

# Putative Targets as Vaccine Candidates with Respect to Biofilm Formation Procedure in Staphylococci

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## ABSTRACT

The amount of multidrug-resistant (MDR) strains, especially methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, as frequent causes of nosocomial and device-related infections have increased. Biofilm formation is an essential requisite in staphylococcal pathogenicity. It is considered as a bacterial surveillance, antibiotic resistance, and transition of antibiotic resistance genes factor. Therefore, biofilm-related macromolecules have been suggested as putative new vaccine candidates to combat staphylococcal infections. Based on the MEDLINE and Google scholar databases, some *Staphylococci* macromolecules are involved in the biofilm formation process and have been reviewed as putative vaccines. Based on experiments, common staphylococcus antigens could prevent the progress of the caused diseases by this genus. Moreover, considering related stages in biofilm formation, a multivalent putative vaccine (protein and polysaccharide) candidate could be enhancing the eradication chance of aforementioned bacterial families.

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## INTRODUCTION

Staphylococcaceae family is non-motile, non-spore-forming, catalase-negative bacteria that grow on most bacteriological culture media at both anaerobic and aerobic conditions. Based on the ability of bacteria to produce coagulase, a protein enzyme that facilitates the conversion of fibrinogen to fibrin, staphylococci are divided into coagulase-positive and -negative *Staphylococcus* (CoPS and CoNS, respectively [1]. Unlike the CoNS, the CoPS often have golden discoid colonies surrounded by a zone of  $\beta$ -hemolysin. Because of increasing cases of antibiotic-resistant patterns in the strains of staphylococci, there is a demand for an effective prophylactic vaccine against these bacteria. Staphylococcaceae family is considered as the etiological agents of several mild and intense disorders, such as sepsis and endocarditis [1]. Based on the preclinical models, various antigens can prevent the spread of staphylococcal diseases either alone or in a combination with other antigens. The development of a protective vaccine to cease the spread of sepsis is one of the most challenging issues in pharmacology research [1].

*S. aureus* is a primary pathogen causing a wide range of diseases, such as mild skin and soft tissue infections, bacteremia, endocarditis, pneumonia, metastatic infections, sepsis and toxic shock syndrome in hospitalized patients. The reason for this wide range of symptoms might be related to

undiscovered factors that make the host susceptible to colonization [2]. The contamination of medical devices with *S. aureus* inserted into the patient's body might be remarkably dependent on the patients' health. There are similarities in the developed infections caused by staphylococci biofilm and usually highly intensive care is needed in such cases. Infections caused by *S. epidermidis* are more difficult to treat by antibiotic therapy in comparison with *S. aureus* [3]. Moreover, medical devices act as a spreading source of several bacterial infections to different parts of the human body. Over the past decades, there has been an increase in the nosocomial infections caused by staphylococcus species, especially *S. aureus* [4, 5]. Since the 1960s, the first methicillin-resistant *S. aureus* (MRSA) strains were detected which have remained a major global challenge [6]. Thus from the molecular pathogenesis perspective, it is essential to know the relevant factors involved in such biofilm formations and to discover their physiological status within the body.

*S. epidermidis* is an inhabitant of human skin. For a long time, it was only considered as a contaminant when cultured from blood or tissue samples [7, 8]. Since *S. epidermidis* is a part of normal skin flora, it probably initiates contamination after implantation of a medical device. In recent years, *S. epidermidis* has been accepted as a leading cause of nosocomial

bloodstream infections, especially in patients with prosthetic medical devices [9, 8]. *S. epidermidis* is an opportunistic pathogen, principally known as the cause of infection in immunocompromised patients [10]. Biofilm formation of *S. epidermidis* is a critical factor in the pathogenesis because it can be colonized on medical devices which makes it resistant to multiple antibiotics and host defenses. There is an essential need to remove or replace the biofilm contaminated medical implants. Moreover, studies are needed to be done to provide new and effective vaccines against staphylococcal biofilm formation [11].

CoNS which inhabit on a person's skin include *S. hominis*, *S. epidermidis*, *S. saprophyticus*, *S. warneri*, *S. cohnii*, *S. saccharolyticus*, *S. haemolyticus*, *S. capitis* and *S. lugdenensis*. They are normally harmless to their host. Most staphylococci including *S. epidermidis* in the cases that the skin is injured might be pathogenic. CoNS colonization seems to be relevant to the specific sites of the infection and its abundance. For instance, *S. saprophyticus* which is a common inhabitant of inguinal and perineal areas, is an etiological agent of urinary tract infections [12].

Biofilm formation and consistency of host immune evasion of *S. epidermidis* and *S. aureus* make them the main concern of the nosocomial infections in hospitals [13, 14]. Despite being a part of human flora, the ability to adhere to the medical device surfaces and developing multilayered structures, known as "biofilm", makes them problematic [15]. Biofilm is defined as a community of cells encased within an exopolymeric matrix and attached to a surface. It has been proved that biofilms are resistant to antimicrobial therapy and host defense [13].

