UNVEILING THE ANTIBACTERIAL POTENTIAL OF NATURE-INSPIRED MATERIAL FOR DESIGNING FOOD-RELATED COATINGS

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Abstract - The escalating challenge of antibiotic resistance has driven the innovation of new antibacterial and antifouling materials. Recent developments focus on nature-inspired topographical engineering and nanostructured surfaces to combat resistant bacteria. This review discusses these advances, emphasizing the potential of nanoantibiotics and biopolymers. Nanoantibiotics revitalize drug effectiveness by encapsulating them in nanoparticles, presenting a new strategy to fight pathogens. Biopolymers, eco-friendly and biodegradable, emerge as a sustainable alternative, with applications in food safety and beyond. The exploration of these materials signifies a leap in design, fabrication, and the possibility of cost-effective, large-scale production, highlighting a promising avenue for commercial applications to tackle antibiotic resistance and biofouling effectively.

Key words - Antibacterial; antimicrobial; hierarchical structures; bio-inspired; biomimetic

1. Introduction

The emergence of antibiotic-resistant bacteria poses a severe threat to human life, with approximately 700,000 deaths annually attributed to infections caused by these microbes [1], [2]. If antibiotic resistance continues to evolve at its current rate, this number is projected to go up to 10 million by 2050 [1], [2]. In addition, the formation of biofilms on surfaces, whether biotic or abiotic, generates another critical threat to human health. Biofilms are formed when surfaces are attached, proliferated and colonized by bacteria, producing extracellular polymeric substances (EPS) that protect them from toxins and antimicrobial agents. Therefore, EPS can lead to the development of antimicrobial resistance [3], [4]. To solve these challenges, the development of antibacterial materials and surfaces has become a critical engineering pursuit, as they can prevent bacterial adherence and biofilm formation, reducing the reliance on antibiotics and disinfectants. This is particularly relevant in the food packaging industry, where the use of antibacterial materials can help extend the shelf life of food products and prevent the spread of foodborne illnesses.

Surface functionalities can be used to kill bacteria, as demonstrated in several studies [1], [2], [3], [5], [6]. Surfaces play an important key in impacting the viability and adhesion of bacteria on surfaces, and particularly synthetic and natural materials have been discovered and developed. Additionally, modulating surface chemistry and physics are essential in order to stop bacterial attachment [7], [8], [9]. Furthermore, the modification of surface chemistry and nanotopography of materials like poly(ethylene terephthalate) (PET) by marine bacteria has been shown to affect bacterial adhesion. Covalent attachment of certain polymers to glass surfaces has also been found to make the surfaces lethal to bacteria. These findings highlight the potential of surface chemistry and topography in developing antibacterial coatings and materials.

This review article provides an overview of bio-inspired materials and their capability to stop bacterial adhesion and viability. It explores natural materials (either surface architecture or biopolymers) that either prohibit bacterial attachment or directly disrupt bacteria cells that manage to attach to the facial material. The methodologies employed in mimicking these naturally occurring bactericidal surfaces are presented, and a discussion of the impact of physical and chemical parameters on the antibacterial efficacy is conducted. The review also examines the factors affecting the contact-killing antifouling mechanisms of synthetic surfaces. We also delve into the application of these technologies in food packaging, discussing the current state of the art, the challenges faced, and the future directions of this important field.

2. Overview of bacterial attachment: from surface interactions to microcolony development

Bacterial attachments are critical factors in the persistence and spread of foodborne pathogens on various surfaces within food processing environments [10]. The process of bacterial adherence to food contacting materials progresses through reversible and irreversible stages, culminating in the biofilm formation (Figure 1), which are complex communities of microbial cells embedded in extracellular polymeric substances. These biofilms can adhere to a multitude of surfaces, including those in food processing facilities, and are notoriously difficult to eradicate due to their resistance to cleaning and disinfection methods [11]. The presence of biofilms in the food industry is a significant concern as they can harbor foodborne pathogens like *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli*, leading to the contamination of food products and an increased risk of outbreaks of foodborne illnesses [12], [13]. Moreover, biofilms can protect bacteria from disinfectants, thereby enhancing their survival and the potential for subsequent contamination, which can result in reduced product quality, shorter shelf lives, and serious public health risks.

