Bio-inspired mechano-bactericidal nanostructures: a promising strategy for eliminating surface foodborne bacteria

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Abstract

Contamination of food by bacterial pathogens is one of the biggest concerns in the food industry as it can result in serious human illnesses and death. Approaches to eliminate bacteria from outer surfaces of food products would be an effective way to safeguard against bacterial contamination. Nanopillars found on natural surfaces have been shown to mechanically damage cell membranes of foodborne bacteria. Therefore, fabricating bio-inspired mechano-bactericidal nanostructures into food packaging and processing materials could be a promising strategy to reduce surface bacterial contamination, to improve food safety. In this review, we summarize the formation of natural and synthetic nanopillared surfaces and their mechanism of action, and highlight the factors that influence their mechano-bactericidal activities.

Keywords: mechano-bactericidal, nanostructured surfaces, bio-inspired, bactericidal mechanisms, food safety

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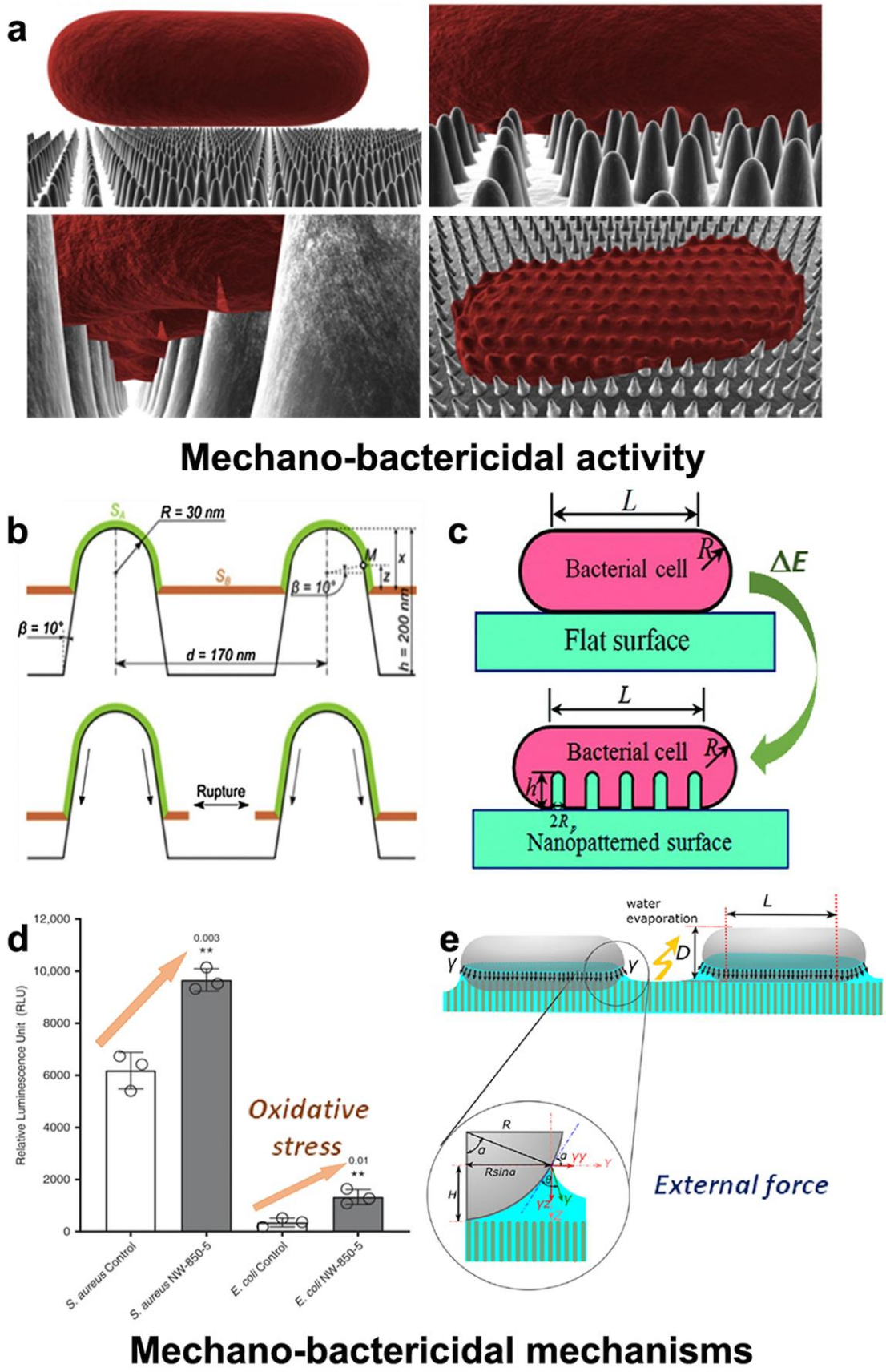
1 Introduction

