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Review

Antibacterial surfaces developed from bio-inspired approaches

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ABSTRACT

Prevention of bacterial adhesion and biofilm formation on the surfaces of materials is a topic of major medical and societal importance. Various synthetic approaches based on immobilization or release of bactericidal substances such as metal derivatives, polyammonium salts and antibiotics were extensively explored to produce antibacterial coatings. Although providing encouraging results, these approaches suffer from the use of active agents which may be associated with side-effects such as cytotoxicity, hypersensibility, inflammatory responses or the progressive alarming phenomenon of antibiotic resistance. In addition to these synthetic approaches, living organisms, e.g. animals and plants, have developed fascinating strategies over millions of years to prevent efficiently the colonization of their surfaces by pathogens. These strategies have been recently mimicked to create a new generation of bio-inspired biofilm-resistant surfaces. In this review, we discuss some of these bio-inspired methods devoted to the development of antibiofilm surfaces. We describe the elaboration of antibacterial coatings based on natural bactericidal substances produced by living organisms such as antimicrobial peptides, bacteriolytic enzymes and essential oils. We discuss also the development of layers mimicking algae surfaces and based on anti-quorum-sensing molecules which affect cell-to-cell communication. Finally, we report on very recent strategies directly inspired from marine animal life and based on surface microstructuring.

1. Introduction

It is now accepted that microbial populations use cell attachment to solid substrates to survive, forming structured communities called biofilms. Biofilms are defined as biopolymer matrix-enclosed microbial populations adhering to each other and/or surfaces [1]. Consequently, two bacterial living states exist in natural environments: planktonic (free floating) and sessile (attached) states. While the planktonic growth mode is important for the bacterial spread, biofilms are necessary to allow bacteria to persist and to resist adverse environmental conditions. Therefore, biofilms occur on inert and living supports in natural environments and in industrial installations [2] (Fig. 1). Bacterial biofilms are responsible for a wide range of human infections, including otitis media, osteomyelitis, native valve endocarditis and cystic fibrosis pneumonia [1,3]. Bacterial biofilm infections are particularly problematic because sessile bacteria can withstand host immune responses and are drastically more resistant to antibiotics (up to 1000-fold), biocides and hydrodynamic shear forces than their planktonic counterparts [4]. In humans, individuals with implanted medical devices, e.g. prostheses or catheters, and those with compromised immune systems, are considered to be most at risk of biofilm infections, and even humans with competent immune defenses often fail to resolve these infections independently. The protective mechanisms at work in biofilms appear to be distinct from those that are responsible for conventional antibiotic resistance. Although several mechanisms have been postulated to explain this reduced susceptibility of sessile organisms to antimicrobials, it is becoming evident that biofilm resistance is multifactorial [5]. Poor antibiotic penetration, nutrient limitation, slow growth, adaptive stress responses and the formation of multi-resisting cells are hypothesized to constitute a multi-layered defense [6]. Genetic and biochemical details of the biofilm defenses are now beginning to emerge. The biofilm phenotype of *Pseudomonas aeruginosa* appears to be regulated more at the translational and perhaps post-translational levels than at the transcriptional level, as highlighted by the discrepancies between microarray and proteomic experiments [7]. Whereas transcriptome analyzes led to the conclusion that few genes showed differential expression in planktonic and biofilm cells [8,9] protein-based approaches suggested that a large number of proteins are differentially regulated during biofilm development [10,11]. Considering the high resistance of sessile micro-organisms to inhibitors, the eradication of biofilms needs

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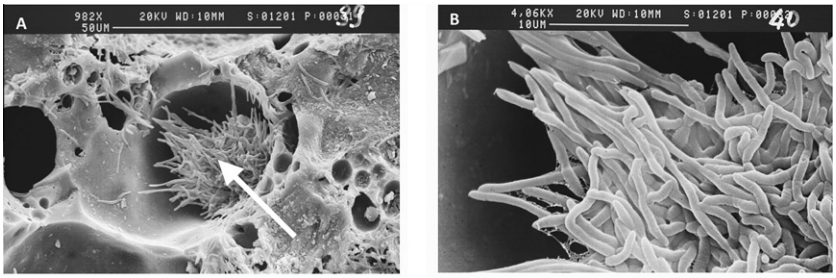

Fig. 1. (A) Biofilm of *Flavimonas oryzihabitans* visualized on a clay bead. (B) Enlarged image showing the morphology of the bacteria morphology on the biofilm.

high concentrations of disinfectants or antibiotics, causing severe environmental damages and the emergence of multi-resistance. In this context, prevention of biofilm formation is clearly preferable to any treatment strategy [12,13].

