Chapter

Formation, Antibiotic Resistance, and Control Strategies of *Staphylococcus epidermidis* Biofilm

Wei Chen, Ting-Ting Xie and Hong Zeng

Abstract

Staphylococcus epidermidis, member of the group of coagulase-negative staphylococci, belongs to an opportunistic pathogen. It is reported that the major pathogenicity of *S. epidermidis* is attributed to its biofilm formed on the surface of infected tissues, which enhances bacterial resistance to antibiotics. Thus, how to inhibit biofilm formation and screening biofilm inhibitors will have great value in reducing bacterial drug-resistance, which is beneficial to prevent and treat biofilm-associated infections. In this chapter, we present the current knowledge on formation, antibiotic resistance, and control strategies of *S. epidermidis* biofilm. First, biofilm formation in *S. epidermidis*, including factors involved in different phases in the process of biofilm, is analyzed. Second, the mechanisms of antibiotic resistance in *S. epidermidis* biofilms, such as poor antibiotic penetration, slow growth, and formation of persister cells, are introduced. Finally, control strategies to *S. epidermidis* biofilm formation are provided.

Keywords: *Staphylococcus epidermidis*, biofilm, antibiotic resistance, biofilm inhibition

1. Introduction

Staphylococcus epidermidis is a commensal inhabitant of human and animal skin that rarely causes disease in healthy persons and animals. In recent years, however, *S. epidermidis* has been the most prevalent species isolated from device-associated infections [1]. The ability of biofilm formation by *S. epidermidis* is an important reason that investigators pay more attention to this emerging pathogen in recent years. It is reported that the major pathogenicity of *S. epidermidis* is attributed to its biofilm formed on the surface of infected tissues, which enhances bacterial resistance to antibiotics [2]. Biofilm formation by *S. epidermidis* involves two major steps. After finishing initial attachment, bacteria accumulate and form a multilayered architecture [3]. Bacteria develop biofilm by producing high-viscosity extracellular matrices including polysaccharides (EPS), proteins, and DNA (eDNA).

There is an increasing amount of biofilm research aimed at exploring how bacteria control their biofilm formation and to discover nontoxic compounds that can attenuate biofilm formation without allowing bacteria to develop drug resistance [4]. Special plants and Actinomycetes are both rich sources of bioactive substances, notably antibiotics, enzymes, enzyme inhibitors, and pharmacologically active agents [4, 5]. Moreover, some Actinomycete species were reported to produce inhibitors against biofilm formation by *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [6–9].

With this background, we aim to present the current knowledge on biofilm formation of *S. epidermidis* and review the control strategies to biofilm.

2. Biofilm formation in *S. epidermidis*

S. epidermidis infections are regarded as prototypic biofilm infections. The process of biofilm formation by *S. epidermidis* is periodically dynamic. Also, surface adhesion between planktonic bacterial cells is a key for biofilm formation. Once several cells succeed in adhering on a surface, named initial attachment of cells, surface motility and binary division result in an aggregation of attached cells. These primary cell aggregates produce exopolymers, including exopolysaccharides and extracellular proteins, which form extracellular matrix. Some of those factors may also originate from lysed cells, such as extracellular DNA (eDNA) [10]. Subsequently, there is development of a multicellular, multilayered biofilm architecture. In the later phase of biofilm formation, biofilm cells and clusters can detach. This detachment process is of key importance for the dissemination of biofilm-associated infection [10].

2.1 Factors involved in primary attachment in *S. epidermidis* biofilm formation

Nonspecific adhesions between bacterial cells, which are mainly attributed to the composition of compounds on the surface of bacterial cells and their hydrophobicities, play an important role in biofilm formation. Additionally, autolysin (AtlE) and teichoic acids have influences on biofilm formation [11, 12]. It is reported that lots of autolysin enhanced the cell surface hydrophobicity and increased the biofilm formation. Also, teichoic acids correlated with increased cell surface hydrophobicity, so they contributed to biofilm formation [11, 12].

In vivo primary attachment occurs to host tissue or host matrix proteins. *S. epidermidis* produces a variety of surface proteins binding host proteins in a specific manner. Bacterial surface proteins with such capacities have been termed microbial components recognizing adhesive matrix molecules (MCRAMM) [13]. The C-terminus of such bacterial surface proteins consists of an LPxTG (Leu-Pro-x-Thr-Gly) motif containing Gram-positive cell wall anchor, which covalently links to the cell wall [1]. According to genomic analyses, *S. epidermidis* has at least 14 MCRAMMs with an LPxTG motif. Many of those belong to the serine-aspartate (SD)-repeat-containing protein family (called Sdr). The SD-repeat region spans the cell wall and extends the ligand-binding region from the surface of the bacteria [14]. Adequate SD repeats within proteins are essential for outstanding from bacterial cell surface, which are covalently anchored to the peptidoglycan of Gram-positive bacteria.

