Bioinspired Antibacterial Surfaces

Subjects: Materials Science, Biomaterials
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Briefly, depending on whether extra interventions are needed, bioinspired antibacterial surfaces can be categorized into passive and active ones

Keywords: biomimetic ; antibiofouling

1. Passive Antibacterial Surface

1.1. Bacteria-Repellent Surface

Boosting the surface repulsion of bacteria, which is mainly inspired by superhydrophobic biological skins, can remarkably minimize the bacterial infection rate. Generally, the factors that can control bacterial repellence include wettability, topography, material stiffness, surface charge, and their combinations.

The most common approach to interfere with the surface–bacteria interaction is to regulate the surface wettability, i.e., hydrophobicity or hydrophilicity. For example, the surface can be rendered hydrophobic by grafting low-surface-energy molecules or infusing liquid lubricant, the result of the latter being named slippery liquid-infused porous surfaces (SLIPSs) [1][2][3]. A novel SLIPS consisting of microporous poly (butyl methacrylate-co-ethylene dimethacrylate) films infused with the perfluoropolyether fluid−slippery poly (butyl methacrylate-co-ethylene dimethacrylate) was demonstrated to prevent different strains of the opportunistic pathogen *P. aeruginosa* from biofilm formation for 7 days [4]. Further combining low-surface-energy components with a large microroughness can amplify the apparent wettability, which tremendously weakened bacterial adhesion [5][6]. One typical example was a lotus-leaf-inspired surface patterned with regularly spaced micro-pillar arrays and packed nanoneedles, allowing a more than 98% antibacterial rate against *Escherichia coli* at high-concentration (10^8 colony-forming unit/mL) and long-term-culture conditions [7][8][9][10][11][12]. A mimic of shark skin with superhydrophobicity also showed a similar inhibition to the adhesion of the zoospores Ulva (~5 μm diameter) and *S. aureus* (1 μm diameter) [13][14][15].

On the other hand, causing surface hydrophilicity can also decrease the total contact and inhibit bacterial adhesion, because the hydrophilicity helps to reduce the number of bacteria proteins attached to the surface. A simple way to make surfaces hydrophilic is directly coating hydrophilic components such as polymers and zwitterions [16][17][18][19][20]. For example, a branched-chain-polymer-based surface with antibiofouling properties was developed by conjugating dioxy-containing polyethylene glycol with gentamicin terminals. The introduction of polyethylene glycol increased the surface hydrophilicity, which inhibited protein adhesion and repelled bacterial fouling. In addition, the transplanted *S. aureus* infection model showed that the branched-chain polymers have good antibacterial and antifouling ability in vivo. [21]. For zwitterions, the introduction of zwitterions onto cotton-texture surfaces significantly increased surface hydrophilicity. The modified cotton texture surfaces can effectively resist initial bacterial adhesion, kill attached bacteria, and release dead bacteria [22][23][24].

Solely topographical modifications also provide a persistent and predictable form for control of bacterial behavior, especially using ordered patterns. M. Yang et al. showed that submicron-scale pillar patterns strikingly inhibit bacterial adhesion, growth, and colonization by physically hindering bacterial cell-to-cell interactions. Furthermore, they investigated the effect of morphology (e.g., honeycomb) and sizes on the adhesion and growth of bacterium with different shapes (e.g., rod *E. coli* and spherical *S. aureus*). The fluorescent image results showed that a 1-μm patterned surface significantly reduced bacterial adhesion and growth while inhibiting bacterial colonization compared with a flat surface. From a dynamic perspective, the selective adhesion of bacterial cells to patterns was synergistically mediated by maximizing cell–substrate contact area and minimizing cell deformation. They established that two main factors, namely energetically favorable adhesion sites and physical confinement, contribute to the antibacterial properties of the honeycomb-like pattern [25].

Adopting soft materials, e.g., hydrogel, with low stiffness, can tune the surface bacterial adhesion. Harder polymer surfaces typically have higher network densities than softer polymer surfaces, resulting in a higher density of functional groups that liquid media and bacterial cells can interact with. Generally, a soft hydrogel surface with low stiffness exhibits better antibacterial performance. A positive correlation between the surface stiffness and adhesion was demonstrated by larger bacteria colonization on the stiffer surface. Polyelectrolyte multilayer membranes from polyacrylamine hydrochloride and polyacrylic acid were prepared with Young's moduli ranging from 1 to 100 MPa. A positive correlation between the surface stiffness and adhesion with *E. coli* and *S. aureus* was found on such surfaces [26]. A cross-linked membrane composed of poly(L-lysine) and hyaluronic acid was prepared, and the number of bacteria on the non-cross-linked membrane at 30 kPa was lower than that on the cross-linked membrane at 150 kPa [27]. Polydimethylsiloxane substrates with a stiffness of 100 to 2600 kPa were found that affected the physiology of *E. coli* and *P. aeruginosa* [28]. A positive relationship between the fouling intensity of *E. coli* and *S. aureus* and hydrogel stiffness was reported by conducting tests of bacterial attachment on three poly (ethylene glycol) dimethacrylate surfaces with low (44.05–308.5 kPa), moderate (1495–2877 kPa), and high (5152–6489 kPa) stiffness, respectively [29].

