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Bioinspired nanostructured bactericidal surfaces

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The rise of antimicrobial resistance as a pressing global healthcare challenge underscores the need to identify novel strategies to tackle pathogenic bacteria. Many naturally occurring nanostructures exhibit an innate ability to deactivate bacterial cells by physical contact. However, several aspects of the underlying mechanisms are poorly understood due to the complex interactions of bacterial cells with nanostructures, which are difficult to simulate using theoretical models. This review describes the experimental reports of the state-of-the-art in designing bioinspired mechano-bactericidal surfaces and theoretical models to elucidate underlying phenomena at the cell–material interface. The different processes used to make nanostructured surfaces and their effects on bactericidal activity are summarized. Recent findings disputing the current understanding are critically discussed. Lastly, the challenges and opportunities in fabricating nanostructures on devices and implants for clinical use are presented.

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Introduction

Growing antibiotic resistance patterns related to overuse in humans and in livestock are an urgent threat to public health and food security, demanding a paradigm shift in our approach to infectious diseases [1]. The World Health Organization (WHO) recognizes antimicrobial resistance (AMR) as one of the gravest threats in modern medicine [2]. The 2021 WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) demonstrates rates of bacterial resistance of up to 54% against commonly used first-line antibiotics for the bloodstream and urinary tract infections and, alarmingly, even higher rates of resistance to some last-resort antibiotics used in complex hospital-associated infections [3]. At the patient level, progressively increasing antibiotic resistances with recurrent infections requires escalation to broader spectrum antibiotics with riskier side effect profiles, including disruption of the systemic microbiome and increased risk of opportunistic infections such as clostridium difficile [4,5]. The alarming rise of superbugs and the relative lag in the development of novel antibiotics has motivated a quest for functional surfaces that can minimize bacterial colonization by either minimizing the attachment (i.e. anti-biofouling) or killing (i.e. bactericidal) through contact to minimize infections. The current pandemic has further increased public awareness of the need for antimicrobial surfaces for the food industry, healthcare, and shared public spaces [6].

Most surfaces are not inherently antibacterial. The highly mobile and competitive nature of bacteria to race to occupy surfaces leads to their colonization by initial attachment. This is followed by cell replication and the eventual formation of biofilms along with the secreted extracellular polymeric matrix. Conventional approaches to preparing antibacterial surfaces include using metal coatings (e.g. Ag, Cu, Zn, or metal oxides) or biochemical modification with antibacterial agents (e.g. antibiotics). However, the antibacterial agents often do not reach effective concentrations, and excess environmental release of antibiotics can lead to the inception of problematic drug-resistant strains.

Nature offers much inspiration in the quest for antibacterial surfaces. Microtopography on lotus leaves, rose petals, and sharkskin is known to impart anti-biofouling character. More recently, the mechano-bactericidal activity of insect wings and gecko skin was discovered [7•]. These surfaces are characterized by ordered or disordered anisotropic nanostructures such as pillars, needles, or hair-like projections. Bacterial cells are ruptured through direct physical contact with these nanostructures [7•,8]. The discovery of this evolutionary advantage in the form of natural mechano-bactericidal activity offers a hitherto unexplored approach for combating bacterial colonization without generating AMR. The developments in micro-manufacturing/nano-manufacturing in recent decades are timely for replicating such architecture on synthetic substrates. Comprehensive reviews on various surface nanotopographies that minimize bacterial colonization of surfaces through

antibiofouling and bactericidal mechanisms are available in the scientific literature [9–11]. However, this is an active area of research where the science (understanding of the mechanisms of antibacterial action) and the strategies to replicate them are rapidly evolving, necessitating a review describing the latest developments in the field. Moreover, only a few reviews describe the latest methods to replicate such topographies on synthetic surfaces. The current review focuses on mechano-bactericidal surfaces. Specifically, this review highlights the key issues in this field, including: i) the bactericidal activity of various natural and synthetic bactericidal surfaces and the role of topography; ii) the latest biophysical models for the bactericidal mechanisms and their limitations; and iii) challenges in fabrication techniques to replicate natural structures on biomedical devices.

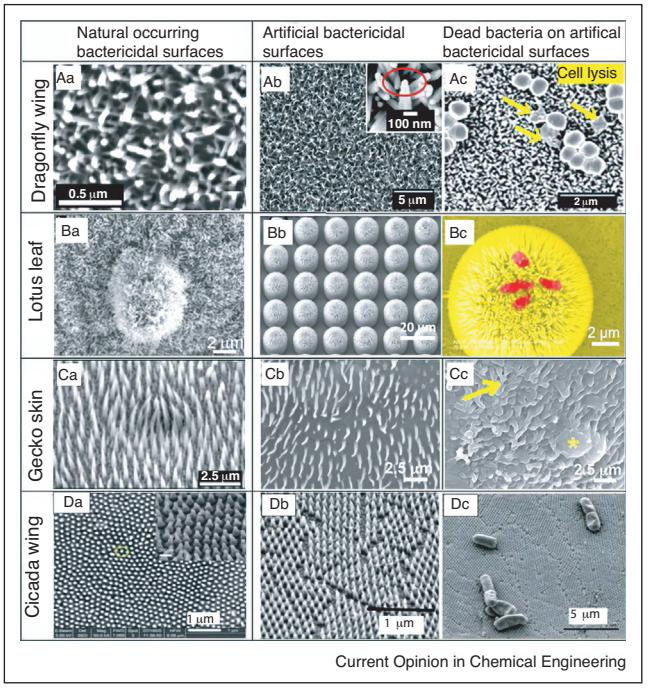
Natural and synthetic bactericidal surfaces