Adhesion of pathogenic bacteria to food-contacting surfaces and subsequent biofilm formation can result in food safety issues. Biofilms facilitate cell-cell and cell-surface attachment and provide a protective environment for bacterial pathogens to withstand cleaning efforts and persist in food-processing environments, leading to foodborne outbreaks [1]. Traditional approaches entail either chemically modifying or physically coating the surface with antibacterial agents in order to eliminate the attachment of bacteria to the surface [2]. However, designs that use antibacterial release to eliminate surface bacteria are problematic since the active agents often do not reach required concentrations [3], while the overuse of antibacterial agents can lead to antimicrobial resistance. Additionally, antibacterial agents may also introduce undesired toxins into food products.

Inspired by the naturally occurring surfaces such as insect wings and gecko skin, a novel mechano-bactericidal approach has been developed in recent years. Hierarchical nanofeatures including pillars, hairs, and needles exist on these surfaces, and bacterial cells are inhibited, injured, and even killed when they directly contact surface nanotopography [4,5]. Synthetic surfaces capable of mimicking this physical biocidal mechanism have been fabricated and some exhibit promising antibacterial properties. Therefore, this mechano-bactericidal approach may overcome the drawbacks of traditional antibacterial treatments and be applied in food packaging and processing to improve food safety. In this review, we have summarized different mechano-bactericidal nanostructured surfaces, found both in nature and synthesized by various fabrication techniques, as well as their theoretical mechanisms of action. Factors that affect mechano-bactericidal activity are highlighted, including characteristics of the bacteria as well as surface nanofeatures such as spacing, contact area, and aspect ratio. Finally, future opportunities, challenges, and research directions are described.

2 Bactericidal mechanism of nanostructured surfaces

The bactericidal effects of nanopillars require bacterial cells to adhere to the surface (Figure 1a). Bacterial surficial attachment is mediated by microbe structure, surface charge, hydrophobicity, production of extracellular substances, and cellular appendages such as fimbria or pili [6]. Higher adhesion forces between bacteria and nano-textured surfaces induce a higher probability of cellular rupture [7]. Two models, the biophysical model and the analytical thermodynamic model, have been developed to describe the mechanism of cell death on nano-patterned surfaces [8]. However, neither of these two models explain the up-regulation of oxidative stress proteins in bacterial cells exposed to nanopillared surfaces, so a third model regarding oxidative stress has been proposed [9*]. In addition to these commonly accepted models, other possible mechanisms such as external forces have also been proposed to further explain the interactions between bacteria and nanopillared surfaces [10].



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65 **Figure 1.** Schematic diagram of mechano-bactericidal activity on nanopillar surface (a) and

different bactericidal mechanism models (b-e): (b) biophysical model; (c) thermodynamic model; (d) H_2O_2 production in response to nanopillar surfaces in oxidative stress model; and (e) sketch of the capillary meniscus on bacterium during water evaporation and analysis of capillary forces [9,11,12,14].

2.1 Biophysical model

The bactericidal mechanism of nano-textured surfaces is the physical stretching of the cell membrane upon direct contact with the surface resulting in rupture. This effect is dependent on the rigidity of bacterial cell membrane [11]. The exact interactions of bacterial adsorption on a nanopillared surface can be divided into two regions: areas in direct contact with the pillars (S_A) and areas between pillars (S_B) (Figure 1b). Mathematical modeling describes cell wall stretching dynamics within the two regions by calculating the total free energy (E):

$$E = \frac{\varepsilon n_0 S_A}{1+\alpha_A} + \frac{k}{2}\left(\frac{\alpha_A^2 S_A}{1+\alpha_A} + \frac{\alpha_B^2 S_B}{1+\alpha_B}\right) + \lambda k\left(\frac{S_A}{1+\alpha_A} + \frac{S_B}{1+\alpha_B} - \frac{S_i}{1+\alpha_I}\right) \quad (1)$$