The SD repeat family protein Sdr G in *S. epidermidis,* which is very similar to a fibrinogen-binding protein (Fbe), is necessary and sufficient for binding to fibrinogen-coated material. SdrG knock-out mutant showed less adhesion on fibrinogen-coated surfaces. It is reported that in vivo anti-SdrG antibody decreased the numbers of *S. epidermidis* cells adherent to biomaterials [14]. One of Sdr proteins, SdrF, mediates binding to type I collagen via one or both α1 chains, named collagen-binding protein [15].

Some of surface proteins on bacterial cell wall are adherent to host cells via non-covalent interaction, such as hydrophobic bonds and Van der Waals' force, which of process are involved into the polymers on bacterial cell surface, e.g., teichoic acids. Teichoic acids are main components consisting of the cell wall of Gram-positive. They bind to peptidoglycan of cell wall and influence the activity of autolysin (AtlE). AtlE, encoded by the atlE gene, is a bifunctional autolysin: one is able to mediate bacterial adhesion, and the other is to promote bacterial cell autolysis, which releases DNA out of cells, named extracellular DNA (eDNA) [16].

2.2 Factors responsible for cellular aggregation in *S. epidermidis* biofilm formation

Following the primary attachment of cells to a surface, bacterial cells occur to accumulate with the help of a variety of associated-accumulation factors, such as polysaccharide intercellular adhesin (PIA), accumulation-associated protein (Aap), and so on.

In the process of biofilm formation by *S. epidermidis*, PIA plays an important role in cell aggregation. Studies with *S. epidermidis* mutant revealed that the accumulation-defective mutants were unable to form a biofilm as they were unable to display intercellular aggregation or to produce PIA [17]. Further characterization of this *S. epidermidis* mutant showed that a deletion of icaR gene was found to upregulate PIA expression, providing evidence that this gene negatively regulates the PIA expression [17]. However, it is reported that there is no ica operon in some of clinical *S. epidermidis* strains, which have capacity of biofilm formation, named ica or PIA-independent type. In these strains, the accumulation-associated protein (Aap) is a major factor contributing to exopolysaccharide-independent biofilms of *S. epidermidis* [1]. Aap protein promotes cell-cell adhesion via a Zn^{2+}-dependent mechanism [18]. It is reported that 90% of isolated *S. epidermidis* strains contain aap gene, which is implicated in both PIA-dependent and PIA-independent biofilm formations of *S. epidermidis* [18]. *S. epidermidis* ATCC 35984 is a ica^{+} strain and a biofilm former, whose biofilm formation mainly depends on PIA consisting of reducing polysaccharides in which dihydroxyl groups are unsubstituted. However, exopolysaccharides in ica^{-} *S. epidermidis* mainly consist of nonreducing polysaccharides [19].

2.3 Biofilm formation and maturation

Cellular aggregation constantly occurs and subsequently forms biofilm. Disruptive molecules create channels in the biofilm, which are essential for nutrient accessibility in deeper biofilm layers and give the biofilm its characteristic structure, often described as mushroom-like shapes [10]. The characteristic structure of mature biofilms with mushroom-like shapes and channels is dependent on the production of phenolsoluble modulins (PSMs) in *S. epidermidis*.

Of primary importance for dissemination of biofilm-associated infection, cells or cell aggregates may detach from a mature biofilm to reach the next infection sites. This may occur by mechanical forces under flow, such as present in a blood vessel, in a process often called sloughing [10]. Additionally, the bacteria can trigger detachment by PSM production. These surfactant-like molecules work by decreasing noncovalent adhesion between bacterial cells.

3. Mechanisms of antibiotic resistance in *S. epidermidis* biofilms

Several in vitro studies have demonstrated that bacteria within biofilms are more resistant against antibiotic treatment as compared to planktonic cultures of the same strains [20].

S. epidermidis and other bacterial species produce an extracellular matrix called glycocalyx or slime, which is a highly hydrated complex composed of teichoic acids, proteins, and exopolysaccharides. In biofilms, poor antibiotic penetration, nutrient limitation and slow growth, and formation of persister cells are hypothesized to be responsible for drug resistance.