The additional benefit of the polymeric modification is its static charge, which can interact with bacterial membranes. In general, most bacterial cells are surrounded by a layer of peptidoglycan (composed of sugars and amino acids) that are negatively charged, which can be trapped or even killed on positively charged polymeric surfaces, or repelled by negatively charged surfaces. However, this repellency is largely dependent on the species of bacteria. For example, Gram-positive bacteria with a polycationic glycocalyx were more likely to adhere to negatively charged surfaces than Gram-negative bacteria with a polyanionic glycocalyx. Due to the larger discharge capacity, the direct current positive charging method had a better antibacterial effect than the alternating current charging method. In addition, the capacitance-based platform can effectively prevent the formation of biofilms by means of cyclic charging. Extracellular electron transfer between bacteria and charged titania nanotubes doped with carbon-disrupted bacterial morphology and induced intrabacterial ROS burst, leading to bacterial death upon charging [30].

1.2. Contact-Killing Surfaces

It has to be admitted that bacteria-repellent surfaces cannot always successfully prevent bacteria from attaching to them. In this case, people need another effective strategy to resist bacterial infection, namely contact-killing surfaces, where bacteria are killed once they come into contact with the surface. Contact-killing surfaces can be designed and engineered via coating with bactericidal layers or tuning mechanical properties.

Bactericidal substances such as antibacterial metal, antibacterial polymers, and antibacterial peptides can be covalently immobilized on the surfaces [31]. Antibacterial metals should be toxic to a broad spectrum of bacteria, such as Zn^{2+}, Na^{+}, Mg^{2+}, Ca^{2+}, K^{+}, Ag^{+}, Hg^{2+}, and As^{3+}, most of which have been used as antibacterial agents since ancient times [32]. While how antibacterial metals kill bacteria has not yet been fully understood, two possible mechanisms have been proposed to try to explain this phenomenon. First, antibacterial metal could generate oxidative stress to form reactive oxygen species that can kill bacteria. For example, Au was added to the Pd catalyst to promote the release of oxygen-based radical species. It was found that this method was more bactericidal and virucidal and inhibited biofilm formation compared to other methods based on chlorination or pre-formed H_2O_2 alone [33][34]. Second, the release of free metal ions from metal surfaces was responsible for bacteria inactivation [35][36][37][38][39][40][41][42][43][44][45]. For example, compared with a pure strontium calcium phosphate coating, the addition of Zn^{2+} increased the killing rates for *S. aureus* and *E. coli* from 61.25% and 55.38% to 83.01% and 71.28%, respectively. Bacteria on such surfaces with Zn^{2+} underwent partial shrinking, twisting, and even dissolving before death (red arrows) [46].

Negative charges enrich surfaces and generate an attraction force that destroys the integrity of cell membranes and inactivates bacterial enzymes. Most negatively charged materials are polymers, which can be covalently bonded with surfaces with long-term effectiveness. Commonly used antibacterial polymers include quaternary ammonium compounds (QACs), quaternary phosphoniums, and N-chloramines. Taking QACs as an example, they have strong contact-killing activity against both Gram-positive bacteria and Gram-negative bacteria by destroying their membrane. A QAC (s-poly (2,3-dimethylmaleic anhydride) (melittin)-b-poly (2-hydroxyethyl methacrylate) was modified on a surface as a multistage polymer brush to combat bacterial infection [47]. However, these QAC-based surfaces tended to induce irritation and inflammation, which hindered their practical application in the biomedical field [48][49][50][51][52][53][54][55]. In contrast to antibacterial polymers, antibacterial peptides hold great potential to solve the issues of irritation and inflammation and reduce the possibility of induced resistance. When interacting with negatively charged bacterial membranes, antibacterial peptides that usually carry a positive charge in the physiological environment would self-assemble into secondary structures such as α-helical structures, β-sheet structures, ring structures, extended structures, and mixed structures. These shape changes of antibacterial surfaces induced by self-assembly exposed their initially hidden amino acid, which

can destroy the integrity of a cell membrane and further kill bacteria [56][57][58]. For example, WRWRWR-G_4-(dihydroxyphenylalanine)$_4$ was allowed to first self-assemble, and then screws with this coating were implanted into femurs near the joints of Sprague–Dawley rats to evaluate their antibacterial performance in vivo. This animal experiment indicated that the number of bacteria on both the screws and the surrounding tissues were reduced compared with those on bare screws, indicating good antibacterial properties [59].