Where ε is the energy gains per adsorption site on nanopillar surface; n_0 is the surface density of the sites on relaxed layer; α_A is the stretching of adsorbed region of layer; α_B is the stretching of suspended region of layer; k is the stretching modulus; λ is Lagrange multiplier; S_i is the total initial area of unperturbed membrane; and α_I is the initial stretching degree [11]. Once α_B reaches a critical value, the membrane ruptures.

When a bacterial cell contacts nanopillars, it adsorbs onto the nanopillars, resulting in the increased surface area and the stretching of the regions between pillars. Once beyond the maximum stretchable potential, the cell membrane ruptures and leaks cytoplasmic contents, leading to eventual cell death [11]. The shortcoming of this model is that it does not incorporate information on bacterial shape or composition, or the mechanical properties of the nanopillars.

2.2 Thermodynamic model

This model suggests that the bactericidal activity is related to the balance between the deformation energy and contact adhesion energy [9*]. Interactions between

bacterial cells and surfaces are defined through analyzing the total free energy change of adherent bacteria (Figure 1c) [12]. It is calculated by comparing a nanopillared surface to a flat surface where the primary differences are the deformation of the cell membrane and the contact adhesion interface in the attached area. The total free energy change (ΔE) on a nanopillared surface is given by:

$$\Delta E = \frac{1}{2}\delta\frac{\Delta S^2}{S_0} + \left(E_{edge}^{Bend} + E_{ad}^{Bend} - E_0^{Bend}\right) - \gamma S_{ad} \quad (2)$$

Where δ is the stretching modulus of membrane; S_0 and ΔS are the initial area and the area change of cell membrane; E_{edge}^{Bend} is the deformed bending energy of the membrane at the edge of bacterial cell; E_{ad}^{Bend} is the deformed bending energy of contact adhesion membrane; E_0^{Bend} is the deformed bending energy of initial cell membrane; γ is the contact adhesion energy density between flat surface and cell membrane; and S_{ad} is the contact area [12].

This model also assumes the bacterial membrane is a thin elastic layer and neglects the membrane composition. The higher density and larger radius and height of nanopillars increase the contact adhesion energy, resulting in a large degree of bacterial membrane stretching [12]. If the degree of stretching is sufficient, it will lead to rupture and death of bacterial cells. According to this model, bacterial cells with larger stretching capacity will have greater resistance to the bactericidal action of nanopillars [13].

2.3 Oxidative stress model

Although cell membrane deformation and penetration have been observed in many studies, such events do not lyse bacteria. Thus, interactions mentioned above cannot solely account for the reduction in bacterial viability. The oxidative stress model is proposed to explain why up-regulation of oxidative stress proteins and time-dependent decrease in cell viability are observed in both Gram-positive and Gram-negative bacteria when the cells come to contact with nanopillared surfaces (Figure 1d) [9*].

Due to the effect of nanopillars, increased levels of reactive oxygen species (ROS) and hydrogen peroxide were observed. Upregulation of ROS and hydrogen peroxide triggered bacterial oxidative stress leading to damage of DNA, lipids, and proteins. Such damages altered the bacterial cell envelope morphology and might enhance cell rupture [9*].

2.4 Other models

No consensus has been reached on the bactericidal mechanism, and more factors such as external forces have been considered. Li et al. proposed the mechanism to explain the death of small, long, and thin bacteria on nanopillared side edges. Surface adhesion, gravity, or a combination of both accounted for the stretching, compression, tearing or disrupting of bacterial cell wall [13]. Bandara et al. [10] found that the strong adhesion and shearing stress were imposed by nanopillars, contributing to the damage of bacterial membrane. A recent study demonstrated that bacteria on multiple hydrophilic "mechano-bactericidal" surfaces remained viable unless exposed to a critical level of external force that could rapidly deform and rupture bacteria (Figure 1e) [14].

