficacy of superhydrophobic surfaces in reducing bacterial adhesion and preventing biofilm formation even under submerged conditions, when they are properly designed at the nanoscale.

3. Perspectives

Much work has been carried out to date on superhydrophobic surfaces, allowing us to understand a little more clearly about the mechanism behind their antimicrobial properties. These surfaces have demonstrated great potential in preventing bacterial adhesion and biofilm formation. Many techniques have been proposed for their preparation and application in different fields. Even so, there is still much work to be done, such as improving the surface mechanical properties over time, and mainly toward our understanding of the underlying mechanism behind their antimicrobial and antifouling capability. With the work so far, we can conclude that micro- and nanostructures have great influence on their antibacterial properties, so this requires further in-depth studies. As discussed in this chapter, there is a lack of standard methodology for evaluating antibacterial properties; however, we can highlight the need to evaluate not only bacterial adhesion but also the prevention of biofilm formation with longer incubation times. Much work has been done so far, but there is still a long way to go from the laboratory to reach real-life applications. We strongly believe in the potential of superhydrophobic surfaces and we encourage continued research on its magnificent properties, especially for their advantages over other antimicrobial surfaces.

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