Skin and other exterior tissues of animals and plants have evolved unique architectures for specific functionality, such as strong adhesion (e.g. gecko feet), superhydrophobicity (e.g. lotus leaf), antireflection property (e.g. moth-eye), change in color due to iridescence (e.g. peacock feathers), and anti-biofouling property (e.g. sharkskin) [12]. Among these unique natural surfaces, bactericidal behavior against pathogenic microorganisms is believed to have evolved to minimize infections for improved survival. For example, dragonfly and cicada wings [13,14], lotus leaf [15], and gecko skin [16] are known for their characteristic nanopillars (NPs), which have offered much inspiration to researchers for biomimicry. These natural surfaces and synthetic bioinspired counter-surfaces are illustrated in Figure 1.

Biomimicry on synthetic surfaces

Biomimetic NPs have been fabricated on surfaces such as silicon [17–19], titanium [20,21•], and polymer surfaces [23,24] by etching or growing carbon nanotube arrays [22] to impart remarkable bactericidal capabilities. Black silicon was the first synthetic nanostructured surface consisting of 500 nm long pillars with a 30 nm tip diameter mimicking the topography of dragonfly wings [19]. The nanopillar density is a key influencing parameter; a higher density kills more bacterial cells, whereas if the NP density becomes too sparse, a bacterium will settle between the pillars without mechanical rupture. A variety of fabrication processes have been used to mimic natural NPs on different substrates and are detailed below [23–26]. Recent works on fabricating bactericidal nanostructures are compiled in Table 1. Titanium-based surface nanostructures prepared by the hydrothermal process demonstrated between 76% (for NPs) and 96% bactericidal activity (for nanowires) against *Staphylococcus aureus* in static culture conditions [20,21•]. The NPs can be converted into isotropically grown nanowires (niche type) by extending the hydrothermal process to eight hours.

Figure 1



Scanning electron micrographs of naturally occurring bactericidal surfaces **(Aa)** Dragonfly wing, **(Ba)** Lotus leaf, **(Ca)** Gecko skin, and **(Da)** Cicada wing. The corresponding synthetic bactericidal surfaces are illustrated showing: **(Ab)** hydrothermally induced spike-like nanostructures on titanium surface with **(Ac)** flattened dead bacteria (indicated by arrows) after 2 hours of incubation [13]; **(Bb)** Hierarchical structures on silicon using photolithography followed by reactive ion etching and **(Bc)** dead red pseudocolored bacteria [14]; **(Cb)** Micrographs of chitosan spinules prepared by casting with **(Cc)** smeared bacteria (with * symbol) and dead bacteria covers spinules (indicated by arrows) [15]; **(Db)** Nanocones on polyethylene terephthalate fabricated by nanosphere-mask colloidal lithographic with **(Dc)** associated deformed bacterial cells [16].

Nanowires are an order of magnitude taller when compared to nanopillars. Importantly, larger mammalian cells proliferate more than on the flat surface. In contrast, the much smaller bacterial cells are trapped in nanowires. The trapped bacterial membrane deforms and subsequently ruptures while the cell tries to escape. Moreover, up to 90% and 99% bactericidal efficiency was observed on a hydrothermally etched titanium nanostructured surface after 6 hours against *S. aureus* and *Pseudomonas aeruginosa*, bacteria that are commonly implicated in medical implant-associated infections; however, there is considerable scope for optimizing nanopillars for enhancing cytocompatibility [27]. Moreover, the bactericidal efficiency in this experiment was limited to the initial few hours. Subsequent growth of bacterial cells was observed on the NP surfaces as the sharpness of the nanostructures was compromised. NPs covered with dead cells were less effective in physically rupturing additional cells. Similarly, nanostructured polymeric surfaces that were