Many studies have demonstrated that biofilm developed in a 2-step physiologically process; primary adherence of the cells to the site and the maturation of the biofilm. Phase-specific factors are needed for each of these steps. In general, there is no agreement about different steps of biofilm formation in staphylococci. We review here three main stages, namely attachment, maturation/aggregation and detachment [15].

## 1- Attachment

The first stage of biofilm formation is attachment. That is, bacteria attach to their host cell membrane by bacterial appendages which are cell-surface components that facilitate adhesion to other cells. Matrix proteins play a critical role in both adherence and the evasion of the host immune system. This makes matrix proteins as important virulence factors in Staphylococci. The Gram-positive bacterial proteins are divided into two families; microbial surface components, recognizing adhesive matrix molecules (MSCRAMMs), and serine-rich repeat proteins (SRRPs) [16].

One of the most important factors of colonization is the interaction between the matrix proteins of the host and MSCRAMMs. A set of MSCRAMMs with a capacity to link to protein matrix in humans, such as fibrinogen, fibronectin, and several matrix proteins are synthesized by *S. epidermidis* and *S. aureus* [17]. The common structure of MSCRAMMs consists of an exposed ligand-binding domain, a membrane-spanning domain (mostly with a repeated structure) and a domain responsible for the covalent and non-covalent attachment to the bacterial surface. Sortases are a family of prokaryotic enzymes that catalyze the covalent attachment of the MSCRAMMs LPXTG (Leu-Pro-any-Thr-Gly) motif, which is split between the threonine and glycine residue [18]. Sortases anchor up to 21 and 12 different LPXTG proteins to the cell wall in *S. aureus* and *S. epidermidis*, respectively [19, 20].

MSCRAMMs can mediate indirect binding to host-plasma-covered surfaces with fibronectin (Fn), collagen (Cn) and fibrinogen (Fg) as matrix proteins. Cell surfaces are covered with a different macromolecules, such as proteins including Embp, GehD, SdrG, SdrF, AtlE and Aae autolysins as well as polysaccharides (i.e. cell wall teichoic acid (TA) and polysaccharide intercellular adhesion; PIA) and matrix-binding determinants [21, 22]. Serine-aspartate repeat (Sdr) protein family members are categorized into two distinct species; however, their function is the same [23]. Both species use autolysin AaP proteins to form their noncovalent bonds, maintaining the three-dimensional structures of the macromolecules [24]. Autolysins are the most frequent proteins on staphylococcal cell surfaces, non-covalently linked to teichoic acid [25]. These enzymes have a considerable role in the rate of cell wall- turnover and are critically important for the bacterial attachment. Moreover, they facilitate the attachment on plastic surfaces and harbor binding sites for human matrix proteins [26]. The GehD lipase plays a more important catalytic role than the autolysins and it has an additional adhesive function [27]. Given attachment is the first step of biofilm formation, any of the surface-located macromolecules could be considered as a putative vaccine candidate [7].

## 2- Maturation/Aggregation

The maturation phase has two main characteristics in the biofilm formation; A) intercellular aggregation by a wide range of molecules including sticky macromolecules; B) formation of the three-dimensional structure of mature biofilm.

### Adhesive Forces

Poly-N-acetylglucosamine (PNAG) is the most important PIA because its chemical composition is the most responsible molecule for adhesion in the Staphylococcal aggregation [28]. The extracellular matrix of staphylococcal biofilm is often called "slime" which is consisted of several polymers including PIA, proteins and teichoic acids. The core polymer of PIA has a  $\beta$ -1, 6-linked N-acetylglucosamine structure [29]. Homologs of PIA have been recently found in different biofilms of pathogens, which suggest its broad function in biofilm formation and biofilm-associated infections. PIA biosynthesis depends on the expression of the *icaADBC* operon. The expression of *icaADBC* is regulated by an array of environmental factors and regulatory proteins [30, 31]. The Intercellular Adhesion (*ica*) locus contains an N-acetylglucosamine (GlcNAc), a PIA deacetylase (*icaB*), a putative PIA exporter (*icaC*) and a regulatory gene (*icaR*) [32, 33]. Some strains without the *ica* genes have been isolated from biofilm-associated infection which suggests that PIA is not generally essential for biofilm formation in staphylococci [34, 35]. The proteinaceous intercellular adhesion is involved in cell accumulation of those strains that do not produce PIA polymer [7]. Accumulation-associated protein (Aap), is the most important protein involved in PIA-independent biofilm formation and contains various domains including domain A, linked to corneocytes, making it of great importance for skin colonization [36]. To induce biofilm formation, Aap interacts with PIA, and then a 220 kDa Aap protein needs to be proteolytically broken down to a smaller 140 kDa form [37, 38]. The function of the staphylococcal surface proteins, SSP-1 and SSP-2, might be similar to Aap role in terms of biofilm production [39]. *S. epidermidis* surface (Ses) proteins have been proven to be formed by SSPs; therefore, providing cell-cell adhesion over longer distances which explains how these

proteins contribute to the aggregation step of the biofilm development.

