REVIEW ARTICLE

Mechanobactericidal nanotopographies for food industry: A promising strategy for eradicating foodborne pathogens - progress and challenges

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Abstract

Nowadays, food preservation, quality maintenance, and safety are major emerging concerns in the food industry. Methods for removing pathogens from the outside surfaces of food products would be an effective way to prevent bacterial contamination. Nanotopographies found on natural surfaces have been shown to mechanically damage the membranes of foodborne bacteria. Thus, using bioinspired mechanobactericidal nanostructures in food packaging and processing materials has the potential to lower surface bacterial contamination while increasing food safety. However, putting this concept into practice remains a challenge. This review discussed recent advances in understanding mechanobactericidal mechanisms, issues concerning the durability of nanotopography under external forces, and the scalability of nanostructures over larger areas. Furthermore, this review provides insight into critical research on the long-term efficiency of mechanobactericidal nanostructures and their potential for implementation in the food industry.

Keywords: Antimicrobial, Food packaging & safety, Mechanobactericidal surfaces, Nanofabrication, Nanomaterials

1. Introduction and need for alternate strategies for food safety application

Bacteriocins have been used in food packaging films to combat deterioration caused by food-pathogenic microorganisms for decades. Antimicrobial packaging in films prevents microbial growth on the food surface by direct contact of the packaging material with the surface of foods. Food packaging aims at shelf-life extension, maintenance of quality, and assurance of safety of the food product. However, currently, food security is a huge issue, so the antimicrobial packaging system is specifically created to prevent bacteria that negatively affect the above three goals [1]. Bacterial infections are predicted to cause 60% of foodborne illnesses and 65% of deaths worldwide (about ~187,000) [2,3]. According to Machell and group microbial infection and product expiration account for 33%−50% of worldwide food losses each year [4]. The need to use antimicrobial compounds to prevent food contamination is increasingly gaining scientific attention around the world, leading to substantial research on the subject. As a result, these problems necessitate the development of more effective food quality management systems to secure, preserve, and deliver healthful food to customers. The rise in customer demand for natural, local, and organic products needs effective food preservation against microbial contamination [5−7]. Conventional methods for sustaining food quality over time have failed to satisfy customers, who prefer items derived from natural sources [8,9]. As a result, there is a need in the food business to investigate alternatives to commonly used chemicals. Bacteriocins, or bacterially synthesised antimicrobial peptides produced from grapefruit extracts and mustard oil, are the latest developments in this sector [8−10]. Several natural chemicals, such as antimicrobials and antioxidants, have been shown to be successful in laboratory applications, but their efficiency in real-world applications is still limited by the specific properties of the foods and the conditions of application [11,12]. The Food and Drug Administration regulates the addition of antimicrobials directly into food, known as formulation, and food

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wrapping films, by specifying the safe amounts of antimicrobial compounds that may be added to food. The addition of antimicrobials to food packaging film compositions often results in the immediate suppression of undesirable microbes. However, the survivor population will continue to grow until the antimicrobials provided are depleted. This is mostly owing to complicated interactions with the food matrix or natural deterioration over time, resulting in a reduction of shelf life [11−14]. Moisture, oxygen, heat, and microbial contamination all contribute to food products deteriorating before their expiry date, increasing food waste [15−17]. In the case of food shipments, if an analysis reveals bacterial development in one or more of the products after packaging, the entire shipment is rejected [17]. The food packaging industry has directly benefited from the development of antimicrobial nanocomposite materials for packaging applications, which are intended to decrease microbial growth on food surfaces [18−20].

Antimicrobial functionality has recently been added to plastic products and polymer materials through the use of metal micro-nanoparticles such as silver (Ag), copper (Cu), titanium dioxide (TiO_2), and zinc oxide (ZnO), quaternary ammonium salt compounds, and biomass materials such as catechin and chitosan. These antibacterial agents are either directly added to the items. However, safety concerns have been raised since nanosized materials utilised as composite additives or coatings typically display cytotoxicity *in vitro* and *in vivo* investigations [21]. Exposure to nanoparticles found in food packaging can occur by skin contact, inhalation, or ingestion of nanoparticles that have migrated into food. Furthermore, nanoparticles may be released into the environment and subsequently into the food chain. Several studies have already reported on the toxicological aspects of antimicrobial nanomaterials [22−24], and, while there is limited scientific data on the migration of nanomaterials from packaging materials into food, it is prudent to consider that, once present in the food packaging material, nanoparticles may eventually migrate into food or the environment [25−29].

Nature provides much inspiration in the search for antimicrobial surfaces. The micro-nanotopography of lotus leaves, rose petals, and shark skin is known to have antibiofouling properties. Insect wings and gecko skin have recently been shown to have mechano-bactericidal action [30]. These surfaces are distinguished by ordered or disordered anisotropic nanostructures such as pillars, needles, or hair-like protrusions. Bacterial cells are ruptured upon direct physical contact with these nanostructures [30,31]. The finding of this evolutionary advantage in the form of spontaneous mechano-bactericidal activity provides a hitherto unknown strategy for countering bacterial colonisation without causing antimicrobial resistance. This material-centric strategy of creating nanostructures on metallic, ceramic, and polymer surfaces is aimed at interfering with the early phases of the biofilm life cycle, slowing or even stopping the transition to mature, persistent biofilms. Nanofabrication and nanoengineering breakthroughs have resulted in the construction of nanopatterned surfaces with natural topographies, which limit microbial invasion through physical disruption rather than biochemical effects [32]. Despite the promising antibacterial properties of mechanobactericidal nanotopographies on diverse surfaces, their implementation for food safety remains in its early stages due to several technical challenges. In this review, we will critically assess the evolution of mechanobactericidal techniques and potential solutions with food safety implications. We also highlight the key steps for future translational research, which could speed up their practical application and hence improve food safety and quality.