Table 1

Bactericidal activity (BA) of bioinspired synthetic nanostructured surfaces

Engineered surface	Preparation method	Features and dimensions	Bactericidal efficiency	Remarks
Black silicon	Reactive ion etching (RIE) [17]	Height (H): 280 nm, Diameter (D): 62 nm, Period (P): 62 nm	85% after 24 hours, Static culture condition (SCC)	Denser NPs exhibit higher BA.
	Plasma etching [18]	H: not reported, D: 150–200 nm, Spacing (S): 100–250 nm	86% after 24 hours, SCC	Effective against gram-negative, gram-positive, and spore-forming bacteria.
	Plasma etching [19]	H: 182−241 nm, D: 156−207 nm, P: 165−251 nm	∼15−23% after 1 hour, SCC	Need to check BA for longer incubation interval. Earlier lysed bacteria may help to attach and grow upcoming bacteria.
Titania nanowires [20]	Hydrothermal process	Brush like nanowires of 100 nm average diameter	50% after 1 hour, Dynamic culture condition (DCC).	Effective against *P. aeruginosa*, *E. coli*, *B. subtilis*, less effective against drug resistant bacteria (*E. faecalis* and *K. pneumonia).*
Titania NPs [21•]	RIE	H: 1000 nm, D: 80 nm, Not equally spaced	95−98% after 24 hours, SCC	Cytocompatible for human mesenchymal stem cells.
Vertically aligned carbon nanotubes [22]	Chemical vapor deposition followed by plasma etching	H:1,000 nm, D: 10 nm;	89.6% after 24 hours, SCC	Flexible, high aspect ratio NPs can also kill bacteria. Stored elastic energy releases upon bacterial contact.
Nanopatterned Poly(methyl methacrylate) [23]	Nanoimprint lithography	H: 460 nm, D: NA, S: 300 nm	50% after 24 hours, NA	Smaller and closely packed NPs more effective. Need to test both classes of bacteria.
Polymeric NPs [24]		H: 400 nm, D:80 nm, P:170 nm	100% after 2 hours, SCC	
	Nano-pattern transfer technique	H: 200 nm, D:80 nm, P:170 nm	98% after 2 hours, SCC	Optimum NP density: ∼40 pillars μm^{-2}, Increased height increases cell stretching.
		H: 300 nm, D:80 nm, P:300 nm	26% after 2 hours, SCC	
Diamond nanocone surface [30]	Electron cyclotron resonance mode RIE	H: 3−5 μm, D at top: 10−40 nm, Width (W): 350−1200 nm,	67% after 1 hour, SCC	Needle-shaped structures more suitable. Need to test more bacteria.
Zeolitic imidazole framework, Nanodaggers [31]	Zeolitic imidazolate coating followed by thermal annealing	H: 1000 nm; W: 2000 nm; P: <2000 nm	99% after 24 hours, DCC	Biophysical interaction is significantly dominant over biochemical effect.

initially bactericidal appeared less effective as the accumulated debris of the dead cells reduced the sharpness and height of NPs [28].

Interestingly, insect wings exhibit self-cleaning behavior, which may be critical for retaining bactericidal efficiency. Self-cleaning nature retains the sharpness of NPs by removing the debris from the dead bacteria. Hence, mimicking insect wings structures on deployable biomedical devices with self-cleaning properties can prolong the bactericidal activity [7•]. In recent work, sharp NPs with self-cleaning ability have been prepared by using photolithography followed by reactive ion etching [14] and anodization followed by chemical treatment [40]. Rosenzweig and team used nanoimprint lithography to prepare bactericidal NPs on polymer surfaces to minimize the growth of *P. aeruginosa* [29]. The nanostructured polymer surface was more effective against the bacteria under flow conditions as compared to in static culture. Dimitrakellis *et al*. fabricated multiscale (micro/nano) structures on poly (methyl methacrylate) (PMMA) through plasma etching followed by deposition of hydrophobic fluorocarbon (CFx) and copper thin film to impart both anti-adhesive and bactericidal activities. These surfaces were tested for antibacterial properties under dynamic conditions [56]. Minimal liquid–surface interaction and release of copper ions resulted in 100% efficiency in the first hour. However, the release of CFx and copper ions from the coating has to be optimized to minimize toxicity.

A newly proposed strategy using a metal-organic framework (MOF) nano-daggers was realized through a chemical deposition. These are NPs with a sharp tip like a knife tip and hence, called daggers. These nano-daggers exhibited excellent bactericidal activity (99% after 24 hours) in dynamic culture conditions owing to the positive charge on the nano-daggers that electrostatically attracts bacteria (that contain negatively charged phospholipids in the membrane) and ruptures mechanically; however, the sharp nano-dagger tips may also damage mammalian cells [31]. This experimental observation underscores the importance of the biochemical nature of cell membranes,

which drives the interactions between bacteria and nanostructured surfaces.