An efficient approach to prevent the biofilm formation consists in depositing a bactericidal layer on the material's surface. Various synthetic approaches based on immobilization or release of bactericidal substances such as metal derivatives, poly(ammonium salts) and antibiotics have been extensively explored to produce antibacterial layers [14–18]. However, depending on the application, they are not completely satisfactory because of their limited efficiency, their toxicity or their role in the emergence of multi-resisting pathogens. In addition to these synthetic approaches, there are fascinating strategies developed by nature over millions of years to prevent bacterial colonization on living tissues. Indeed, plants and animals have adaptively developed a vast array of defense mechanisms in response to an ever-present pathogen pressure. These natural strategies have recently emerged as a new source of inspiration to create antibiofilm coatings. This review attempts to report some of these bio-inspired coatings designed to prevent bacterial colonization.

2. Coatings based on antimicrobial peptides

2.1. Overview of antimicrobial peptides

Antimicrobial peptides (AMPs) are secreted by numerous living organisms (micro-organisms, vegetables, insects, fishes, amphibians and mammalians) to protect themselves against invading micro-organisms [19–21]. These peptides permeabilize membranes and may act specifically on pathogenic micro-organisms (bacteria and/or fungi) [22–24].

As mentioned by Haug et al. in a recent review [25], the field of AMPs has been growing continuously since the pioneering work of Boman's research group [26,27], which first isolated antibacterial peptides from insects. In the 1980s, Lehrer's group discovered defensins from rabbit lung macrophages [28,29] and Zasloff antibacterial magainin peptides from the skin of the African clawed frog *Xenopus laevis* [30]. It is now recognized that AMPs constitute a first barrier against pathogen dissemination in pluricellular organisms (see reviews by Giuliani et al. [31] and Jenssen et al. [32]). During the last decades, more than 800 AMPs have been characterized from a wide variety of eukaryotic species [25]. Dedicated websites strive to list the discovery of new peptides from many natural sources [33]. AMPs represent a vast group of molecules variously active against bacteria, enveloped viruses, protozoa and fungi. They share some common features, such as usually being cationic, although some anionic AMPs have been described [24], with zero net charge and amphipathicity, but are otherwise highly diversified from a structural point of view. Cationic AMPs can be clustered into four main classes [25]: (i) linear helical peptides, (ii) peptides enriched with one amino acid, (iii) peptides with one disulphide bridges, and (iv) peptides with two or more disulphide bridges.

Most activities of AMPs are in the macromolecular range (see the review by Rivas et al. [34]). It has been proposed that AMPs achieve their bactericidal effect in a number of different ways [35] but all data indicate that they act predominantly by disrupting the integrity of cell membranes through interaction with the phospholipid component (see Refs. [31,35–37] and references therein) (Fig. 2). Membrane disruption can occur by a number of different mechanisms. The main models advanced for plasma membrane permeation range from a canonical trans-membrane pore (barrel-stave) to solubilization of the membrane by a detergent-like action, based on the amphipathic character of the AMPs and their massive accumulation into the membrane (carpet-like model) [35–39]. Other lesser known models such as the molecular electroporation or the sinking raft model may also prove to be important to explain the membrane biocidal mechanisms of AMPs [35]. However, not all AMPs seem to exert their action on membranes: an increasing number of peptides have been described as acting on intracellular targets in bacteria inhibiting protein or cell-wall synthesis or interactions with DNA or RNAr [24]. The barrel-stave model of plasma membrane permeation predicts membrane permeation at very low peptide/phospholipid ratios, assuming that peptide–peptide interaction is stronger than the peptide–phospholipid one. This model entails a poor selectivity of pore formation with respect to membrane composition [34]. Moreover, if a strict peptide stoichiometry is required for pore formation, an upper size limit for the leakiness of molecules is imposed, at least in the first steps of the mechanism prior to osmotic lysis [34]. In contrast, the carpet-like model involves massive accumulation of cationic AMPs in the plane interfacial region of the outer leaflet of the membrane where monomer–monomer electrostatic repulsion is quenched by the anionic phospholipids. Once a threshold accumulation is reached, the membrane is solubilized [40].

AMPs present several theoretical advantages but also disadvantages as anti-infective drugs [41]. Advantages include a broad-spectrum activity, a rapid onset of killing, a low level of induced resistance and concomitant broad anti-inflammatory activities. However, the potential local toxicity, the susceptibility to proteolysis, the pH sensitivity, the sensitization and allergy after repeated applications and the cost of synthesis constitute the main disadvantages. Thus, only few AMPs have currently proceeded into clinical trials [42–44] and, to date, none of the described peptides has obtained US Food and Drug Administration (FDA) approval for clinical indications.

An interesting illustration of natural antibacterial coatings based on AMPs is provided by amphibians and fishes which secrete a dermal chemical defense slime, incorporating various antimicrobial peptides to prevent the colonization of their skin by micro-organisms [22,45]. Following the ingenuity of this strategy, different methods based on physical or chemical immobilization of peptide molecules have been explored to tether AMPs on solid

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