2. Mechanobactericidal nanostructured surfaces: mechanism and nano-fabrication techniques

2.1. Understanding of mechanobactericidal mechanism and influencing parameters

Nature spurred the creation of nanotopography, as observed on the Cicada wing [33]. Furthermore, the nanotopography on the dragonfly and gecko skin differs significantly from that on the Cicada wing [34]. However, all three topographies (Cicada wing, Dragonfly wing, and Gecko skin) have mechanobactericidal characteristics. The literature describes two key mechanisms underlying bacterial cell-topography interactions: (i) rupture of the bacterial membrane suspended between two nanostructures [33] and (ii) membrane rupture at the tip of nanostructures [34]. The most effective bactericidal surfaces are thought to have feature dimensions of 10−100 nm. Furthermore, the nanostructures should be tall enough to limit bacterial contact with the substratum while allowing for maximum stretching as the bacterial membrane adsorbs. Eukaryotic cells can survive and colonise nanostructures due to their bigger physical size and better flexibility. These cells can adapt to deformation stress by invaginating surface features [35]. It appears that nanopillars are less effective in killing gram-positive bacteria; nevertheless, there is ample evidence to refute this [36,37]. Additional research is required to demonstrate the strain-dependent bactericidal action of nanostructured surfaces. The varying shapes and sizes of nano-topography may affect the surface's wettability and the same has to be considered. Furthermore, the flexible nanostructures (e.g. nanostructures on polymer surfaces, like PET) store and release elastic energy, causing tension in the bacterial membrane and ultimately increasing stretching. As a result, the elastic energy stored in flexible nanostructures should be equal to or greater than that of the bacterial cell wall [38]. Hydrophilic, high surface energy nanostructures have excellent bactericidal properties; however, hybrid antibacterial surfaces infused with a small amount of bactericidal agent could be highly efficient futuristic antimicrobial surfaces [39].

Given the difficulties of in situ characterization of bacteria's dynamic interactions with nanostructures, biophysical models can help elucidate the underlying mechanisms, and several models have been proposed [40–45]. However, the mechanisms causing bacterial membrane rupture remain hotly debated. The common findings of recent models to enhance bactericidal activity are: (a) sharper and high aspect ratio nanopillars increase the stretching at the tip of the pillars by increasing membrane tension between pillars (Fig. 1a) [40], (b) pitch should be smaller than the size of the bacterial cell [41], and (c) the rigidity of the cell and the thickness of the peptidoglycan layer determine rupture susceptibility [42]. Nonetheless, the nanoscale events that contribute to cell lysis are being contested. None of the models account for the substrate's biochemical effect and instead focus solely on physical interactions, although the combination of biochemical and physical interactions has a major impact on bactericidal activity [43,44]. There is a need for models of bacterial interactions with hierarchical microstructures. Recently, developed models assume a uniform distribution of nanopillars and do not account for hierarchical structures, as depicted in Fig. 1b, allowing for potential improvements to these models. A more realistic model should take into account cell capabilities such as motility, fission, and cell wall composition. The most recent finite element analysis analyses the interaction of spherical-shaped bacteria with topography, which is analogous to laser-induced textures in dynamic fluid flow [45]. Recent research contradicts previous theories by indicating that bacteria on various mechano-bactericidal surfaces remained viable unless subjected to the necessary degree of external pressures required to deform and break the membrane (Fig. 1c) [46]. Fig. 1d demonstrates the effect of asperities of varying heights on bacteria attachment. When protrusions are large enough to allow the cell to sit in valleys between two adjacent protrusions, it is sheltered from hydrodynamic turbulence and hence more likely to adhere to the substrate.

Nanostructures such as nanopillars and nanowires operate primarily through a contact-killing mechanism. This involves the physical interaction between the nanostructures and bacterial cells, leading to membrane rupture and cell lysis. For instance, nanopillars with dimensions typically around 50–250 nm in diameter and 80–250 nm in height have been shown to effectively pierce bacterial cell walls, resulting in cell death [76,78]. Nanopillars have demonstrated significant bactericidal activity against various strains, including *Escherichia coli* and *S. aureus*. Their effectiveness is influenced by parameters such as height, diameter, and spacing. For example, nanopillars inspired by cicada wings can achieve around 50% cell death in *E. coli* while sparing human cells [77,79]. Similar to nanopillars, nanowires also exhibit selective bactericidal activity. Studies have indicated that titanium nanowires can lead to over 50% cell death in *Pseudomonas aeruginosa*, showcasing their potential in medical applications. Nanospikes have been noted for their ability to induce cell lysis through sharper tips, which can more effectively penetrate bacterial membranes. Research has shown that nanospikes can significantly reduce the viability of *P. aeruginosa* [80]. While less commonly discussed in the context of direct bactericidal activity, nanocrystals can contribute to antibiofouling effects through their surface properties and potential chemical interactions. However, their physical impact on bacteria is less pronounced compared to the aforementioned structures [76].