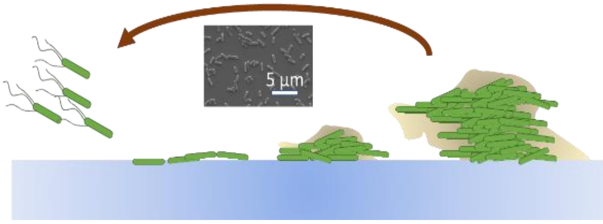

Figure 1. *Stages of biofilm formation on a surface. Initial reversible attachment of planktonic bacteria. Irreversible attachment facilitated by exopolysaccharide production. Early development of microcolonies through cell growth and division. Mature biofilm with complex three-dimensional structure and heterogeneous distribution of bacterial cells, EPS matrix, and water channels. Dispersal of individual cells or clusters, returning to planktonic state and colonizing new surfaces*

Bacterial attachment is a process where bacteria adhere to surfaces, which can be biotic (living) or abiotic (non-living) [10]. The process of bacterial attachment involves specific adhesive molecules on the bacteria's surface that attach to receptors on the host. This attachment occurs in various stages, such as initial reversible adhesion, followed by more permanent adhesion, and eventually leading to biofilm development. The first stage of reversible attachment is facilitated by general physical forces, including hydrophobic and Lifshitz-van der Waals interactions. This is followed by irreversible attachment, usually aided by adhesive molecules known as adhesins [14]. After attaching to a surface, bacteria can develop into biofilms. These are intricate assemblies of microorganisms encased in a matrix made of substances they produce themselves. The formation of a biofilm is a multifaceted procedure that includes stages like adhesion, growth, and cell communication, and it begins with bacteria reversibly attaching to a surface. This is followed by a permanent attachment, the creation of microcolonies, and the eventual growth into a layered, three-dimensional structure. Biofilms create a protective habitat for bacteria, improving their ability to survive under harsh conditions and increasing their resistance to cleaning agents. The ability to attach and form biofilms is an essential survival mechanism for nearly all bacteria, enabling them to reside in environments rich in nutrients.

3. Exploring bio-Inspired antibacterial and antifouling strategies

Bio-inspired antibacterial and antifouling strategies aim to mimic natural mechanisms to prevent bacterial attachment, biofilm formation, and subsequent contamination. These strategies are particularly relevant in the context of increasing antibiotic resistance and the need for effective non-chemical approaches to control microorganisms. Cicada wings possess nanopillar structures that impart antimicrobial behavior [15], [16], [17]. The wings of cicadas possess unique nanostructures in the shape of closely-packed nanopillars with diameters ranging from 156-207 nm and heights ranging from 182-241 nm [17]. The nanopillars are aligned in a near-hexagonal symmetry and have been shown to present a crucial part in controlling the wettability of the cicada wings. Their topographical and dimensional structures were considered to impact the wetting behavior of cicada wings. The arrangement of the nanopillars determines the hydrophobicity or super-hydrophobicity of the wings. Dragonfly wings have also been found to exhibit self-cleaning and antibacterial properties similar to cicada wings. The microstructure on dragonfly wings is also composed of nanopillars with irregular shapes and diameters ranging from 83 to 195 nm [18], [19], [20], [21]. Figure 2 illustrates the surface architecture found on the surface of a dragonfly wing. This surface architecture can eliminate both Gram-positive and Gram-negative bacteria due to the smaller tip diameters of the nanoprotrusions.

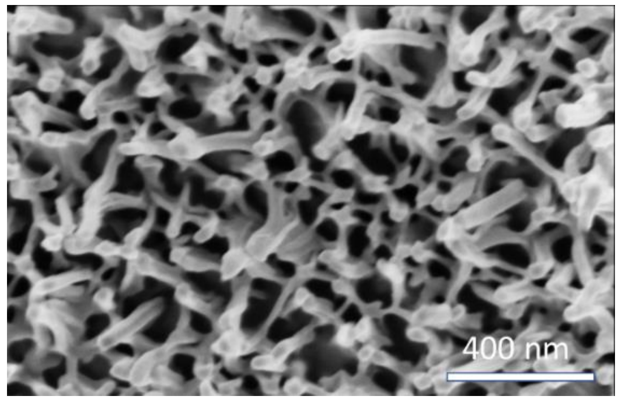

Figure 2. *Scanning electron microscopy (SEM) image of the nanostructure on a dragonfly wing. The intricate array of nanopillars and nanochannels contribute to the hydrophobic properties of the wing, self-cleaning capabilities, and bactericidal effects*