Factors affecting bactericidal property

The shape and size of nanostructures, substrate wettability, roughness, and choice of bacterial species influence the observed bactericidal efficiency of a surface. The shape and dimensions of the nanostructures are particularly critical parameters [32]. The height of nanostructures is a key factor; for example, nanopillars of 50 nm height were ineffective against *S. aureus*, whereas 400 nm tall pillars were bactericidal [33]. High aspect ratio structures are typically effective against a wide variety of bacteria [32]. However, if NPs are too tall (≈1000 nm), they are often not well separated, resulting in low bactericidal efficacy [34], whereas thinner structures of intermediate height have shown improved bactericidal efficacy [35]. Apart from height, spacing between nanofeatures and the diameter of nanostructures also influence the bactericidal activity [36••]. Even for nanostructures of high aspect ratios, spacings larger than the bacterial size diminishes the bactericidal efficacy. In this case, bacteria settle between the structures instead of landing on NP tips. Similarly, NP diameter should be less than or equal to the bacterial size. Very large diameter and closely packed nanostructures can act as a breeding site for bacteria [36••]. Alongside 3D bactericidal nanostructures, 1D and 2D colloidal nanostructures such as carbon nanotubes (CNTs) and graphene nanosheets (GNSs) are comprehensively reviewed by Lin *et al.* [58]. These nanostructures are so thin that they cause bacterial membrane disruption by piercing (e.g. by CNTs) or slicing (e.g. by GNSs). Similar to 3D nanostructures, the high aspect ratio CNT and a high degree of sharpness in GNS (acts as nanoblade) possess higher bactericidal activity [58–60]. The vertically aligned carbon nanotubes with an extremely high aspect ratio (100–3000) impart extreme flexibility, which enhances the elastic energy stored in CNTs as they bend in contact with bacteria [22]. The stored bending energy in CNTs is a substantial factor for the physical rupturing of both Gram-positive and Gram-negative bacteria with 89.6% bactericidal efficiency. The sharp diamond nanocone features fabricated using complex electron cyclotron resonance mode RIE are found to have the excellent bactericidal ability for nonuniform array and decreased density over the more uniform, highly dense nanocone surface after 1 hour of bacterial testing [30]. In agreement with these results, Wu *et al.* identified optimum NP density (40 pillars μm^{-2}) on polymer surfaces, which yields 100% bactericidal efficiency after 2 hours in static culture [24]. The distribution and density of nanostructure are one of the influencing parameters; hence, there is no need to have ordered nanostructures for enhanced bactericidal efficacy, but rather optimal density and distribution is the key.

The surface topography affects its wettability; hence, it is challenging to investigate the influence of the wettability alone on bacterial lysis. Some studies have shown that mechano-bactericidal activity is independent of surface wettability. For example, although a thin gold coating altered the superhydrophobic character of cicada wings, the nanostructures continued to exhibit their mechano-bactericidal activity [37]. The superhydrophobic lotus leaf is well known to repel bacteria. Moreover, micropatterned titanium oxide hydrophobic surfaces exhibited more than 80% antibiofouling properties against gram-positive and gram-negative bacteria [38]. While comparing hydrophobic surface with superhydrophobic, the photocatalysis effect of titanium oxide needs to be considered that significantly contributes to the antibiofouling property. Hydrophobic titanium surfaces are reported to have low antibiofouling activity compared to hydrophobic titanium oxide surfaces [13]. Recent findings by Rujian *et al.* illustrated the synergetic antibacterial property of the lotus leaf, initially through super repellency as well as the effective bactericidal property via physical rupturing after adherence of *Escherichia coli* [14]. Still, the correlation between surface wettability and bactericidal activity remains poorly understood.

Notably, anisotropic nanostructures are more effective against gram-negative bacteria, though some also report good activity against gram-positive bacteria [19]. The experimental design plays an important role in these observations. Aside from NP dimensions, the growth medium, incubation time, and concentration of bacterial cells also influence the bactericidal response of the surface [21•,39,40]. Most tests are performed in static conditions [21•], whereas flowing growth medium to introduce the shear stresses better mimics the conditions experienced when the surfaces are in real-world use. Bacterial adherence is reduced ten folds under flow conditions compared to static culture [41]. Dynamic culture conditions enhance shear stresses at the interface of the NPs and the mobile bacteria, and hence, the nanostructured surfaces are more effective in killing motile bacteria than non-motile strains in flow conditions [42]. This phenomenon was demonstrated on Cicada wings and Si NPs, which were more effective against motile *E. coli* with flagella than the strains lacking flagella. Microbes in certain growth phases also seem more likely to contact the nanostructured surfaces. Damselfly (*Calopteryx hemorrhoidalis*) wings more effectively ruptured bacteria in the early log phase of bacterial growth compared to bacteria in the stationary growth phase [43]. Another important factor that needs to be considered is the bacterial concentration used for antibacterial testing. The antibacterial property of superhydrophobic surfaces is essentially lost with the use of high bacterial concentration ($>10^8$ CFU/mL) for testing owing to a large number of bacteria layers on the surface [57]. There is an upper limit of bacterial concentration threshold above which passive surfaces lose

the antibacterial property. Hence, it becomes imperative to develop a hybrid surface with dual functionality (antibiofouling and bactericidal), which can exhibit antibacterial properties for a longer duration irrespective of bacterial concentration. The minimum metal-enriched superhydrophobic polymer surfaces have exhibited long-lasting antibacterial properties with higher concentrations (2×10^9 CFU/mL) of cyanobacteria [57]. This approach of developing hybrid antibacterial surfaces needs to be explored for a variety of materials, including metallic implants and natural biopolymers.