3.1 Antibiotic penetration of biofilms

Biofilms are typically characterized by dense, highly hydrated clusters of bacterial cells enclosed in a self-produced polymeric matrix that is primarily composed of exopolysaccharides such as polysaccharide intercellular adhesin (PIA) in staphylococci and adherent to a surface. This matrix, also termed slime or extracellular polymeric substance (EPS), impairs the access of antimicrobial agents to the bacterial cells [21]. Additionally, either a reaction of EPS with or its adsorption to the components of the biofilm matrix can delay penetration of the antibiotics through the biofilm matrix. The effective diffusion coefficients of solutes in biofilms average about 40% of the respective diffusion coefficient in pure water [20]. *S. epidermidis* slime has been found to remarkably decrease the activity of the glycopeptides vancomycin and teicoplanin. The efficacy of cloxacillin, amoxicillin/clavulanic acid, imipenem, cefpirome, erythromycin, roxithromycin, clindamycin, fusidic acid, trimethoprim/sulfamethoxazole, doxycycline, gentamicin, tobramycin, netilmicin, amikacin, isepamicin, ofloxacin, ciprofloxacin, and daptomycin is also moderately affected by the exopolysaccharide matrix of *S. epidermidis*. Other studies have suggested that *S. epidermidis* glycocalyx reduces susceptibility to pefloxacin and moderately affects the activity of daptomycin, linezolid, and quinupristin/dalfopristin [22, 23]. The role of biofilm matrix in retarding the penetration of antibiotics is thereby contributed to the drug resistance of *S. epidermidis* biofilms.

3.2 Slow cell growth in biofilms

Slow cell growth of the bacterial has been found in mature biofilms [17]. This phenomenon is responsible for the decreased susceptibility of bacteria in biofilms to antibiotics requiring growing organisms for their bactericidal effects. For example, penicillins and cephalosporins prefer to killing the growing bacterial cells, and the rate of killing cells is proportional to the growth rate [17]. It is well known that most antimicrobial agents act on certain types of macromolecular synthesis to exert antimicrobial activities, such as the synthesis of enzymes, proteins, and nucleic acids (DNA or RNA). Thus, these antibiotics have little effects on bacteria with stagnant macromolecular synthesis, which leads to bacterial drug resistance.

Nutrition restriction is one of reasons that are responsible for slow cell growth. The mechanism of nutrition restriction is closely related to the osmotic restriction. Due to the existence of biofilm osmotic restriction, nutrients are not easy to pass through biofilm, which leads to the lack of nutrition in biofilm and slows down the growth rate of inner layer bacteria. This slow growth state of inner layer bacteria also forms a protective mechanism, which reduces the susceptibility of bacteria to antibiotics [24].

When the biofilm cells are exposed to antibiotics, the bacteria on the surface of the biofilm are killed by the drug, and the cells in the middle and deep layers of the biofilm are not affected. After the antibiotic treatment stops, the remaining bacteria will use dead bacteria as nutrients to reproduce rapidly, which can only take a few hours to reproduce [25, 26].

3.3 Formation of persister cells

Delayed penetration of the antibiotics through the biofilm matrix and slow rate of bacterial reproduction in biofilm cannot explain entirely the resistance of biofilms to one important class of antibiotics, namely the fluoroquinolones. This class of antimicrobial agents equilibrates across bacterial biofilms and exerts bactericidal effect on nondividing cells [17]. Although a dose-dependent bactericidal action was observed in *P. aeruginosa* biofilms by the fluoroquinolones ofloxacin and ciprofloxacin, a further increase in the antibiotic concentration or a prolonged drug action period did not improve killing rates after an initial 3- to 4-log drop bacterial counts. This result suggested that a small portion of "persister" cells occurs after administration of fluoroquinolones [17, 27, 28]. The most significant difference between persisters and mutant resistant strains is that the drug resistance of persisters is only a phenotypic variation without gene mutation, so this phenotype is not genetic. These strains were collected, recultured, and detected the drug resistance. It was intriguing that the drug resistance disappeared, and the minimum inhibitory concentrations (MICs) were the same level as those of parent strains. Meanwhile, the resistant strains caused by mutation showed a stable genetic drug resistance, and MICs were higher than those of parent strains [28].

Persister cells in biofilms are considered to the key in the extraordinary survival properties of biofilms. The dynamic features of biofilm formation and shedding of cells from one biofilm to form a new biofilm may also explain the chronic nature of biofilm infections and the need for extending antimicrobial agent treatment to disturb the dynamics of biofilm formation [17].

