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 staphylococcal 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.

INTRODUCTION

Staphylococcaceae family is non-motile, non-sporo-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 β-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*, *saprophyticus*, *S. warneri*, *S. cohnii*, *S. saccharolyticus*, *S. haemolyticus*, *S. capitis* and *S. lugdunensis*. 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 fibrinogen (Fn), collagen (Cn) and fibrinogen (Fg) as matrix proteins. Cell surfaces are covered with a different macromolecules, such as proteins including Empb, GehD, SdrG, SdrF, AtfE 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 β-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 protelytically 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 SSSP; therefore, providing cell-cell adhesion over longer distances which explains how these
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 (Emph) which are engaged in the initial phase of biofilm production as well as autolysin E (AItE) 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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