2.2. Fabrication of mechanobactericidal surfaces

Although nanostructure fabrication techniques have been successful in the electronics industry, they have limitations in the food industry due to high costs and low throughput when nano-patterning non-flat substrates or complex geometries, as well as the requirement for specialised equipment and clean-room facilities. Furthermore, Hayles and colleagues optimised the operating parameters for semiconductor materials rather than those commonly used in culinary applications [47]. Nanofabrication technologies that strike a

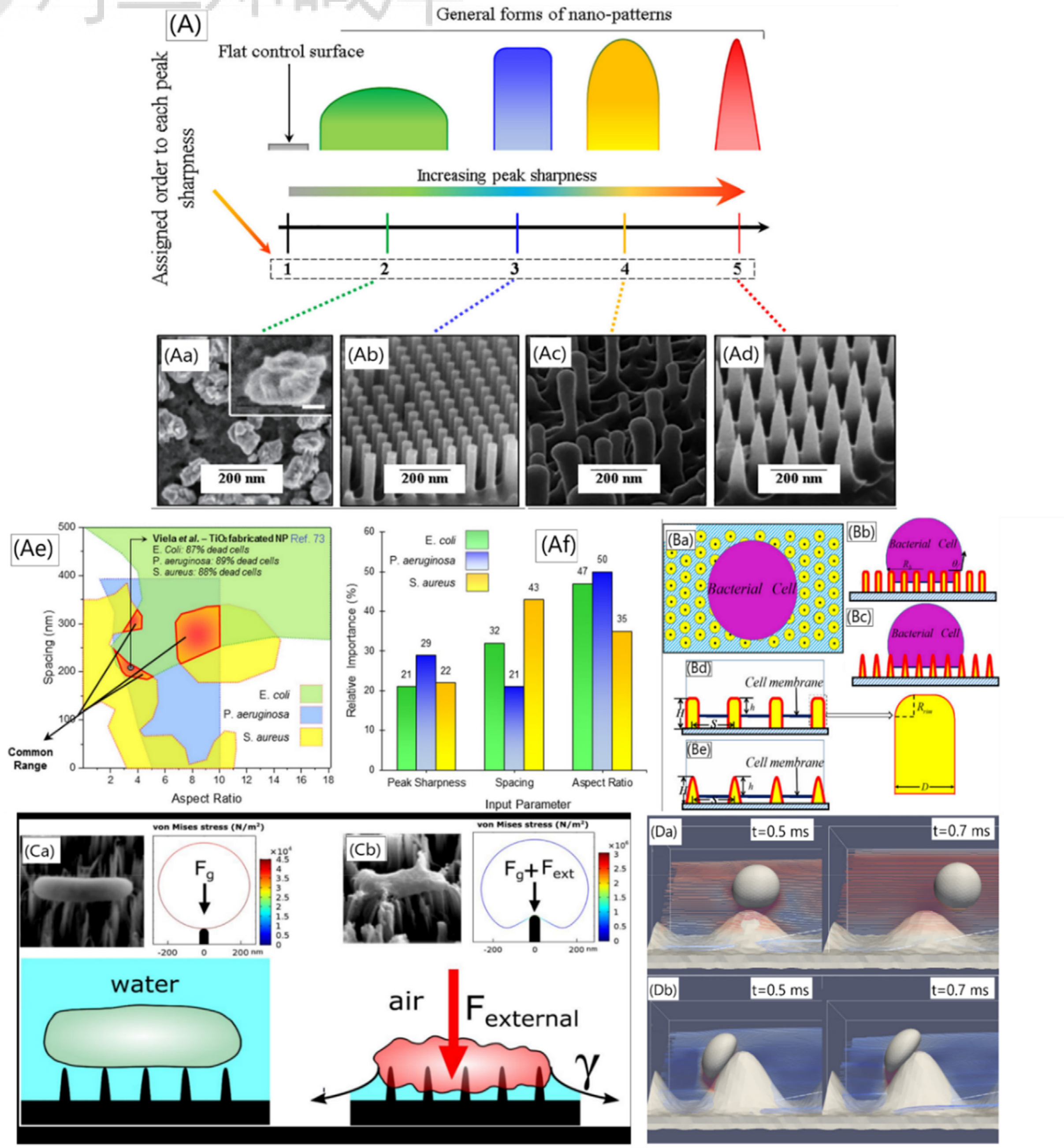

Fig. 1. *The different natural bactericidal surfaces have different shapes and size of nanostructures: (A) illustrations of different forms of the peak sharpness and their assigned orders used in an artificial neural networks model and examples of the corresponding shapes; (Aa) nano-nuggets, (Ab, Ac) 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 [40]. 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 [34]. (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 [46]. (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 [45] (Reproduced with due permission from Refs. [34,40,45,46]).*

compromise between precision and functionality are more likely to be used in the food packaging industries. Common methods for producing bactericidal surfaces include plasma and hydrothermal etching (for silicon, metal, glass, and polymers) [35,48–50], electrochemical etching (for metal and silicon) [35], laser treatment (for metals, and ceramics, polymers) [51], and nanoimprint lithography (for polymers) [38,52]. However, producing nanostructures that can be scaled up while remaining cost-effective is a significant barrier to the widespread adoption and deployment of food packaging materials.

A variety of nanostructures (e.g. brush and niche types) exhibit outstanding bactericidal activity when hydrothermally processed [35]. However, the structures produced by these techniques have random orientations and are extremely sensitive to slight parameter fluctuations, which can jeopardise the repeatability of high-performance bactericidal nanostructures between batches. Nanoimprint lithography and soft moulding techniques give tens of nanometer resolution and hierarchical architectures, resulting in superior bactericidal characteristics for polymer surfaces [53]. Nanoimprint lithography is efficient, precise, scalable, and cost-effective provided large copies are made from a master mould; nevertheless, it is only applicable to flat surfaces. Furthermore, the procedure requires multiple steps and is confined to thermoplastics. Chemical etching could be used with nanoimprint lithography for metal surfaces, however this may not be cost-effective. The majority of these methods are unsuitable for surface nanostructuring of complicated geometries, 3D surfaces, and polymeric scaffolds. There is a significant unmet demand for creative approaches to manufacture bactericidal nanostructures on 3D polymer and metallic surfaces. Laser interference (LI) lithography is a scalable approach to yield ordered structures down to submicron (<300 nm) resolution [54]. The modified LI approach employs an axicon lens to achieve a narrow intensity distribution. It can produce nanostructures of varying sizes by adjusting the depth of focus and beam propagation. Direct nanopatterning of food-grade SS (304 or 316) is especially appealing given its ubiquitous use in the food industry. Electrochemical anodization is another effective technique for metallic materials. It happens when metallic substrates are subjected to oxidising electrolytes and an electric field, resulting in cylindrical pores. Anodization allows for nanostructuring of metallic substrates with arbitrary curvatures and geometries over a large surface area. Tunable anodization parameters enable precise and independent control of numerous nanotopographical features, such as pore diameter (6–500 nm), length (10–100 nm), and shape. Porous anodic materials (e.g., alumina, titania) have been investigated for antifouling properties against food pathogens, although their mechanobactericidal activities are unclear [55,56]. To ensure the reliability and consistency of the fabrication process, researchers employ in-situ monitoring techniques and feedback control systems [78]. This allows for real-time adjustments to the process parameters, ensuring that the desired nanostructure dimensions are consistently achieved across large areas and multiple samples. Such control strategies are crucial for scaling up the fabrication process for industrial applications in food safety and quality management.