4. Control strategies to *S. epidermidis* biofilm formation

Because the expression of toxins and other virulence factors is less in *S. epidermidis*, the biofilm forming capacity is its major virulence factor. Biofilm growth is characterized by high resistance to antimicrobial agents and host immune responses, making biofilm eradication tremendously difficult. The increasing prevalence of multidrug-resistant *S. epidermidis* strains additionally hampers antimicrobial therapy. Therefore, targeting factors expressed at different phases in biofilm formation might offer new tools to combat *S. epidermidis* infections.

4.1 Inhibition of initial attachment

The first step of biofilm formation is bacterial adherence to the host cell surface. Direct binding to host cell surface is mediated by electrostatic and hydrophobic interactions and van der Waals forces and affected by physicochemical variables [29].

Found in our research, after investigating the antibiofilm activities of spent media from 185 Actinomycete strains using two *S. epidermidis* strains (ATCC 35984 and a clinical strain 5-121-2) as target bacteria, three strains of tested Actinomycete (TRM 46200, TRM 41337, and TRM 46814) showed a significant inhibition against *S. epidermidis* biofilm formation without affecting the growth of planktonic cells. Effect of Actinomycete supernatants on cell surface hydrophobicity (CSH) of *S. epidermidis* was measured by Microbial Adhesion to Hydrocarbon (MATH) assay. The adhesion of staphylococci to n-hexadecane was used to measure the hydrophobicity of *S. epidermidis*. All the crude proteins from spent media showed a reduction in the CSH against *S. epidermidis* ATCC 35984 and 5-121-2, which explain at least in part the inhibitory effect of Actinomycete supernatants on biofilm reduction [19].

Moreover, apart from physico-chemical determinants, it was demonstrated that the major autolysine AtlE is involved in attachment to polystyrene surfaces. Therefore, AtlE may be indirectly involved in cell adhesion via releasing DNA. Treatment of *S. epidermidis* cells with DNaseI was found to inhibit biofilm formation at an early time point, suggesting that release of DNA also contributes to the attachment of *S. epidermidis* to artificial surfaces [30]. In our research, we performed the degradation of the crude proteins from spent media against *S. epidermidis* DNA. The crude protein from spent media of TRM 46200 showed a significant DNA-degradation activity. Importantly, the crude protein from spent medium of TRM 41337 possessed the highest DNA-degradation activity as that of the positive control, 10 μg/ml of DNaseI [19].

S. epidermidis foreign-associated infections occurring early are thought to involve direct interactions of the bacterial surface with host extracellular matrix (ECM). Specific binding to surface ECM proteins involves cell wall-associated adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) [31, 32]. The recent studies have shown that antibodies against cell surface components of *S. epidermidis* can affect the rate of biofilm formation or adherence of these bacteria to medical devices in vitro. Using polyclonal antibodies against a fibrinogen-binding protein from *S. epidermidis* (Fbe) could block adherence of *S. epidermidis* to fibrinogen-coated catheters in vitro [33, 34]. Consequently, all these surface-located components are good candidates for vaccine development aiming at the inhibition of the initial attachment step of biofilm formation.

4.2 Inhibition of bacterial accumulate

After adherence to the host cell surface, biofilms develop through intercellular aggregation. The major factor involved in intercellular adhesion is polysaccharide intercellular adhesin (PIA). The de-acetylation of PIA is not only essential for biofilm formation but also crucial for *S. epidermidis* virulence [29]. Hence, PIA was one of the first targets evaluated in view of biofilm-inhibiting *S. epidermidis* vaccine development. Pier and coworkers have significantly contributed to the evaluation of PIA as vaccine target. Following the evidence, high-molecular-weight PIA could elicit an antibody response accompanied by opsonophagocytic killing of the PIA-dependent biofilm-forming *S. epidermidis* M187 and three *S. aureus* strains. The PIA-specific antibodies can prevent biofilm formation or retard already initiated biofilm development [35].

PIA biosynthesis depends on the expression of the icaADBC operon, which is controlled by a complex regulatory network. Gomes et al. studied the effect of rifampicin+gentamicin and rifampicin+clindamycin combinations on the expression of icaA and rsbU genes, responsible for poly-N-acetylglucosamine/polysaccharide intercellular adhesin (PNAG/PIA) production. The results demonstrated that this combinatorial therapy can cause a lower genetic expression of the two specific genes tested and consequently can reduce biofilm formation recidivism [36, 37].

Nevertheless, *S. epidermidis* strains lacking icaADBC but still producing biofilm were isolated, indicating the existence of an ica-independent mechanism of cell accumulation. A proteinaceous intercellular adhesin involved in cell accumulation during biofilm formation was discovered. The accumulation-associated protein (Aap) can functionally substitute PIA as an intercellular adhesin, and there is good evidence that additional proteinaceous intercellular adhesins must exist. They showed that monoclonal antibodies against Aap can significantly reduce the accumulation but not initiation phase of *S. epidermidis* biofilm formation in vitro [38].