Physical model

Considering the challenges of *in situ* characterization of the dynamic interactions of bacteria with nanostructures, biophysical models help elucidate the underlying mechanisms, and several models have been proposed [44–48]; however, the mechanisms leading to the rupture of the bacterial membrane continue to be highly debated. The accuracy of the models remains questionable as they fail to explain many experimental observations [45–48]. Recent mechano-bactericidal physical models are listed in Table 2, along with a summary of the proposed rupture mechanism, driving forces involved, bactericidal efficiency, limitations, and suggestions to further refine the model. Initial models proposed rupture of bacterial membrane between the NPs, but subsequent models suggested rupture at the NP tips [46]. Bacterial membrane ruptures when maximum stress at the interface of NP tip and bacterial membrane exceeds the local maximum allowable membrane strain [48]. Furthermore, this model suggests that a sinusoidal arrangement of cylindrical nanopillars on a patterned surface is more advantageous to gain bactericidal efficacy over an ordered NP array. The model assumptions need further improvement. The bacterial membrane was modeled as a thin elastic layer ignoring actual membrane composition. The biophysical model based on experimental observation by Bandara and their team reported that strong adhesion followed by shear stress due to bacterial movement imposed by the wall of nanopillars results in cell lysis [44]. However, this model does not incorporate

Table 2

Summary of recently developed biophysical models to predict bactericidal efficacy of nanostructures

Type of model	Bacterial shape considered	Mechanism of rupture	Driving force	Findings of the bactericidal model and recommendations for improvement
Physical model based on minimization of total surface energy [36••]	*E. coli*, and *P. aeruginosa* (cylinder with hemispherical caps on both ends), *S. aureus* (sphere)	Adhesion and rupture of the cell membrane at NP apex when the spacing between NPs is less than the size of a bacterium	Hydrostatic pressure and gravitational force	**Bactericidal activity (BA) improved by:** High aspect ratio NPs with spacing less than a bacterium size. **Suggestions**: Adhesion forces, Fatigue performance of NPs, Force exerted by NP walls on bacteria when NP spacing > bacterium size needs to be considered.
Physical model based on experiments [45]	*S. aureus*, *P. aeruginosa*	Bacteria adhere to NPs, and are physically ruptured when they try to move on	Shear force due to fluid flow	**BA improved by:** Increase in shear force, increase in van der Waals forces. Less spaced NPs exert more shear force on the bacterial membrane. **Suggestions:** Need to simulate for flexible NPs.
Biophysical model based on surface energy gradient [46]	Considered planar area near the pillars, no specific shape considered	Stretching of the membrane between the regions of contact of a bacterium with nanostructures — mechanical stress overcomes the elasticity of the membrane	Did not mention specifically	**BA improved by:** reducing NP height (from 612.1 nm to 213.3 nm). **Suggestions:** Need to combine chemical and physical approaches to determine bactericidal activity.
Thermodynamic-based model [47]	*E. coli*, and *P. aeruginosa* (cylinder with hemispherical caps on both ends), *S. aureus* (sphere)	Attractive forces between NPs and bacterium provide energy to disrupt the membrane; active response by a bacterium	Passive forces (van der Waals forces, polar interactions) and active forces (e.g. bacterial movement)	**BA improved by:** Tall NPs, no influence of pitch and pillar radius (contradicts findings from [33,42]) **Suggestions:** Only simulated a very thick membrane, a more realistic thin membrane needs to be simulated.
Thermodynamics-based model [48]	Only spherical shape *S. aureus* considered	Interfacial energy gradient leads to cell movement on nanostructures, negative along the direction of the entering NPs	Difference between Gibbs free energy and deformation surface energy at the interface	**BA improved by**: Radius of NPs and decreasing height (contradicts [33], agrees with [43]). **Suggestions:** Include external forces (e.g. shear force).

information on bacterial shape and composition, which affects bacterial movement. Recent findings contradict the old models by demonstrating that bacteria on different mechano-bactericidal surfaces remained viable unless exposed to the critical level of external forces required to deform and rupture the membrane (Figure 2c) [49••].

The common findings of recent models to enhance the bactericidal activity are: (a) sharper and high aspect ratio NPs increase stretching at the tip of the pillars by increasing membrane tension between pillars (Figure 2a) [36••], (b) pitch should be smaller than the size of the bacterial cell [45], (c) the rigidity of the cell and the thickness of peptidoglycan layer decides susceptibility for rupture [47]. Nevertheless, the nanoscale events leading to cell lysis are still debated. None of the models include the biochemical effect of the substrate and only focus on physical interactions even though the combined result of biochemical and physical interactions significantly influences bactericidal activity [31,52••]. There is a need for models for bacterial interactions with hierarchical nanostructures. Current models assume a uniform distribution of NPs and do not account for hierarchical structures, as can be seen in Figure 2b, which provides opportunities for further improvements in these models. The cell functionalities such as motility, fission, and composition of the cell wall need to be considered for a more realistic model. The latest finite element analysis interprets the interaction of spherical shape bacterium with topography similar to laser-induced textures in dynamic fluid flow [50]. Figure 2d depicts the effect of asperities with different heights on bacterial attachment. When protrusions are large enough to allow the cell to occupy valleys between two adjacent protrusions, the cell is protected from hydrodynamic turbulence and hence more prone to adhering to the substrate.