3. Challenges and opportunities for food packaging application

The growing worry over currently employed antibacterial food packaging is on a much bigger scale than laboratory results. Laboratory-grade tests are frequently conducted with food simulants, which are considered to be significantly less complex than actual food systems [8]. In reality, the diet contains more salt, less water activity, minerals, and fats or proteins, which have been shown to interact with antimicrobials [57,58]. Furthermore, food packaging circumstances and transportation methods may have a significant impact on the efficacy of leaching-based antimicrobial coatings. The rate of antimicrobial release is an important element to consider, as is the time required to saturate the area. Will excessive leaching damage the meal remains a matter of optimisation for all leaching-based coatings or edible thin films [10]. Despite the promising alternative of mechanobactericidal nanostructures, it still faces challenges such as compromise in nanostructure sharpness due to accumulated debris of dead bacteria, nanostructure durability, the feasibility of making nanostructures on large areas, and the need to standardise testing protocols for mechanobactericidal surfaces. The upcoming subsections elaborate on these constraints and propose plausible solutions.

3.1. Compromise in sharpness of nanostructures

The bactericidal activity of nanostructured surfaces has been tested for only several hours. Bacterial cells then grew on the nanostructured surfaces when the nanostructures' sharpness was impaired [59–61]. Nanostructures wrapped in dead cells were less effective at physically rupturing new cells.

Similarly, nanostructured polymeric surfaces that were initially bactericidal became less effective as dead cell debris diminished the sharpness and height of the nanostructures [62]. Interestingly, insect wings demonstrate self-cleaning behaviour, which may be important for maintaining bactericidal efficacy. The self-cleaning characteristic of nanostructures keeps their sharpness by eliminating detritus from dead bacteria. As a result, replicating insect wing structures on deployable metallic or polymeric materials with self-cleaning capabilities can extend bactericidal action [30,63].

In this direction, Jiang and colleagues demonstrated that the functionalities of bactericidal nanopillars can be greatly expanded by creating a hybrid thermoresponsive polymer@nanopillar-structured surface that keeps all of the characteristics of pure nanopillars while releasing dead bacteria (see Fig. 2a) [64]. The researchers created such surfaces by coaxially coating mechano-bactericidal ZnO nanopillars with thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm) brushes. By combining ZnO nanopillars and PNIPAAm chains, antibacterial efficacy may be controlled between strong mechano-bactericidal action (~99%) and high bacteria-releasing efficiency (~98%). Another group of researchers created a mechano-bactericidal nanopatterned surface with salt-responsive bacterial releasing activity by grafting salt-responsive polyzwitterionic (polyDVBAPS) brushes onto a bio-inspired nanopattern surface (see Fig. 2b) [65]. The concentration of salt in water affects the configuration of grafted polymer brushes. The surface demonstrated high mechano-bactericidal effectiveness in water (low ionic strength circumstances), however dead bacterial residues were easily lifted by the stretched polymer chains and removed from the surface in 1 M NaCl solution (high ionic strength conditions).