The contact-killing function can also be achieved by the mechanical rupturing of cells using fine surface structures, such as nanopatterns [119–[60], nanowires [61], nanotubes [62], and nanopillars [63][64][65][66][67][68][69][70][71][72][73][74][75]. The rupturing of the bacterial membrane occurs when the cell membrane is not elastic enough to bear the exerted tensile force. For example, a dragonfly-wing-inspired surface patterned with nanopillars possessed a mechanical bactericidal effect. As a result, this surface was highly bactericidal against all Gram-negative and Gram-positive bacteria. It showed an estimated average kill rate of up to 450,000 cells/min cm^2. The cell integrity of the bacteria was mechanically disrupted by the patterned nanopillars on the surface. Moreover, the viability analysis of bacteria using confocal laser scanning microscopy (CLSM) confirmed that all bacteria were dead after attachment (red color). This biomimetic work demonstrated promising prospects for the development of a new generation of antibacterial surfaces [76].

1.3. Responsive Surfaces

The aforementioned passive antibacterial surface shares a bottleneck: the killing efficiency of antibacterial surfaces may become weakened while the toxicity to normal cells and tissues may be exacerbated during long-term use. A potential alternative candidate is responsive surfaces that can be switched on/off in an on-demand manner [77]. Surface responsiveness is attributed to the use of agents that can be stimuli-triggered by the change in certain bacterial chemical cues (i.e., pH and enzymes) or external triggers (i.e., temperature, ions, light, and magnetism).

Bacterial infections are always accompanied by acidification of the environment (the pH of the infection site drops to 5.5). Such a change in pH can act as a powerful trigger to turn on the antibacterial function by exposing a surface-bound bactericide or releasing preloaded antibacterial agent [78]. The acidic effect mainly stems from low-oxygen fermentation triggering the production of organic acids, such as lactic acid secreted by *S. aureus* or acetic acid secreted by *E. coli*.. Such characteristics can be harnessed to selectively release the antibacterial substances, killing bacteria in real time. For example, a hierarchical antibacterial surface was constructed with a top layer of pH-responsive polymer brush and a bottom layer of bactericidal agents. Decreases in pH could collapse the top layer and induce the exposure of the bactericidal agents, and ultimately activate the bactericidal function. More importantly, the recovery of pH could reconfigure the top layer and switch off the bactericidal function, demonstrating reversibility [79]. When the pH drops from 7.4 to 5.0, the killing efficiency of the proposed surface changes from 9.3% to 77.5%.

In addition to pH, substances secreted by bacteria during metabolism, such as enzymes, can also act as a powerful trigger for killing activity. For example, an enzyme-responsive peptide biointerface was designed based on the saliva-acquired pellicle bioinspired polypeptide DDDEEKRWRWRWGPLGVRGD (SAP-MP196-G-1) that consists of the enzyme-responsive sequence GPLDV and the antimicrobial peptide RWRWRW. When the biointerface was invaded by *S. aureus*, the enzyme response sequence GPLDV was cleaved by the secreted enzyme from *S. aureus*. As a result, the antimicrobial peptide RWRWRW was exposed to kill bacteria. By measuring the number of bacteria in the different groups through quantification of OD_{600}, it was found that bacterial growth was markedly lower on the proposed surface than in a control. [80].

An ion-responsive surface can be achieved by grafting specific ion-pair polymers on the surface, which can endow surfaces with conformational change, high surface wettability, and electrostatic repulsion under the action of external ions. The additional ion-responsive polymer consists of anionic and cationic ionizable units in each repeating unit. The strong hydration of anionic and cationic ionizable units makes the surface excellent in antibiofouling properties. With such beneficial characteristics, the ion-responsive surface appeared a good candidate for achieving antibiofouling function through ion variation [81][82]. As a three-function surface, the reusable antibacterial surface was prepared with comprehensive antibiofouling, antibacterial, and self-cleaning properties. This antibacterial surface comprised (1) poly-n-hydroxyethyl acrylamide hydrophilic polymer as an ultralow-pollution background that can prevent long-term bacterial colonization; (2) triclosan, which can effectively kill attached bacteria; (3) a salt-sensitive polymer, namely, poly(3-(dimethyl(4-vinylbenzyl) amino) propyl sulfonate), which was used to release attached bacteria in the salt solution. The antibacterial surface exhibited three functional antimicrobial activities: poly-n-hydroxyethyl acrylamide resisted bacterial attachment, triclosan killed about 90% of the bacteria on the surface, and poly(3-(dimethyl(4-vinylbenzyl) amino) propyl sulfonate) released about 90% of the dead bacteria on the surface [83].