Fabrication techniques and challenges to realized nanostructures

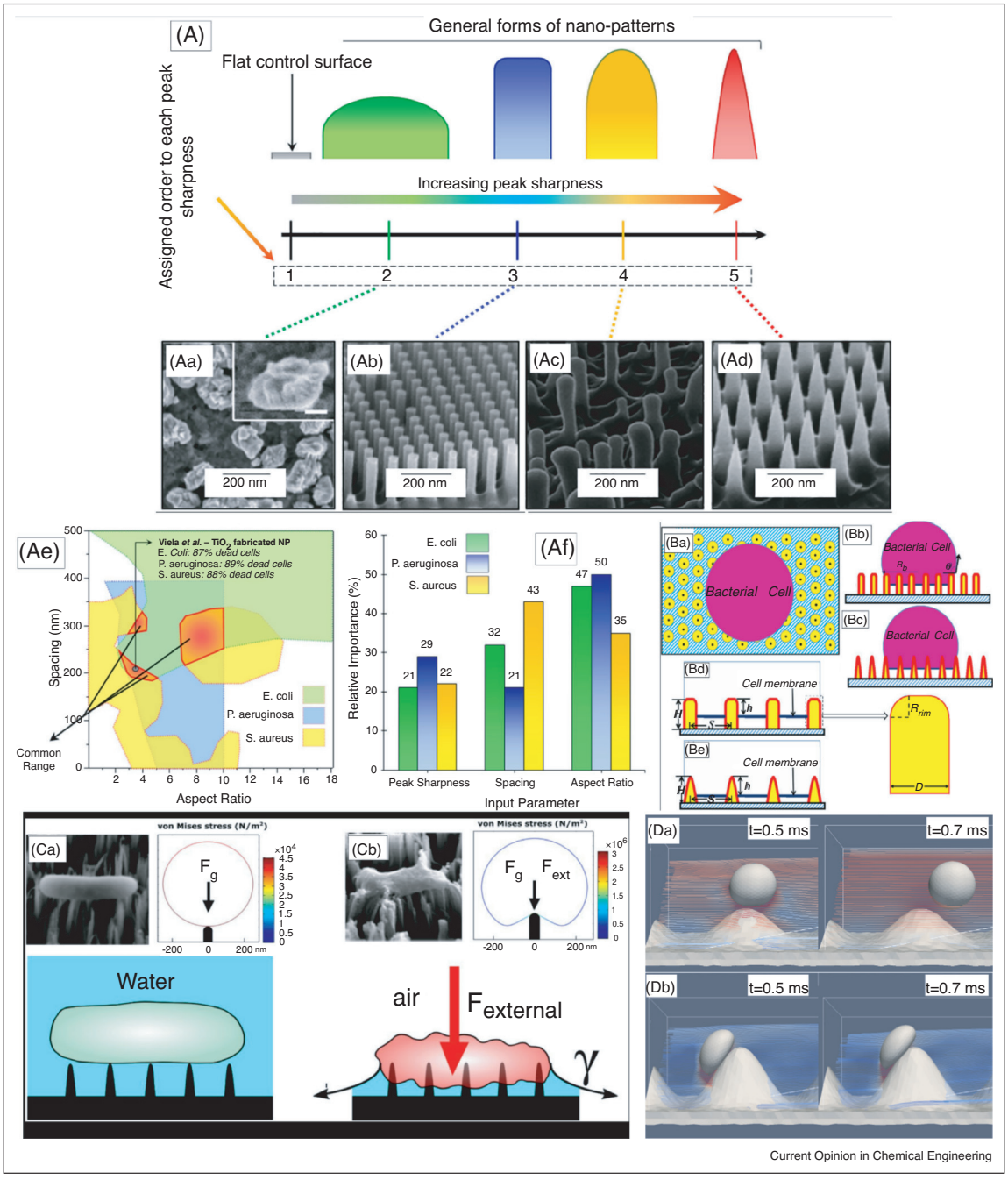
Popular processes to prepare bactericidal surfaces include plasma and hydrothermal etching (for silicon, metal, glass, and polymers) [18–20], electrochemical etching (for metal, and silicon) [25], laser treatment (for metals and ceramics) [26], and nanoimprint lithography (for polymers) [23,24]. However, the fabrication of nanostructures that affords scale-up and is cost-effective is a critical bottleneck in the widespread adoption and deployment of such surfaces. Given the variety of biomaterials used clinically, the fabrication method must suit the particular class of material. Plasma etching is a popular and promising technique to produce large-area surfaces exhibiting excellent bactericidal activity with minimal cost and applicable to metals and polymers [18–20,56]. Moreover, the post-processing techniques such as physical or chemical vapor deposition to alter the wettability of plasma etched surfaces significantly increase the cost [56]. Potential application of bactericidal dental and orthopedic titanium implants can be realized by hydrothermal synthesis and anodization processes [51,52••,53•]. A range of nanostructures (brush and niche type) that show excellent bactericidal activity while permitting the growth of tissue cells can be easily reproduced using the hydrothermal process [20]. However, the structures fabricated by these processes have random orientation and are highly susceptible to minor variations in parameters, which can compromise the reproducibility of high-performance bactericidal nanostructures across batches. Nanoimprint lithography and soft molding techniques provide resolution in tens of nanometers and produce hierarchical structures to induce excellent bactericidal properties for polymer surfaces [54]. Nanoimprint lithography is efficient, precise, scalable, and can be cost-effective if replicas are produced from a master mold; however, it is limited to flat surfaces. Moreover, the process is multistep and limited to thermoplastics. Chemical etching could be combined with nanoimprint lithography for metal surfaces but may not be cost-effective. Most of these processes are not suitable for surface nanostructuring of implants with complex geometries or 3D porous metallic implants and polymeric scaffolds. There is a large unmet need for novel strategies for fabricating bactericidal nanostructures on 3D porous structures. Laser interference (LI) lithography is a scalable technique to yield ordered structures down to submicron (≈300 nm) resolution [55]. The modified LI technique utilizes an axicon lens to produce a narrow distribution of intensity. It can yield nanostructures of different sizes by changing the depth of focus along with the beam propagation. In addition to these lenses, the 5-axis movement affords additional degrees of freedom to fabricate hierarchical structures and can enhance both self-cleaning and bactericidal properties (Figure 3a).