3 Naturally occurring nanotopography

Natural nanotextured surfaces evolve as a strategy to inhibit bacterial growth and biofilm formation. For example, cicada [15], damselfly [16] and dragonfly [10] wings, and gecko skin [13] are coated with nanopillars (Figure 2). Such micro- and nano-structures on biological surfaces endow characteristic properties of self-cleaning, superhydrophobicity [17], antireflection [18], and bactericidal activity [19]. Among these properties, bactericidal behavior towards *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, common pathogens that cause foodborne infections, has drawn significant amount of attention [20]. Bactericidal activities of various natural nanostructured surfaces are shown in Table 1.

3.1 Insect wings

Nanopillars on the surface of cicada (*Psaltoda claripennis*) wings are able to structurally deform *P. aeruginosa* cells beyond repair within 3 min and are therefore bactericidal [15]. To identify the role of surface chemistry in the bactericidal effects, cicada wings were coated with a 10 nm-thick gold film to decrease the hydrophobicity without changing topography. The bactericidal effect of gold-coated wings was preserved, indicating that the physical structure, rather than the surface chemistry, was responsible for bacterial inactivation [15]. Subsequent studies revealed that subtle nanoscale differences could cause substantial changes in bactericidal action [21]. Long sharp nanopillars had broader antibacterial activity against both Gram-positive and Gram-negative bacteria, while short blunt nanopillars were only bactericidal against specific species [22*]. The bactericidal selectivity based on different nanopillars was also observed in nature. Dragonfly wings nanopillars have random shapes, sizes, and distribution, and are effective against both Gram-positive and Gram-negative bacteria, while the nanopillars on cicada wings are more consistent and are only effective against Gram-negative bacteria [11,15,21].

3.2 Gecko skin

Geckos have a uniform array of nano-tipped spinule (hairs) hierarchical structures on their skin, capable of killing bacterial cells [23]. The hairs are approximately 1-4 μm in length and taper into 50 nm nanotips that are composed of two length tiers. They can curve significantly due to their flexibility and elasticity [19] and have displayed a high efficiency in eliminating pathogenic bacteria on the skin even after multiple exposures [13]. Multi-tiered nanostructures also play an important role in the death of bacteria, due to synergistic stresses on cells [13].

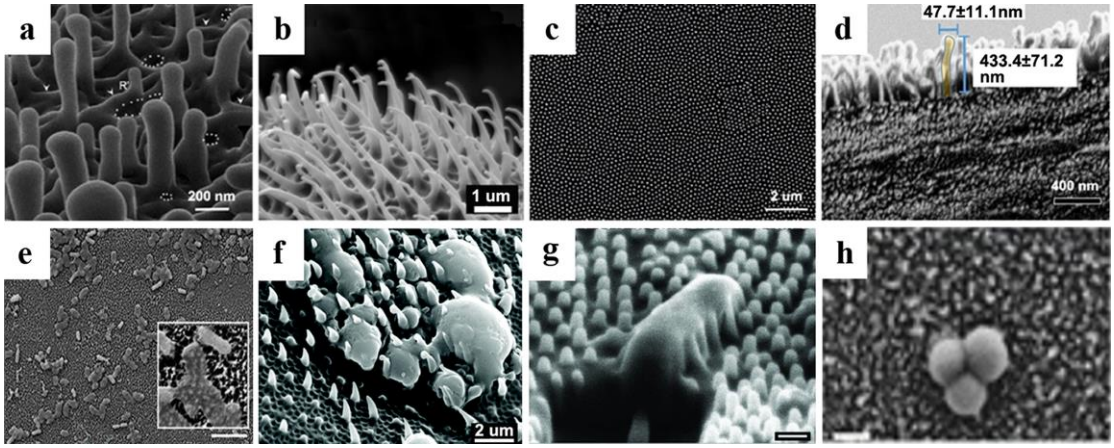

Figure 2. SEM images of natural bactericidal nanotopographies (top) and bacteria on surfaces (bottom): (a,e) *E. coli* on dragonfly wing; (b,f) *Porphyromonas gingivalis* on gecko skin; (c, g) *P. aeruginosa* on cicada wing; and (d,h) *S. aureus* on damselfly wing [10,13,15,16].

4 Synthetic bactericidal surfaces

To mimic nanostructured surfaces found in nature, many synthetic nanopillars (Figure 3) have been fabricated from various materials including black silicon [24], graphene [25], titanium [26*], copper [27], and gold [28], and have exhibited remarkable bactericidal capabilities (Table 1).