The temperature has been widely used to control antimicrobials on solid surfaces made of thermally responsive polymers. One typical example of thermally responsive polymers is poly(n-isopropylacrylamide) (PNIPAAm), which can be utilized to achieve a temperature-responsive surface with wettability for bacterial adhesion and separation [84][85]. When the temperature rises higher than the lower critical solution temperature of PNIPAAm, the hydrogen bond between PNIPAAm and water is severely broken, resulting in the hydrophobicity of PNIPAAm. A new thermally responsive surface consisting of thermally responsive hydrogel regions and mechanically supported elastomer regions were prepared. The alternative microscale arrangement of these two regions enabled the surface morphology to have a significant effect on disrupting bacterial colonization and dispersing heat-sensitive individual bacteria. This can effectively prevent bacterial infection without inducing the cohesive loss of human epidermal tissue, thereby serving as an extracellular biointerface for precise local antimicrobial therapy [86].

Many surfaces are sensitive to light, including ultraviolet, visible, or near infra-red light. In practical applications, visible or near infra-red light is more attractive for clinical applications due to its low toxicity and deep tissue penetration. For example, light-responsive surfaces with antibacterial strategies such as antimicrobial photothermal therapy (APTT) and antimicrobial photodynamic therapy (APDT) rely on the generation of local antimicrobial properties to kill cells driven by different frequencies of light. APTT is a physical antibacterial strategy, in which the photothermal agent can continuously heat up under specific light, and the high temperature induces cell-membrane rupture, protein/enzyme denaturation, cell cavitation, and cell-fluid evaporation [87]. For example, two-dimensional Nb_2C Mxene nanosheets as a photothermal agent with implanted medical titanium plates were prepared. The temperature of modified titanium plates was raised steadily to 70 °C within 2 min under the irradiation of a high-power density near-infrared laser. The bacterial survival rates for *S. aureus* and *E. coli* dropped sharply from 100.4% ± 3.12% and 100.02% ± 2.76% in the control group to 1.19% ± 0.93% and 1.06% ± 0.58% in the modified titanium plates + near-infrared laser group, respectively [88]. APDT is a minimally invasive strategy that uses light-responsive photosensitizers to generate reactive oxygen species through photochemical reactions, resulting in irreversible damage and cell death [89][90]. For example, smart nanoplatforms with photosensitizer molecule chlorin e6 were prepared. When light irradiated the above platform, the ratio of anaerobic *P. gingivalis* and *Fusobacterium nucleatum* was reduced from 66.21% in the control group to 51.91% [91]. Combing APTT and APDT, a red phosphorus/zinc oxide heterojunction was prepared that has excellent solar photothermal conversion and photocatalytic efficiency, further leading to the death of bacteria through hyperthermia and reactive oxygen species. The bacteriostatic effectiveness on *S. aureus* at 5 min was 99.96 ± 0.03%, and that on *E. coli* at 4 min was 99.97 ± 0.02% [92].

A magnetic responsive surface is also a good antibacterial surface. The application of the magnetic field induced the magnetic metal to spin, deform, and exert physical forces on the bacteria, which resulted in the disruption of the dense biofilm matrix and simultaneous lysis of the cells. Once exposed to a low-intensity rotating magnetic field, the liquid metal droplets are physically driven to change shape, creating sharp edges. When in contact with bacterial biofilms, the particle motion created by the magnetic field, coupled with the presence of nanoedges, physically ruptures the bacterial cells and disrupts the dense biofilm matrix. For example, magnetic galinstanc-based liquid metal platforms can also kill bacteria under an external magnetic field. After introducing two major pathogens biofilms, specifically *P. aeruginosa* and *S. aureus*, the system was exposed to a dynamic magnetic field of 775 mGs. Following 90 min of exposure to the magnetic field with the gallium-based liquid metal ferrofluid platforms, it was observed that the average colony-forming unit /mm^2 was reduced for both biofilms of *S. aureus* (99.85%, *p* 0.001) and *P. aeruginosa* (96.51%, *p* 0.01) when compared to controls [93].