The modified LI technique can be adapted for complex 3D and curved surfaces; however, it may be limited to higher melting substrates such as metals and silicon and may not be ideal for low melting biodegradable polymer scaffolds. Figure 3b schematically presents the challenge of preparing nanostructures all over 3D porous polymeric scaffolds that are bactericidal and yet cytocompatible for tissue regeneration. With recent advancements in hydrogels and 3D printing, novel strategies are needed.

Current outlook

Despite the advent of progress in the biomedical field, implant-associated infections remain one of the devasting surgical complications in the clinic. Controlled release-based antibacterial strategies lose their efficacy rapidly over time owing to the depletion of eluting agents. As an alternative, fabrication processes that are scalable and cost-effective for realizing antibacterial and biocompatible surfaces are of much technological significance. The fabrication of highly ordered mechano-bactericidal nanostructures is not trivial. Lithography techniques can

Figure 2



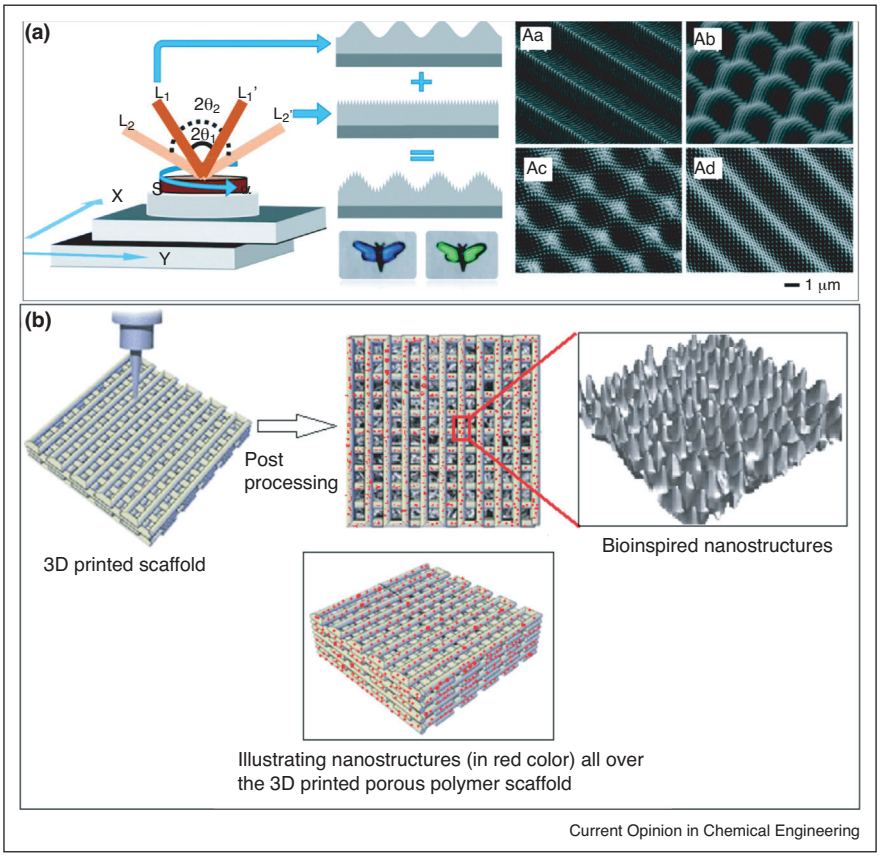
The different natural bactericidal surfaces have different shapes and size of nanostructures: **(A)** illustrations of different forms of the peak sharpness and their assigned orders used in an artificial neural networks model and examples of the corresponding shapes; **(Aa)** nano-nuggets, (**(Ab)** and **(Ac)**) nanopillars, and **(Ad)** nano-spikes. **(Ae)** Illustration of the effect of aspect ratio with respect to pillar spacing on the bactericidal activity against three bacterial species. Three isolated regions (indicated by arrow) show 70% bactericidal efficacy. **(Af)** Results of sensitivity analysis illustrating the effect of inputs parameters on bactericidal effects of NPs. Aspect ratio of NPs becomes more dominant than sharpness and spacing [36••]. Formulation of bactericidal model based on total free energy for different shapes of NPs. **(Ba)** Spherical cell adhered to NPs. Lateral cross-section cells interacting with **(Bb)** cylindrical and **(Bc)** sinusoidal pillars illustrating base radius R_{base} and contact angle θ surface in a hexagonal pattern. **(Bd–Be)** Illustrated dimensions of NPs. Pillar density, radius, and height of the pillars are the most influencing parameters irrespective of the shape of NPs [48]. **(C)** A numerical model that predicts cell lysis under gravity; in the **(Ca)** absence and **(Cb)** presence of external forces and corresponding scanning electron micrographs [49••]. **(D)** Interaction of bacterium with topography in dynamic fluid flow using finite element analysis: **(Da)** Time scale snapshot showing spherical shape bacterium with dot-like projection (blue to red shows increasing velocity streamlines); **(Db)** Time scale snapshot showing spherical shape geometry with higher projected height compared to Da. Large deformation in cellular mesh and large contact area was observed and may increase adhesion probability [50].