4.1 Black silicon and silicon materials

Black silicon (bSi) was the first material used to mimic nanopillars found on *Diplacodes bipunctata* dragonfly wings [29]. Various techniques such as plasma etching [24,30], electrochemical etching [31,32], and laser treatment [33] have been used to fabricate bSi nanotextured surfaces, which were bactericidal against foodborne bacteria such as *S. aureus*, *P. aeruginosa,* and *E. coli* [24,34]. May et al. [24] fabricated a boron-doped diamond (BDD) electrode material using bSi as a substrate. The bSi surface consisted of short needles and was bactericidal against approximately 13% of *P. aeruginosa* cells. In another study, it was found that the reactive-ion etching process endowed Si structures with high aspect ratio nano-protrusions [34]. Nanostructured Si prepared by tetrafluoromethane/hydrogen plasma technique could significantly decrease the bacterial loads of *E. coli*, *S. aureus*, and *Bacillus cereus* with >5 log reductions. By controlling the processing time, plasma etching can form various

surface topographies. Linklater et al. [35] produced different surface nanostructures with increasing heights of 280, 430, and 610 nm through plasma etching using 15, 30, and 45 min etching intervals, respectively. It was shown that shorter and denser nanopillars exhibited better bactericidal properties with 85% and 89% inhibition of *S. aureus* and *P. aeruginosa*. Using plasma etching and deep UV immersion lithography, a Si nanopillar array with an average height of 380 nm and a diameter of 35 nm was engineered [36*]. The bactericidal efficacy towards *P. aeruginosa* was 85%, but only 8% against *S. aureus*.

4.2 Graphene and graphene-derived materials

Graphene and graphene-derived materials (*e.g.* graphene oxide (GO) and reduced GO (rGO)) possess high bactericidal efficacies. Sengupta et al. [37] found that GO inhibited *S. aureus* and *P. aeruginosa* by 93.7% and 48.6%, while rGO killed 67.7% and 93.3%, respectively. Highly wrinkled GO films can be produced by simple vacuum filtration and drying of GO suspension through a pre-strained filter [38]. The sharp edges found on atomically thin layers of graphene are responsible for the deformation of bacterial cells and the subsequent release of cytoplasmic material [5]. Moreover, the oxidative environment induced by nanosheets could likely damage surrounding bacteria cells.

Nanostructures with improved mechano-bactericidal activities can be fabricated by decorating GO with nanoparticles. Selim et al. [39] developed GO sheets with blade-like structure decorated by nano-Cu_2O and SiC nanocomposites, where GO/Cu_2O exhibited higher bactericidal activity than GO/SiC towards Gram-negative bacterial strains (*P. aeruginosa* and *E. coli*). Graphene sponge decorated with copper nanoparticles also displayed high antibacterial efficiency against *E. coli* [40].

4.3 Titanium surfaces

Titanium-based nanotextured surfaces produced by a hydrothermal etching process exhibited the bactericidal activities of 80.7% and 86.8% against methicillin- and gentamicin-susceptible and resistant *S. aureus* strains, respectively [41]. Since the

nanosheet topology produced by hydrothermal etching is random, processing parameters (*e.g.* etching time) were controlled to obtain optimal surface structure for bactericidal activity [26*]. The results showed that titanium treated for 6 h had the best bactericidal capacity against *P. aeruginosa* (99%) and *S. aureus* (90%) [42*].

In addition to hydrothermal etching, other methods were also used to develop nanotextured titanium surfaces. Sjöström et al. [43] used thermal oxidation to fabricate nanospikes on a titanium alloy and observed a 40% reduction of *E. coli*. The reduction was attributed to the sharp tips or edges of nanospikes on alloy surface. Similarly, Hasan, et al. [44] fabricated a black titanium surface through a chlorine-based reactive ion etching processing. The anisotropic nanostructures trapped the light to make the surface appear black and displayed antimicrobial efficiency of killing 95% of *E. coli*, 98% of *P. aeruginosa*, 92% of *Mycobacterium smegmatis*, and 22% of *S. aureus* within 4 h of contact. Furthermore, titanium surfaces with hierarchical nanostructures have been produced by maskless plasma etching [45*], where micron-sized pillars were likely responsible for trapping the cells and the second tier of pillars acted to kill the cells. The bactericidal efficiencies against *P. aeruginosa* and *S. aureus* were 87.2% (30 min of etching) and 72.5% (40 min of etching), respectively.