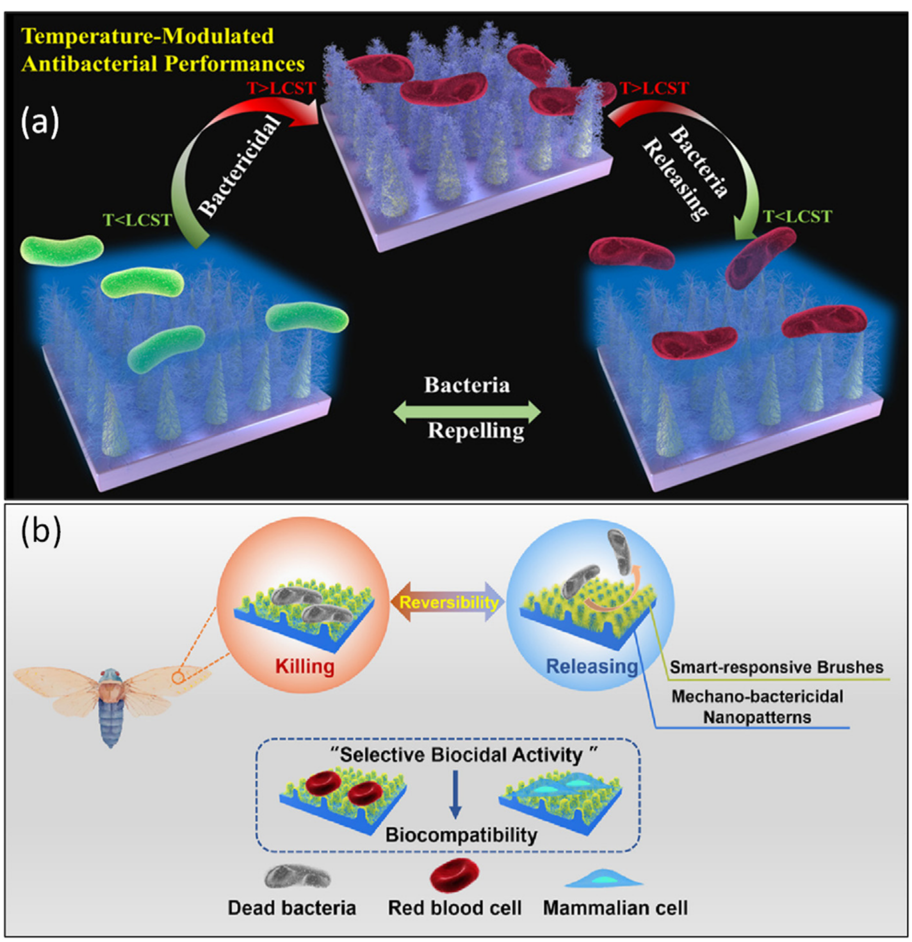

Fig. 2. (a) Schematic Illustration of the Temperature-Mediated Switchable Antibacterial Performances of the Hybrid PNIPAAm@ZnO Nanopillar-Patterned Surface [Reproduced with due permission from Ref. [64], Copyright© 2021, ACS]. (b) Schematic illustrates the selective biocidal activity between bacterial cells and eukaryotic cells. The smart-responsive brushes kill bacteria upon attachment and releases the dead bacteria in a salty environment. Moreover, the red blood cells and mammalian cells were attached and grown further on polymer brushes (Reproduced with due permission from Ref. [65], Copyright© 2022, Elsevier).

3.2. Durability of nanostructures

Surfaces used in food processing, packaging, and protective coverings may be subjected to a variety of stressors, including mechanical (abrasion, shear), chemical (acidic, alkaline), and thermal (steam, freezing). Long-term durability and the capacity to withstand damage under modest pressures (such as the pressure exerted by human contact) are critical for the practical application of touch surfaces. Recently, Roy and group tested the mechanical stability of etched nanostructures on Polyethylene terephthalate (PET) against external pressures by subjecting the etched topographies to 100 kPa pressure, which is the normal force applied by hand on phone screens or while working with tools (see Fig. 3a) [61]. Simulations show that compressive loading with 0.1 MPa pressure results in stresses of around 15 MPa at the pillar base, significantly lower than the stated yield strength values for PET (around 50 MPa). This lends support to the experimental finding that regular forces do not affect nanostructures. Stresses escalated to as high as 185 MPa during succeeding shear stages when pillar tips deflected in the shear direction. Based on modeling and experimental data, we infer that mechanobactericidal nanostructures are most vulnerable to destruction when subjected to both normal and shear loading (Fig. 3b and c). Normal loading causes minimal damage, but bending or twisting causes sub-micron cracks but does not harm the nanostructures themselves (Fig. 3b). Durability under shear loading may be improved by adopting materials with superior mechanical qualities (greater yield strength) or ensuring that the surface is not subjected to shear loading at high pressures. Loss of fidelity over time caused by phenomena such as creep may provide a challenge for these soft and flexible nanopatterns, which should be investigated further.

3.3. Scalability of nanostructures: how to fabricate nanostructures on large areas and curved surfaces?

To move forward with the fabrication of nanostructured surfaces, the existing manufacturing technology must be better understood, and newer production approaches must be developed. Fabrication routes must be accurate, adaptable, and repeatable. Another significant problem in the creation of nanostructured surfaces is that the produced structure must cover a considerable area for the structured surface to have practical uses. Chemical manufacturing procedures, while capable of covering a huge area, produce structures that are difficult to manage in size and spacing. It is also difficult to precisely recreate the constructed structure, and thus the observed feature [72]. Physical fabrication approaches, on the other hand, can produce highly reproducible structured surfaces, but they frequently have a limited area coverage. Physical manufacturing approaches frequently include numerous processing steps that result in a highly reproducible surface; nevertheless, the area covered by the structured surface is frequently less than that covered by chemical processes.

Researchers attempted a successful approach to avoid the drawbacks of chemical methods by developing a micro-imprinting setup. A sequential manufacturing route for making microstructures on metallic surfaces on a large area has been presented (see Fig. 4a and b) [66,67]. The structures have been fabricated in two steps; i) fabrication of mold using focus ion beam machining ii) the structures being transferred to the metal sheet using incremental stamping. This is a plate-to-plate method of transferring the structures on a metal surface. Fig. 4a shows the schematic representation of the process adopted and the developed hardware setup is shown in Fig. 4b. Nano plastic forming (NPF) is one of the recent micro grooving techniques used for nanopatterning of deposited thin films or patterning soft metal sheets [68]. 10 mm × 12 mm quartz slides of 1 mm thickness were coated with 10 nm of Au film through DC Sputter Coating. A specially designed wedge-shaped diamond tool is used for the NPF process. The edge angle, edge radius, and width of the tool are 60°, 50 nm, and 600 μm, respectively. The process of patterning through nanoplastic forming is direct and scalable but it suffers from limited flexibility in terms of the shape of patterns that can be made. The types of patterns that can be made are severely dependent on the nature of the tool. Although the wear rate of the tool is very low however in the case of patterning of substrates like Quartz, Silicon, etc. the nanotip of the diamond may get damaged after repeated use. Using a tool with a damaged tip for patterning can result in discrepancies in the size and shape of the structures.