Biofilm formation is a result of bacterial interactions and group behavior. Quorum sensing (QS) is one of the regulatory mechanisms suggested to be involved in coordinating biofilm formation. The QS system is a cell-to-cell communication system used by many bacteria to assess the cell density. Quorum sensing inhibitors (QSI) could be a novel way to fight biofilm-associated infections. The study has identified furanones and thiophenones as inhibitors of quorum sensing and biofilm formation. In this study, the effect of both the furanone and the thiophenone could be abolished by the synthetic Autoinducer-2 (AI-2) molecule (S)-4,5-dihydroxy-2,3-pentanedione (DPD), indicating that furanone and thiophenone affect biofilm formation through interference with bacterial communication [39].

4.3 Promotion of biofilm detachment

For the biofilm that has been formed on the surface of the host, if the biofilm can be separated by antibacterial oranti-biofilm substances, the bacteria in the biofilm can be released, and the planktonic bacteria are more easily to be killed if the biofilm is exposed to antibiotics. Biofilms are composed primarily of microbial cells and extracellular polymeric substance (EPS). EPS may account for 50–90% of the total organic carbon of biofilms and can be considered the primary matrix material of the biofilm. The components of EPS include polysaccharides, nucleic acids, lipids, and proteins [36].

We initially determined the dependent type of biofilm formation by *S. epidermidis* ATCC 35984 and 5-121-2. The biofilm formation by *S. epidermidis* ATCC 35984 mainly depends on EPS consisting of reducing polysaccharides in which dihydroxyl groups are unsubstituted. Thus, sodium-meta-periodate, which specifically destroys sugars containing unsubstituted dihydroxyl groups, significantly decreased biofilm formation in *S. epidermidis* ATCC 35984. However, not only EPS but also proteins, eDNA, are responsible for the biofilm formation of *S. epidermidis* 5-121-2. Moreover, EPS in *S. epidermidis* 5-121-2, which mainly consists of nonreducing polysaccharides, is distinct with those in *S. epidermidis* ATCC 35984. Thus, three enzymes specific to nonreducing glycosides, amylase, β-glucanase, and β-glucosidase, worked effectively in the degradation of EPS, resulting in biofilm reduction in *S. epidermidis* 5-121-2 [19].

Since extracellular polysaccharides are the main compounds in biofilm matrices, namely in *S. epidermidis*, antimicrobial substances able to disrupt or inhibit EPS are of major interest. N-acetylcysteine (NAC) is an amino acid with strong antioxidant, antimucolytic, and antibacterial properties. As observed by researchers, NAC decreased biofilm formation and reduced the formation of extracellular polysaccharide matrix while promoting the disruption of mature biofilm. NAC has demonstrated not only to reduce adhesion but also to detach bacterial cells adhered to surfaces and to inhibit bacterial growth in vitro. The possible action of NAC in the biofilm matrix can result in the release of cells either individually or in cell clusters, becoming the biofilm and loose cells more exposed and susceptible to the host immune system and to other antimicrobial agents [40]. Kaplan et al. found an enzyme called dispersin B, which can promote biofilm detachment from *Actinobacillus actinomycetemconitans*, which rapidly and effectively removes biofilms formed by *S. epidermidis* on the host surface. Dispersin B is a β-1,6-N-Acetylglucosaminidase that causes *S. epidermidis* to detach from the biofilm matrix by degrading PIA [41].

Our results showed that EPS in *S. epidermidis* ATCC 35984 and 5-121-2 was degraded by crude proteins from three Actinomycete strains (TRM 41337, TRM 46200, and TRM 46814) supernatants. Specifically, for the strain ATCC 35984 when treated with crude proteins from spent medium of the strain TRM 41337, arabinose (Ara) was absent in the monosaccharide composition compared with the control. Furthermore, the proportion of mannose (Man) was decreased, while the proportions of glucosamine (GluN), galactosamine (GalN), and galactose (Gal) were increased. When treated with crude proteins from spent medium of the strain TRM 46814, three new monosaccharides, rhamnose (Rha), glucuronic acid (GluA), and galacturonic acid (GalA), appeared. Additionally, the proportions of Man and glucose (Glu) decreased obviously. For the strain 5-121-2, when treated with crude proteins from spent media of TRM 41337 and TRM 46814, a new monosaccharide, Rha, was present [19].

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Conflict of interest

No conflict of interest declared.

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