Bioinspired nanoantibiotics work by encapsulating antibiotic molecules within engineered nanoparticles [22]. These nanoparticles can restore the efficacy of the antibiotics due to their nanoscale functionalities. The nanoantibiotics can cross the bacterial cell membrane, interfere with cellular components, and damage the metabolic machinery, thereby effectively delivering the antibiotics to the target sites inside a bacterium, as shown in Figure 3. This approach is particularly useful for combating intracellular infections, where conventional antibiotics often struggle to reach and eliminate the bacteria. Bioinspired materials can mimic the uptake of biological particulates and release antibiotics inside the cells, thereby overcoming the challenges associated with treating intracellular infections. In some cases, bioinspired nanoantibiotics can also produce reactive oxygen species [1] and disrupt bacterial membranes, serving as additional antibacterial mechanisms. The development of bioinspired nanoantibiotics is a promising strategy to counteract the surge of antibiotic resistance, offering a novel approach to repurpose conventional antibiotics and enhance their effectiveness. Some examples of bioinspired nanoantibiotics include solid lipid nanoparticles, dendrimers, aptamers, protein nanoparticles, and viral nanoparticles [23]. These bioinspired nanoparticles are designed to mimic natural systems and can be formed into various structures such as particles, fibers, and rods. They are used to deliver drugs either passively or actively to combat bacterial infections and are particularly useful in overcoming antibiotic resistance. These nanoantibiotics can encapsulate antibiotic molecules and deliver them effectively to the target sites inside bacteria, potentially restoring the efficacy of conventional antibiotics.

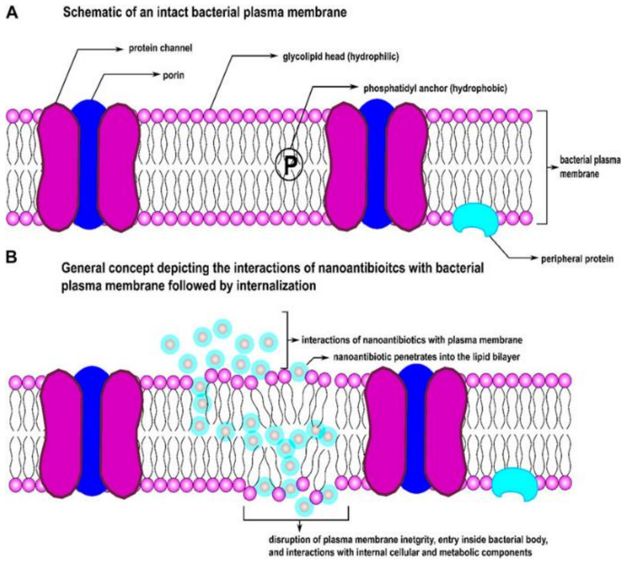

Figure 3. *Schematic showing the intact bacterial cell membrane (A) before; and (B) after interacting with nanoantibiotics (adapted with permission from [22])*