The roll-to-roll UV imprinting is a famous technique for making micro-nanostructures on a large area. Typically, it has five main components: (i) Unwinding unit (ii) Dispenser (iii) Forming unit including pattern roll (iv) Exposer of UV light (v) Winding unit as can be seen in Fig. 4c. The flexible substrate is unwinding from the roller and UV-curable resin is coated on the substrate. The thickness of the resin depends on the needle size, ejection pressure, feed rate, and viscosity of the

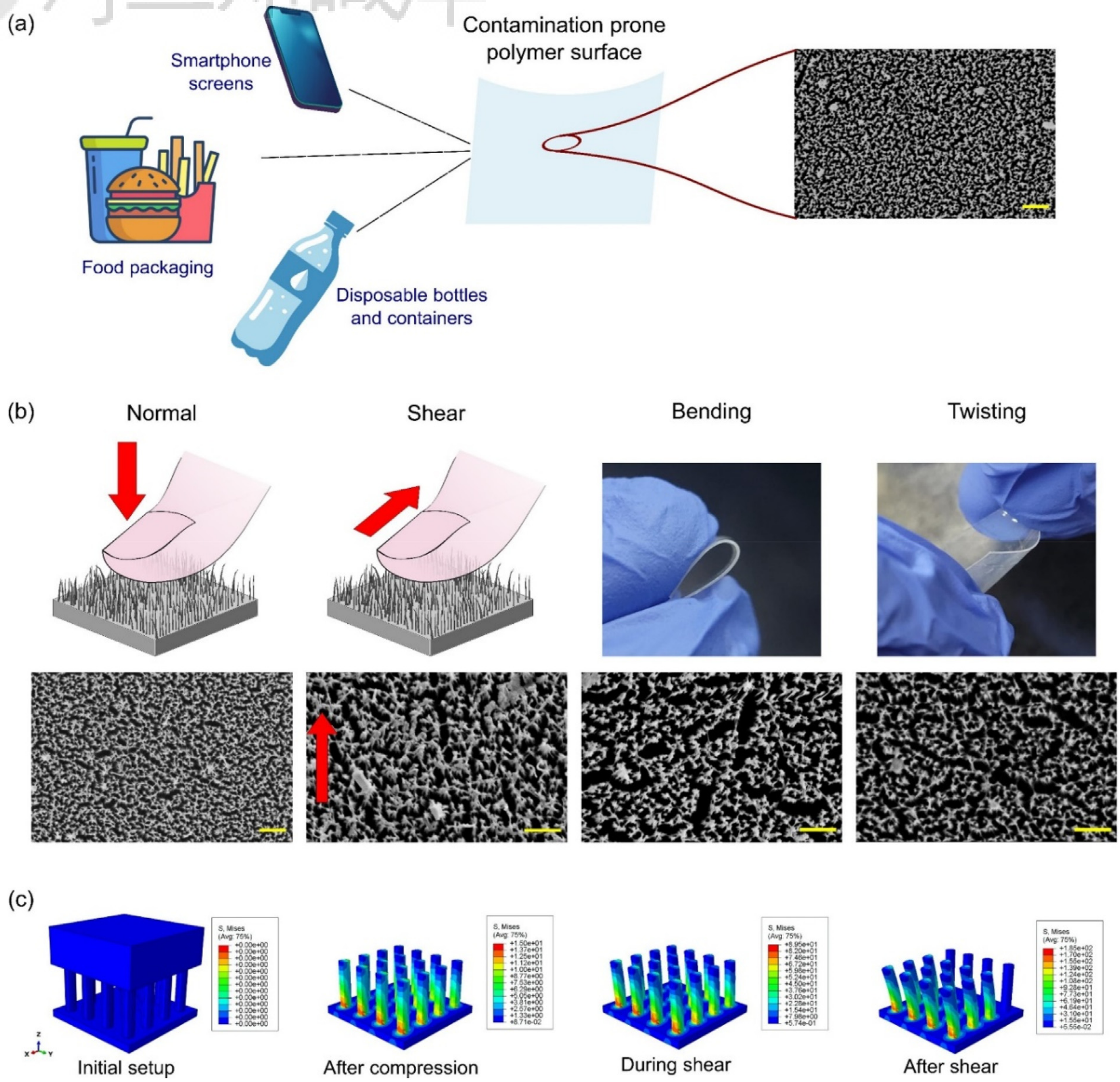

Fig. 3. (a) Schematic of different applications of polymers where they are prone to contamination and spreading of infections; the SEM micrographs on the right show the nanostructured PET surface before loading. (b) Scanning electron micrographs (SEM) of nanostructured PET sheet after normal loading at 100 kPa, shear loading, bending, and twisting. Scale bars are 1 μm. (c) FEM simulations of compressive and shear loading of the nanostructured surface (T1 geometry) at different process stages. Stresses are represented in MPa. (Reproduced with due permission from Ref. [61], Copyright© 2023, Elsevier).

resin. While passing through the forming unit, the tensioned substrate pushes the coated resin into the structured cavity made on the roller surface. At the same time, the UV light is illuminated from the bottom at the contact region and cures the resin within the cavities of the roll template. The winding roller winds the structured surface.