2. Active Antibacterial Surface

Unlike the biological antibacterial surfaces that are adaptive and flexible to diverse harsh environments, an artificial surface usually displays relatively short-term bacterial resistance. To prevent bacterial adhesion and biofilm formation, new physical removal strategies relying on external sources such as mechanical force or energy waves have emerged as alternatives to an antibacterial agent. Generally, these external sources include shear force, interfacial tension, mechanical waves, dynamic actuating motions, and plasma treatment. Unlike passive antibacterial surfaces, the antibacterial process of the active antibacterial surface is controllable and acts directly on bacteria.

The shear-force-based method is very effective to remove bacteria directly by generating shear force sufficient to balance the bacterial adhesion force. Shear forces can be produced by the external application of force parallel to the surface. The inner wall of the microfluidic device is composed of the copolymers, 2-methacryloyloxyethyl phosphorylcholine, 3-methacryloxypropyl trimethoxysilane and 3-(methacryloyloxy) propyl-tris(trimethylsilyloxy) silane, with two typical thicknesses (20 and 40 nm), forming a cross-linked film. Shear forces were generated by friction between the fluid and the

inner wall of the microfluidic device. Under the same shear stress, thicker surfaces could weaken the adhesion of S. aureus, which leads to more bacteria detachment [94].

Surface antibacterial performance can also be improved by adjusting the interfacial tension with surfactants. Among those, one effective way to control interfacial tension is the use of biosurfactant, a kind of surface-active biomolecule produced by many microorganisms. It has been reported that the aggregation of biosurfactants at the interface can reduce the interfacial tension of the solution and form a microcellular structure, which can disrupt the bacterial cell membrane to produce antibacterial properties. Moreover, the properties of the biosurfactant itself, such as its concentration, also influence the antimicrobial performance. Taking sophorolipids (a type of biosurfactant) as an example, at a concentration above 5% *v*/*v*, they can inhibit the growth of Gram-negative *Cupriavidus necator* and Gram-positive *Bacillus* sp. with a bactericidal effect. Below this concentration, the antibacterial properties are greatly reduced [95]. In addition, Zein/gum Arabic nanoparticles were prepared to stabilize the oil–water interface of Pickering emulsions, which strikingly inhibited the growth of *E. coli*. The stabilized emulsion exhibited a controlled release and the antibacterial activity of thymol due to the protective effect from its stable interfacial layer [96].

Mechanical waves such as ultrasound waves could induce surfaces made of piezoelectric materials to generate reactive oxygen species to make them antibacterial. For example, a piezoelectric surface was prepared by using barium titanate nanocubes whose Schottky junctions were modified with gold nanoparticles. This surface could sense exogenous ultrasound waves and produce highly reactive oxygen species as a response to obtain antibacterial ability. It was demonstrated that this surface exhibited high antibacterial efficiency against both typical Gram-negative and Gram-positive bacteria, offering a promising method for efficient ultrasonic therapy [97].

Dynamic actuating motions of surfaces can prevent bacterial attachment to suppress surface fouling. This phenomenon has been widely found in nature, such as red blood cells, arteries, blood vessels, starfish, seaweed, mussels, and the skin of batoidea and pilot whales. Particularly, batoidea manipulate their body in an undulatory style to generate vortices to repel bacteria. Inspired by this, a flexible multilayer responsive surface was designed to integrate dynamic undulatory motion with bactericidal nanospine arrays. Under an applied magnetic field, this surface behaved with a batoidea-like undulatory motion, which generated strong vortices to repel bacteria. Moreover, the integration of a dynamic undulatory motion and static nanospine array enabled this surface to repel and kill bacteria simultaneously, effectively inhibiting biofilm formation for an extended period of 7 days [98].

Plasma treatment is commonly used to make surfaces antibacterial owing to its ability to change the surface wettability. For example, after being treated by non-thermal atmospheric pressure plasma jets (NTAPPJs), the titanium surface became antibacterial against two bacteria with different cell-wall structures, including Gram-positive and Gram-negative bacteria. The adhesion and biofilm formation rates of bacteria on NTAPPJ-treated titanium surfaces were significantly reduced compared to untreated samples. Surfaces treated with NTAPPJ can induce oxidation in bacteria, which were more susceptible to Gram-negative bacteria due to differences in the cell-wall structure. In samples treated with NTAPPJs for a longer time, the adhesion rate and biofilm formation rate of Gram-negative bacteria were significantly lower than those of Gram-positive bacteria [99].

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