4.4 Other bioinspired nanostructured surfaces

Singh et al. [27] prepared a copper nanowhisker surface deposited by molecular beam epitaxy and proposed the behavior ‘pinning effect of water drops’ on the surface would encourage mechanical bacteria killing. Rosenzweig et al. [46*] fabricated the nanopillar surfaces by nanoimprint lithography, which were able to reduce the motility and attachment of *P. aeruginosa*. The technique is time-efficient, scalable, precise, and low-cost, since it can produce large numbers of replicates from one master mold. The same method was also used to prepare polydimethylsiloxane (PDMS) pillar arrays against *E. coli* and *S. aureus* [47], and a similar rapid and accurate bio-templating technique was developed by casting material onto the surface of gecko skin as a negative mold [19]. Furthermore, Yuan et al. [48] reported positively charged metal organic framework (MOF) nano-dagger surfaces that displayed superior bactericidal

activity, because the positive charge enhanced bacteria adhesion and sharp nano-dagger tips promoted bacteria killing.

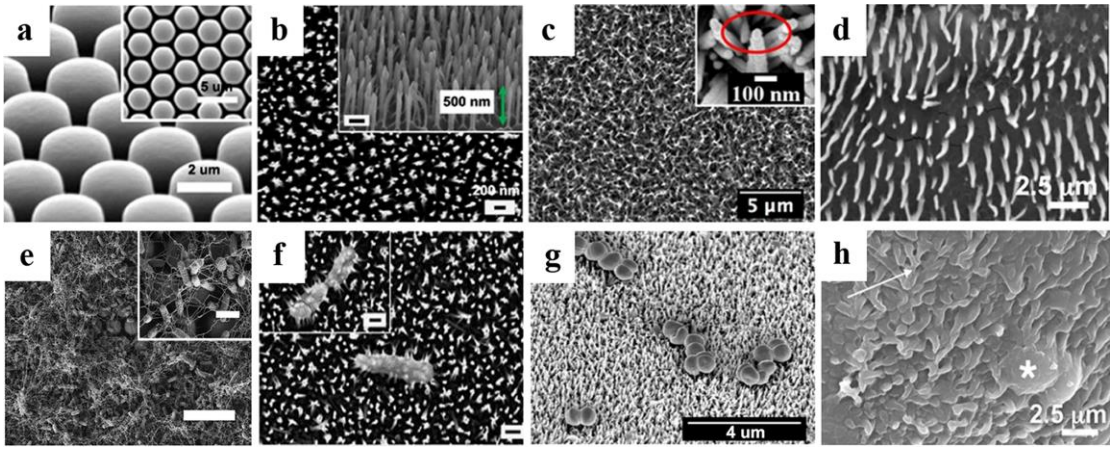

Figure 3. SEM images of synthetic bactericidal nanotopographies (top) and bacteria on surfaces (bottom): (a,e) *E. coli* on polydimethylsiloxane; (b,f) *P. aeruginosa* on black silicon; (c,g) *Staphylococcus epidermidis* on titanium; and (d,h) *Lactobacillus* on chitosan [19,29,49,50*].

Table 1. Bactericidal activities of various natural and synthetic nanostructured surfaces.

Nanostructured surfaces	Bactericidal mechanisms	Bacterial species	Bactericidal effects
Gecko hairs [13]	Surface adhesion / external forces	*S. mutan*	90-95% cell death
Dragonfly wings [10]	Adhesion / shear force	*E. coli*	Reduction of 4.99 $\times 10^5$ cells/min·cm^2
Damselfly wings [16]	n.d.	*S. aureus* *P. aeruginosa*	89.7% cell death 97.7% cell death
Titanium dioxide nanopillars [9]	Oxidative stress	*S. aureus* / *E. coli*	n.d.
Spear-type titanium surface [50*]	Sharp tips of nano spears caused cell	*S. epidermidis*	37% cell death