There are two basic ways to create structures on the roller surface namely direct-structuring and lithography-based techniques. Each method has its unique characteristics hence suitable for fabricating different types of molds. The popular technique to engrave structures on the roller is the single-point diamond turning (SPDT) method because of its high accuracy, large area patterning, and easy to operate. The copper-plated roller mold using the SPDT method has been fabricated for roll-to-roll imprinting [70]. The pitch, depth, and shape angle of the micro-prism patterns were 25 μm, 12.5 μm, and 90° respectively. The lithography-based technique includes stepped rotating lithography. The material from the cylindrical surface can be removed with

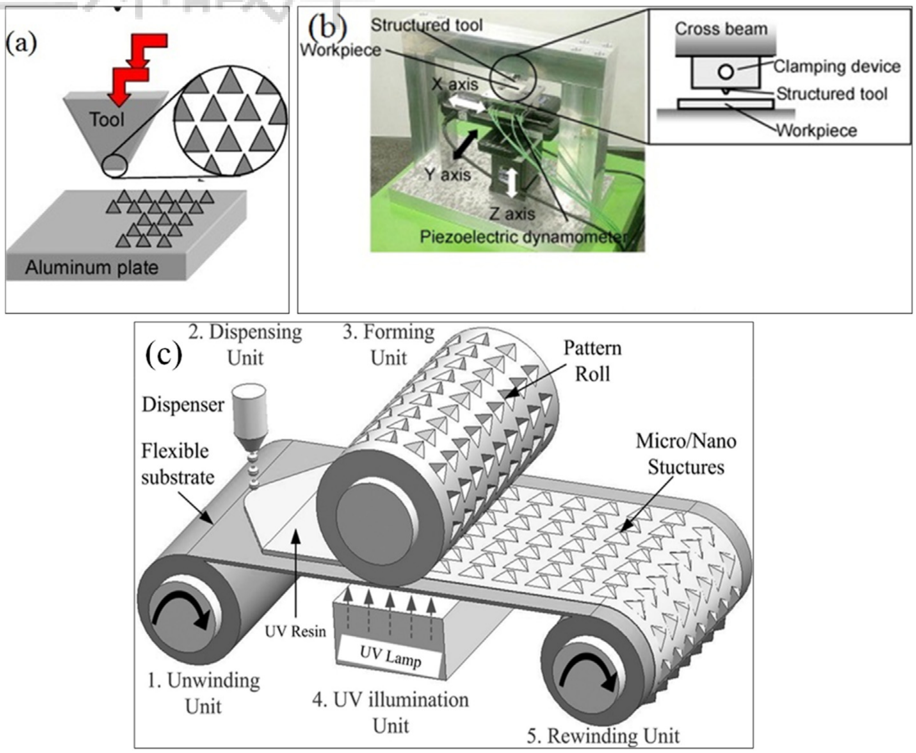

Fig. 4. (a) Structures created on tool face using FIB sputtering and transfer on an aluminum thin plate (b) developed setup for incremental stamping on thin plates [Reproduced with due permission from Ref. [67], Copyright© 2012, Elsevier] (c) Schematic representation of a typical roll-to-roll UV imprinting system (Reproduced with due permission from Ref. [69], Copyright© 2015, AIP Publisher).

the help of a laser beam, electron beam, or UV-light. Leet et al. developed a stepped rotating lithography to fabricate submicron-size features on a metal cylinder [71]. The micro-pillars with diameters of 0.6, 0.8, 1.2 μm were achieved. Fig. 5 shows the method of making a seamless mold roller using UV-lithography. The fabrication of a photo mask and the use of a reflection mirror makes the process very complex and time-consuming. However, this can be easily compensated by producing a larger number of components in a short time.

3.4. Need for standardised testing protocols

Future studies should necessarily adopt a standardized approach for evaluating the mechano-bactericidal nature of nanostructured 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 nanostructures in static conditions. Therefore, for accurate estimation of the bactericidal efficiency of nanotopography, the surfaces should be tested in dynamic flow conditions with maximum bacterial concentration. Moreover, 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. Only a few studies on the bactericidal efficiency of nanostructures under flow conditions have been reported. This provides a lot of research opportunities, as there are many factors contributing to the bactericidal performance of a surface under flow conditions [61]. Hizal and the group stated that the antibacterial effect of nanostructured surfaces with culture medium under flow has not been previously studied, and no further studies have been conducted on this topic since then [73].

4. Conclusion and outlook

Mechanobactericidal nanostructures of various topographies have been tested against bacterial cells. However, only a few nanoscale topographies, such as nanopillars, nanowires, nanospikes, and nanocrystals, have demonstrated bactericidal activity, while others have contributed to a lesser bactericidal or antibiofouling effect. Though it has long been assumed that nanostructures are less effective at killing Gram-positive bacteria, there is a large