Leaves of various plants and flower petals possess superhydrophobic and antimicrobial properties, making them a valuable source of inspiration for developing bio-inspired surfaces. Taro leaves, for example, exhibit hydrophobic, self-cleaning, and anti-biofouling properties, thanks to the well-ordered micro/nanostructures present on their surface. These structures are elliptical-shaped bumps measuring 10 to 30 µm in diameter, which are then coated by a thin film of waxy polygonal epidermal cells, creating a superhydrophobic surface [24]. Natural surfaces like lotus leaves and taro leaves have evolved to display superhydrophobic and antimicrobial behavior due to the presence of well-ordered micro/nanostructures on their surfaces. Lotus leaves have hierarchical structures consisting of microscale elliptical bumps covered by nanoscale crystals. The presence of air pockets between these structures makes lotus leaves superhydrophobic, ensuring water droplets roll off the surface, removing dirt and any foreign material from the surface [25]. Rose petals show as a case of naturally presenting surfaces with hierarchical structures that provide super-hydrophobicity and antimicrobial characteristics. The rose petals are constructed from microstructure including densely packed micron-sized bumps, approximately 20 µm in diameter, with multiple nanometer-sized folds on top of each bump [26]. The micro-size papillae, along with the nanosize folding on the rose petal contribute to its surface roughness to the rose petal, enabling the petal to exhibit superhydrophobic properties. The skins of certain animals, such as sharks, also exhibit antibacterial and anti-biofouling properties [27], [28]. This is due to the appearance of micro-topographical structures that are uniquely arranged on their skin. Moreover, the skin of sharks is equipped with denticles that possess longitudinal grooves. These grooves are oriented in alignment with the direction of water flow and their concave surfaces are characterized by nanostructured protrusions. These structures reduce the surface area available for microbial adhesion and aid in self-cleaning.

4. The role of surface intermolecular forces to disrupt bacteria

The antibacterial properties of natural surfaces, such as the wings of cicadas and dragonflies, are attributed to their nanostructures that physically disrupt bacterial cell membranes [21]. These nanostructures can stretch and rupture bacterial cell membranes, leading to cell death. The adhesion of bacterial cells to these surface protrusions is driven by intermolecular forces, including van der Waals, electrostatic, hydrophobic, and steric forces [16]. As bacterial cells attach to these surface protrusions, they are subjected to an escalating stretching force, which amplifies with the sustained adsorption process, resulting in cell death. The degree of bacterial stretching is determined by the surface nanostructures, which can modulate the extent of physical rupturing and cell lysis [15]. The bactericidal mechanism of cicada wings was found to be independent of the surface coating, indicating that the nanostructures are the primary driving force behind their antibacterial properties. Additionally, the thickness of the bacterial cell wall plays a significant role in determining the extent of antibacterial activity observed on natural surfaces. Gram-positive bacteria, with thicker and more rigid cell walls, require significantly more tensile force to rupture than Gram-negative bacteria with thinner cell walls. Expanding on this, the structure of dragonfly wings creates a hostile environment for bacterial cells. The nanopillars, due to their size and density, penetrate the bacterial cell walls, causing physical damage. Additionally, when bacteria attempt to move or detach, the increased shear forces further damage the cells.

5. Effects of surface free energy on bacterial adhesion

Biofouling, the process where microorganisms attach to surfaces and form a biofilm, is influenced by surface properties such as wettability and topography. Bacterial cells tend to adhere to hydrophilic surfaces when their surface energy is higher than the surrounding liquid medium, while they adhere to hydrophobic surfaces when their surface energy is lower. Different bacterial species show preferences for different surfaces; for instance, *Staphylococcus aureus* and *Escherichia coli* favor hydrophilic surfaces, while *Staphylococcus epidermidis* and *Pseudoxanthomonas taiwanensis* prefer hydrophobic ones [29], [30].

Superhydrophobic surfaces exhibit a markedly lower level of bacterial adhesion compared to surfaces that are moderately hydrophilic or hydrophobic. On these superhydrophobic surfaces, bacteria predominantly stay scattered, even after extended periods, in contrast to hydrophilic surfaces where they tend to aggregate. This aggregation on hydrophilic surfaces suggests an increased propensity for biofilm development, highlighting the effectiveness of superhydrophobic surfaces in hindering such formations [31].

Natural surfaces with micro/nanostructures, such as lotus leaves and rice leaves, exhibit super-hydrophobicity and low surface energy, which reduce microbial attachment and inhibit biofilm development. The nanostructures limit the binding sites for microbes, reducing the chance of bacterial colonization. The interaction of bacterial cells with these surfaces depends on the bacterial morphology and the surface topography. Surface charge density also plays a crucial role in biofilm formation, as bacteria are more likely to adhere to surfaces with a positive charge. However, high charge density can lower cell viability, inhibiting biofilm growth.