	lysis		
Pocket-type titanium surface [50*]	Cell deformation between nano spears inside the pocket	*S. epidermidis*	47% cell death
Polystyrene and chitosan duplicates from gecko skin [19]	Compression, stretching, tearing and piercing	*Lactobacillus*	95% cell death
Positively charged MOF nano-dagger arrays mimicked from cicada wing [48]	Oxidative stress; electrostatic and hydrophobic interactions	*E. coli*	*Log* reduction of 7
		S. aureus	*Log* reduction of 8
Silicon nanopillars [36]	Stretching	*P. aeruginosa*	85% cell death
		S. aureus	8% cell death
Silicon nanopillars [14]	External force / nanopillared surface	*P. aeruginosa*	Over 99.9% cell death
Graphene oxide nanosheets [38]	Electrostatic / van der Waals forces	*E. coli*	80% cell death
		M. smegmatis	70% cell death
		S. aureus	80% cell death

"n.d." not determined.

5 Factors influencing bactericidal activity

In addition to the fabrication parameters, properties of certain bacteria and nanofeatures including protrusion diameter, height, and spacing can also influence the bactericidal efficacy.

5.1 Bacterial cell properties

The bactericidal activity of a nanotextured surface is dependent on bacterial adhesion, which involves an initial attraction and subsequent attachment of bacterial cells. Many factors can affect the attachment of bacteria to a given substrate, namely cell surface hydrophobicity (CSH) and cell surface charge [51]. CSH is a physical force

that can increase or decrease the propensity of microbial adhesion, depending on the surface and bacterial cell wall composition [52]. Bacteria are generally hydrophilic, and therefore can adhere more readily to hydrophilic surfaces [53]. However, microorganisms can switch between hydrophilic and hydrophobic phenotypes depending on environmental conditions and growth phases, meaning that they may not always attach [52]. Cell surface charge may also be a factor responsible for influencing bacterial attachment. While the degree of charge varies between species and strains, the majority of bacterial cells are negatively charged due to an excess of carboxyl and phosphate groups within their cell walls [54]. A positively charged nanostructured surface tends to display higher bactericidal efficiency and selectivity due to the stronger interaction with negatively charged bacterial cell membranes [48].

Differences in Gram-positive and Gram-negative bacteria cell wall compositions can affect their rigidity. The type of bonds (covalent vs. electrostatic) between the peptidoglycan and outer membrane layer also contributes to overall cell rigidity [22*]. Gram-negative bacteria contain a 2-3 nm peptidoglycan layer, while Gram-positive bacteria have a 20-80 nm peptidoglycan layer, giving Gram-positive bacteria more rigidity. Higher rigidity results in higher resistance towards deformational stresses imparted from protrusions on the surface [55].

Bacterial size and shape can dictate interactions with nanotextured surfaces. Larger bacterial cells settle on top of pillars and penetrate gradually, while smaller bacteria tend to settle in the interspace between two pillars, interacting more with edges [13]. It has been proposed that, if the spacing among pillars was comparable to the size of bacteria, the cells would orient themselves in order to maximize the contact area [56]. For example, rod-shaped bacterial cells were found to settle perpendicularly between pillars, and the contact area increased when the spacing decreased. When cell membrane attach and adsorb onto the surface extensive stretching causes the rupture of membrane and finally cell death, albeit only when external forces exceed the maximum elasticity of membrane [34].

Bacterial growth cycle plays a key role in the bactericidal activity of nano-pillared surfaces. Truong et al. [16] investigated the bactericidal action of damselfly wings to lag, log, and stationary *S. aureus* and *P. aeruginosa* cells. It was found that *S. aureus* (89.7% at log phase) and *P. aeruginosa* (97.9% at stationary phase) were most inhibited.