Considering the PIA-independent biofilm formation, other involved proteins are biofilm-associated proteins (Bap) and biofilm-associated homolog proteins (Bhp) [7]. Bap family might be essential in biofilm production because of the presence of Bap homologs in other bacteria and the vital role of this large protein in *S. epidermidis* derived from mastitis [40, 41]. Recent studies have identified that extracellular matrix binding protein (Embp) and fibronectin-binding MSCRAMM facilitate biofilm formation as a proteinaceous intercellular adhesive [42]. Many Gram-positive bacteria have TA polymers, such as *S. aureus* and *S. epidermidis*. There are two sorts of TA, namely cell wall-linked TA (WTA) and lipoteichoic acid (LTA) which is linked to the cell wall by a lipid anchor [43, 44]. TA has a polyanionic character and has been described as a stabilizing factor [7]. D-alanylation of TA in *S. aureus* is a vital factor in biofilm formation [45]. Moreover, a probable role for TA in *S. epidermidis* virulence could be its attachment to the fibronectin-coated surfaces [46].

### Biofilm Structure Disrupting Force

When the biofilm matures, a specific 3D structure is formed through the fluid-filled channels [47]. Based on the findings, modulin proteins as quorum-sensing (QS) mediators play a key role in the mechanisms leading to the channel formation and biofilm structures [7]. Phenol-soluble modulins (PSMs) are a class of surfactant-like peptides, mainly assigned as pro-inflammatory molecules in *S. epidermidis*. They are subdivided by an amphipathic alpha-helical structure into two classes: the shorter type is called  $\alpha$  type, which has a length of approximately 20 amino acids (PSM $\alpha$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$ ) and the longer type that is called  $\beta$  type with a length of approximately 40 amino acids (PSM $\beta$ s) [7]. Shifting  $\beta$ -type and PSMs in PSM expression have been observed when biofilm constructed. In other hand, the expression of PSMs likely constitutes a key factor contributing to the switch between an aggressive and a silent form of *S. epidermidis* physiology during the infection. Detachment of biofilms, dissemination of pathogen and the attraction of immune cells are related to PSMs expression. Whereas suppression of the production of PSMs in the biofilm stage enables the cells to stick together and to evade the host immune defense. [48]. The development of biofilm in *S. epidermidis* is directly related to the down- and up-regulation of PSM expression [7]. At a lower concentration, the PSM $\beta$ s might form "holes" in an early biofilm and lead to the formation of spaces and channels in the biofilm structures [7, 49].

### 3- Detachment

Disperse of bacteria to connect to another colonization site during the establishment of mature biofilm in staphylococci is known as detachment. It may happen by either detachment of single cells or larger cell aggregates. Cell dispersal not only leads to embolism, sepsis and hospital-acquired pneumonia it also leads to biofilm formation at other sites [50]. In staphylococci, agr QS system controls factors that will change the biofilm surface when the rate of associated factors is relatively high [51]. The increase of PSM $\beta$  leads to cluster detachment of the biofilm. As long as the biofilm matures, it results in a systemic spread of its fragments [49]. It has also been suggested that PSM $\gamma$  (identical to  $\delta$ -toxin) acts as a cell-cell disruptive factor [52].

#### The Biofilm-Based Putative Vaccine Candidates

Biofilm formation is a clinical challenge. It increases the antibiotic resistance patterns and bacterial evasion from the host defense [14]. Biofilm formation has great importance in a wide range of infections and has been accepted as a bacterial mode of growth. According to the National Institutes of Health (NIH), approximately 80% of human biofilm-related infections are common [13]. Medical device-associated infections caused by biofilm formation of *S. epidermidis* and *S. aureus* have led to challenging and complicated medical processes. The emergence of antibiotic-resistant strains of staphylococci, mainly MRSA, emphasizes this matter [19].

So far, several bacterial surface-located components including serine-aspartate repeat protein G (SdrG), serine-aspartate repeat-protein F (sdrF), clumping factor A (ClfA), GehD lipase and extracellular matrix-binding protein (Embp) which are engaged in the initial phase of biofilm production as well as autolysin E (AtlE) have been evaluated as putative staphylococcal vaccine candidates [53- 56]. Furthermore, the MSCRAMMs/surface proteins have also been considered in this regard [53- 56].

In conclusion, vaccine development against staphylococcal infections is still in its infancy. As it was previously mentioned, biofilm has resistance against antibiotics and could escape from the host immune system. Recently, several studies have been accomplished based on the selection of antigens to eradicate the biofilm-related infections. General immunization along with using short-term medical implants such as venous catheters seems to be more cost-efficient than removing and replacing the contaminated devices. For permanent medical device users, removing the contaminated device might be risky because of the long hospitalization time and increase in healthcare costs. Thus, justifiable and cost-effective methods must be considered.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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