In addition to plant-based surfaces, natural materials like shark skin and butterfly wings have unique surface structures that exhibit self-cleaning and antifouling mechanisms. These natural examples inspire the development of new antifouling materials. By understanding the mechanisms of these natural surfaces, researchers can create materials that effectively inhibit biofouling and bacterial adhesion, which is crucial in fields such as biomedicine, marine science, and industrial manufacturing.

6. Effects of natural polymers on bactericidal activities

Biopolymers such as chitosan and fucoidan have been found to exhibit antibacterial properties, which can be utilized to combat bacterial infections. Chitosan, a polysaccharide derived from chitin, can kill bacteria through several mechanisms [32]. One of the primary mechanisms involves the electrostatic interactions between the positively charged chitosan and the negatively charged bacterial cell walls. This interaction alters the permeability of the microbial cell, leading to the release of intracellular material and ultimately causing cell death. Chitosan can also block RNA and protein synthesis, inhibiting bacterial growth [33]. However, this mechanism requires a reduction in the size of chitosan to allow penetration into the bacterial cell. Furthermore, chitosan can form a film on the porins of the cell surface to block the exchange of nutrients, leading to microbial cell death. Lastly, the unprotonated amino groups of chitosan can chelate metal ions on the cell surface to disrupt cell function. Fucoidan, a sulfated polysaccharide found in the cell walls of brown algae, has demonstrated significant bacteriostatic effects on the growth of various bacteria. The antibacterial activity of fucoidan is influenced by its degree of sulfation and the level of uronic acid in its composition. Fucoidan inhibits bacterial growth by interacting with the bacterial cell wall and disrupting essential processes [34], [35]. It has been found to be particularly effective against *Staphylococcus aureus* and *Escherichia coli*.

Biopolymers, as antimicrobial agents, present numerous benefits over traditional antibiotics [36]. They are sourced from renewable materials and are biodegradable, which makes them a more eco-friendly option compared to traditional antibiotics that are often derived from non-renewable resources and can have long-lasting environmental impacts. Biopolymers also generally exhibit lower toxicity levels than traditional antibiotics, making them safe for a variety of applications such as wound healing, food packaging, textiles, and water treatment systems. The advantages of biopolymers extend to their resistance to microbial adaptation, versatility, stability, ease of production, and biocompatibility. Unlike traditional antibiotics, biopolymers can effectively combat bacteria that have developed resistance. They can also be modified for specific applications and remain active under a wide range of conditions. Their ease of production and biocompatibility make them suitable for various applications, including medical ones. Given these advantages, biopolymers are emerging as promising candidates for the development of new antimicrobial treatments and materials, offering a sustainable and effective alternative to traditional antibiotics.

7. Summary and future directions

The recent surge in research interest has been directed towards the development of innovative antibacterial materials, specifically those that are topographically engineered to combat antibiotic-resistant bacteria. These materials are inspired by the diverse range of antibacterial and anti-biofouling surfaces found in nature. This review has illuminated the interaction between antibacterial and bio-inspired surfaces found in nature, along with their fundamental antibacterial properties. Moreover, the discussion on nanostructured surfaces has included an examination of their bactericidal capabilities, specifically through contact-killing bactericidal mechanisms. This presents a significant exploration of the recent advancements in the methodologies employed for their design and fabrication. As we continue to explore the possibilities of these surfaces, it is crucial to optimize their design parameters to ensure that they can be efficiently produced on a large scale at a reasonable cost. By doing so, we can take steps toward commercial applications, which will require scalable and cost-effective production methods capable of processing large quantities of material. In addition to these topographically engineered surfaces, there has been significant progress in the development of nanoantibiotics and biopolymers as antibacterial agents. Nanoantibiotics encapsulate antibiotic molecules with engineered nanoparticles, revitalizing the existing arsenal of drugs and making them effective against a range of clinically significant pathogens. Biopolymers, derived from renewable resources and biodegradable, offer several advantages over traditional antibiotics. They are environmentally friendly, less toxic, and safe for use in various applications, including wound healing, food packaging, textiles, and water treatment systems. Overall, the field of antibacterial surfaces, including nanoantibiotics and biopolymers, holds enormous potential to provide practical solutions for the growing problem of antibiotic resistance and biofouling.

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