produce ordered structures, but high costs limit their techno-commercial viability [54]. Among the popular techniques, plasma etching categorized into RIE (structure depth <1 μm) and deep RIE (depth >1 μm) has shown promise against a wide range of bacteria. However, 3D laser interference lithography is an emerging technique to realize hierarchical NPs on objects of complex geometry. There are several unexplored areas in this field that offer exciting research opportunities, including the fabrication of nanostructures onto biodegradable, shape-memory, or biopolymer surfaces to impart mechano-bactericidal activity.

The most efficient bactericidal surfaces are believed to exhibit feature dimensions in the range of 10–100 nm. Moreover, the height of the nanostructures should be tall enough to avoid bacterial contact with the substratum and to ensure maximum stretching as the bacterial membrane adsorbs. The larger physical size and greater elasticity of eukaryotic cells enable them to survive on nanostructures and successfully colonize. These cells can accommodate the deformation stress by invaginating the surface features [21•]. It is believed that the NPs are less effective in killing gram-positive strain; however, there is also sufficient literature that disputes this [20,24]. Further testing is needed to confirm the strain-dependent bactericidal activity of nanostructured surfaces. The effect of superhydrophobicity on bactericidal activity cannot be generalized. The different shapes and sizes of nanotopography may lead to different wettability of the surface.

Furthermore, the flexible nanostructures accumulate and release the elastic energy that imposes tension in the bacterial membrane and ultimately enhances the stretching. Therefore, the stored elastic energy in flexible nanostructures should match or exceed the elastic energy of the bacterial cell wall [22]. The hydrophilic, high surface energy nanostructures exhibit the excellent bactericidal property; however, to make the surface as efficient as a bactericidal agent, the hybrid antibacterial surfaces having nanostructures infused with a little bactericidal agent can be the highly efficient futuristic antimicrobial

Figure 3



(A) Schematic representation of 3D laser structuring to biomimetic hierarchical structures on silicon surface similar to those found on insect wings. **(Aa–Ad)** Scanning electron micrographs of different hierarchical patterns achieved using 3D laser structuring technique [55]; **(B)** Schematic representation of the challenge to make biomimetic bactericidal structures on a 3D polymer scaffold. The challenge is to develop a fabrication method which can achieve bactericidal NPs on all surfaces within a 3D scaffold.

surfaces [56]. Future studies should necessarily adopt the standardized approach for evaluating the mechano-bactericidal nature of different surfaces, which will afford easy comparison of the results across different groups. The bacteria tend to detach from the surface in dynamic conditions, unlike in static conditions, and dead cells become stagnant on the NPs in static conditions. Therefore, for accurate estimation of bactericidal efficiency of nanotopography, the surfaces should be tested in dynamic flow conditions with maximum bacterial concentration. Moreover, the bacterial motility also affects the killing performance, and it also depends on cultural conditions, but it is still not understood how substrate under different culture conditions affects motility and ultimately determines bactericidal performance.

Conclusion and future perspective

The discovery of penicillin charted the course of modern medical care, but we have made no fundamental innovations in the treatment of bacterial infections since the time of Fleming, and we are losing the race to develop novel pharmacologic agents faster than new antibiotic resistance can arise. Given the enormous clinical and economic burden of the accelerated emergence of resistant bacterial strains, feasible and cost-effective approaches to fabricate mechano-bactericidal structures on complex freeform surfaces at an industrial scale are urgently needed for medical and nonmedical applications alike. Several natural nanostructured surfaces exhibit antibacterial properties against common bacteria. Inspired by natural surfaces, synthetic bactericidal surfaces have been realized using the latest nanofabrication techniques. However, the current experimental protocol and theoretical models need to be modified to simulate practical observations. Influencing factors such as surface features, culture conditions, and bacterial mobility affect bactericidal performance. Further investigation is required to understand the effect of individual parameters on bactericidal performance under dynamic culture conditions to better design and optimize efficient bactericidal surfaces. The current understanding of the bacterium-nanostructure interaction is still in the initial stage, and considerable scope for improvement exists. The assumption of rigid nanostructures is not valid for soft substratum-like polymers; hence, structural deformation under different forces may help to formulate a realistic model. The bactericidal performance can be further enhanced by combining optimized nanostructures, considering external forces and surface chemistry.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Deepak Patil: Conceptualization, Writing – original draft. **Maya Overland:** Writing – review & editing. **Marshall Stoller:** Writing – review & editing. **Kaushik Chatterjee:** Conceptualization, Writing – review & editing, Resources, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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