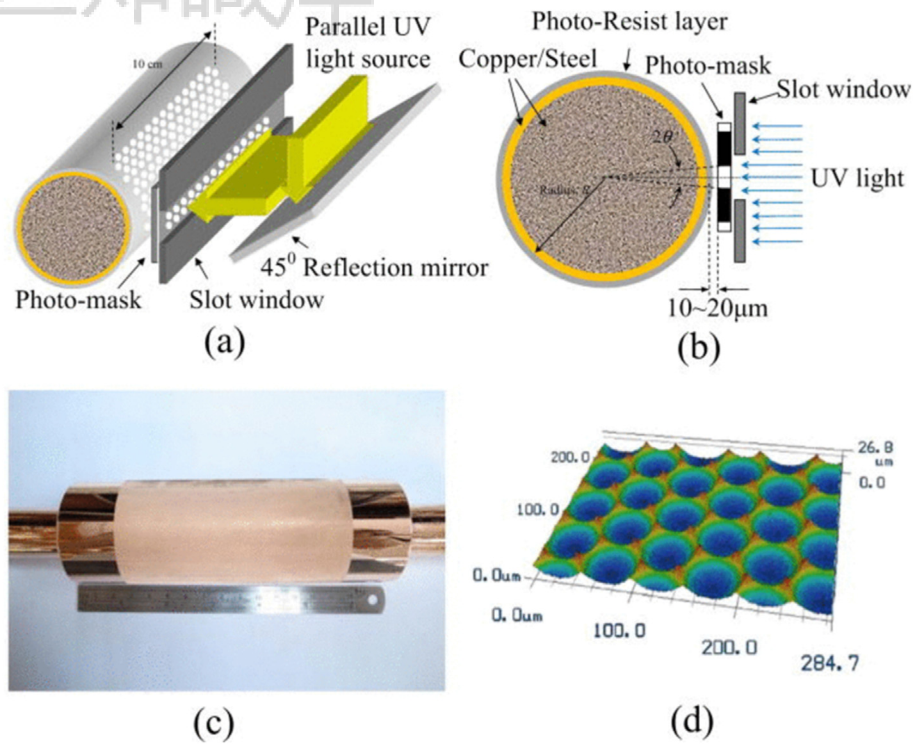

Fig. 5. Advanced approach to fabricate a seamless roll mold: (a) schematic diagram of the step-and-rotate UV-exposure system, (b) detailed cross-sectional view of the system, (c) photograph of the roller mold, and (d) the semi-spherical concave microcavities formed using the mold (Reproduced with due permission from Ref. [71], Copyright© 2011, IEEE).

body of evidence to the contrary. More testing is needed to determine whether the bactericidal effect of nanostructures is based on Gram-stain bacteria. The effect of substrate surface hydrophobicity on the bactericidal activity of nanostructures cannot be generalised either. However, the influence of hydrophobicity on nanostructures' bactericidal efficacy differs depending on the bacterial species tested on the substrate. Hydrophobic surfaces tend to repel water, which can affect how bacteria adhere to and interact with the substrate. This can lead to varying degrees of bactericidal efficacy based on bacterial cell wall composition, surface charge, and nanostructure properties. Different bacterial species possess unique cell wall structures that can influence their adhesion and susceptibility to nanostructures. For instance, Gram-positive bacteria, which have thicker peptidoglycan layers, may respond differently to hydrophobic surfaces compared to Gram-negative bacteria, which possess an outer membrane that can affect permeability and adhesion. Pathogenic strains may have evolved mechanisms to resist certain types of nanostructures, making them less susceptible to hydrophobic effects compared to non-pathogenic strains. Bacteria such as *E. coli* demonstrate a preference for adhering to hydrophobic surfaces. Research indicates that the bactericidal effect of nanostructures increases with the water contact angle (WCA) of the surface, suggesting that more hydrophobic surfaces enhance bacterial adhesion, which can lead to greater bactericidal efficacy [77]. The influence of hydrophobicity on bactericidal activity is not uniform across all bacterial species. For instance, while hydrophobic micro/nanostructured surfaces have shown high bactericidal efficiency against both Gram-positive and Gram-negative bacteria, the degree of effectiveness can differ significantly depending on the specific strain tested [74–76]. Some hydrophobic surfaces have been reported to achieve bactericidal efficiencies greater than 80% against certain strains, while others exhibit minimal or no effect, highlighting the complexity of these interactions [76,77]. The charge of the substrate and the bacteria can interact in ways that either promote or inhibit adhesion, affecting the overall bactericidal effect. Moreover, the size, shape, and material of the nanostructures themselves can interact differently with hydrophobic surfaces, further complicating the relationship. Furthermore, there is a need to standardise the testing process in the same setting as food storage and packaging. For soft and flexible nanostructures, loss of fidelity over time due to processes such as creep could provide difficulty for food-packaging applications and should be researched further. Most nanofabrication processes with promise for food applications are limited to certain materials and their inherent surface features. Thus, the synergetic approach of coating a nanostructured surface with a small amount of leaching-based antibacterial could be both practical and effective in achieving the desired result. After fabricating nanostructures, apply ultrathin antibacterial coatings (<10 nm) to provide desired functional moieties while maintaining advantageous topographical aspects. Antibacterial coatings must be compatible with various food packaging materials, such as polymers and biodegradable options. The coatings should not compromise the mechanical properties or barrier functions of the packaging. Techniques such as electrospinning, spray coating, and layer-by-layer assembly can be scaled for mass production [2]. However, the consistency of coating thickness and uniformity across large surfaces must be maintained, which requires precise control during the fabrication process. The economic viability of producing these coatings at scale is crucial. While nanomaterials can be expensive, the potential for extending shelf life and reducing food spoilage can offset initial costs. Any materials used in food packaging must comply with food safety regulations. This includes ensuring that the coatings do not leach harmful substances into food products. The long-term stability of nanostructured coatings under various environmental conditions (e.g., humidity, temperature) is a concern. Coatings must retain their antibacterial properties throughout the product's shelf life. Moreover, the effectiveness of the coatings may vary against different bacterial strains. It is needed to ensure that the coatings provide broad-spectrum antibacterial activity [12,42].

Whatever may be the antibacterial coating material, the selection of coating thickness is the key point with respect to underlying nanostructures. The coating should not be too thick so that the sharpness of nanostructures gets affected. Moreover, the too thick coating could leach excessive antibacterial agent and could spoil the food. Therefore, the selecting the optimum coating thickness is the key concern. Importantly, removing cellular remains from deceased bacteria from the bactericidal nanofeatures is critical for their renewal and long-term function. This has been accomplished by activating the nanostructures using a variety of environmental stimuli, such as salt exposure [62], alternating between wet and dry states [71], or temperature cycling [61]. Few coatings can be activated on flat surfaces through light exposure or pH adjustment [72]. All of the aforementioned stimuli are commonly found in food-handling contexts and could thus be used to trigger the discharge of bacteria remnants or other debris, extending the lifetime of mechanobactericidal nanostructures. To fully realise the promise of mechanobactericidal nanostructures in the food industry, multidisciplinary efforts in chemistry, microbiology, biophysics, nanoengineering, material science, toxicology, and food science are required, as well as collaborations among academia, industry, and regulatory agencies. Mechanobactericidal surfaces can help fight harmful bacteria and biofilms in the food business, potentially enhancing food safety and quality.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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