5.2 Surface features

5.2.1 Nanostructured surface shape

Besides pillars, alternative structures have also been fabricated in recent years. For example, Cao et al. [50*] produced spear-like and pocket-like structures through hydrothermal treatments. The spear type could not prevent the formation of biofilms, while the pocket type, which was the network of intertwined spears, delayed biofilm growth and killed up to 47% of adherent bacteria. It was proposed that bacteria slid in between arranged spears inside the pocket, resulting in the increased contact area and subsequent cell penetration. Similarly, Linklater et al. [45*] suggested that nanopillar clusters were able to rapidly trap and damage more bacteria than single pillar due to the presence of many sharp and dense tips that enhanced membrane stretching. Moreover, Zou et al. [38] demonstrated that GO nanosheets containing wrinkled structures with a 500 nm roughness grade displayed the strongest antibacterial effect against *E. coli* and *S. aureus*, trapping size matched bacteria with a large contact area. Other studies have shown that the edge of nanostructure is an important factor in determining bactericidal activity. Wandiyanto et al. [26*] fabricated titanium nanosheets with sharp and blade-like nano-edges of about 10 nm, which demonstrated the highest mechano-bactericidal efficacy against *P. aeruginosa* (99%) and *S. aureus* (90%). Zeolitic imidazolate framework nano-dagger arrays were taller, larger, more rigid, and sharper at the tips than common nanopillars [48]. These positively charged nano-daggers led to a stronger interaction with bacteria, and thus increased bactericidal activity. However, the bactericidal efficiencies of nano-pillars, nano-rings, and nano-nuggets with the same height of 100 nm and diameter of 50 nm were similar [28].

5.2.2 Other features

It has been found that spacing, height, aspect ratio, and diameter of nanostructured surfaces can influence mechano-bactericidal activity. By decreasing height, spacing and diameter, the number of bacterial cells attached to the surface decreases [35]. For instance, the pillar arrays with a pitch of 1100 nm only showed antifouling behavior against large bacteria, such as *E. coli*, whereas those with a pitch of 480 nm displayed both bactericidal activity and antifouling behavior [47]. In addition, pillars with spacing under 60 nm could effectively kill *S. aureus* [35]. Smaller spacing was thought to induce more localized forces on cells, causing intensive stretching of membranes and finally cell lysis [11]. However, too small spacing between nanopillars would not allow bacterial cells to settle, providing additional chances for bacterial attachment [49]. This was supported by the result that surfaces with approximately 40 pillars/μm^2 had higher bactericidal effects than that with very high density (~70 pillars/μm^2) [54*].

Bactericidal activity is also related to the height of nanopillars. For example, nanopillars with a height of 50 nm were not effective at killing *S. aureus*, but 400 nm nanopillars were highly bactericidal [28]. Extremely long nanopillars (>7 μm) exhibited superior bactericidal action against various bacterial species, while short ones (<2 μm) killed species selectively [22*]. However, if nanopillars were too long (~1000 nm), they could not be well separated, resulting in a low bactericidal activity [58*].

6 Conclusions and outlook

Several natural surfaces with nanostructures display antibacterial properties against common pathogens including foodborne bacteria. Inspired by these natural structures, synthetic surfaces have been fabricated from black silicon, graphene, graphene-derived materials, and titanium. Factors such as the cell properties of bacteria and surface features (*e.g.* spacing, height, aspect ratio) significantly influence the interactions between surfaces and bacteria. However, current models cannot explain the mechano-bactericidal mechanisms of different nanotopographies.

Compared to traditional antimicrobial strategies, mechano-bactericidal nanostructures have demonstrated exciting potential in the field of food safety and are

promising to be applied in various food supply chain stages. Future research is required to fully realize their applications:

1. The mechanism of mechano-bactericidal properties is key to future development. It is difficult to conclude how nanoscale surface features affect the bactericidal activity due to multiple parameters that need to be considered. Increased nanopillar contact area, density, and aspect ratio, as well as sharper edges and tips possibly contribute to improved bactericidal efficiency. More research is needed to further understand how each parameter influences the bactericidal properties. This will then allow us to better design and optimize the nanotopographies to achieve the broader bactericidal activity.

2. Bacterial adhesion compromises food quality and safety. To address this problem, nanostructures can be produced using various materials (not only inorganic compounds) or formed on different surfaces such as food packaging and food processing devices for applications in food preservation, antibacterial coating, water disinfection, etc. Feasible and cost-effective approaches to fabricate mechano-bactericidal nanostructures on an industrial scale are necessary.

3. The efficacy of most mechano-bactericidal surfaces is not comparable to other bactericidal approaches, which may hinder their applications. The bactericidal activity can be further improved by combining the physical optimization of structures, chemically surface modifications with recognition elements (*e.g.* aptamers, enzymes, and antibodies), and involvement of external forces.

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