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Colonization of Medical Devices by Staphylococci

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Summary

The use of medical devices in modern medicine is constantly increasing. Despite the multiple precautionary strategies that are being employed in hospitals, which include increased hygiene and sterilization measures, bacterial infections on these devices still happen frequently. Staphylococci are among the major causes of medical device infection. This is mostly due to the strong capacity of those bacteria to form device-associated biofilms, which provide resistance to chemical and physical treatments as well as attacks by the host's immune system. Biofilm development is a multi-step process with specific factors participating in each step. It is tightly regulated to provide a balance between biofilm expansion and detachment. Detachment from a biofilm on a medical device can lead to severe systemic infection, such as bacteremia and sepsis. While our understanding of staphylococcal biofilm formation has increased significantly and staphylococcal biofilm formation on medical devices is among the best understood biofilm-associated infections, the extensive effort put in pre-clinical studies with the goal to find novel therapies against staphylococcal device-associated infections has not yet resulted in efficient, applicable therapeutic options for that difficult-to-treat type of disease.

1. Introduction

As a result of the considerable advances made in modern medical technology, medical devices such as artificial implants, pacemakers, prosthetic joints, and catheters, play an increasingly important role in healthcare (Darouiche, 2001; Donlan, 2001; Zimmerli et al., 2004; Gorski, 2010; Califano et al., 2012; Crnich and Drinka, 2012; Gandhi et al., 2012; Nicolle, 2012). However, the lack of self-cleansing capacity makes medical devices vulnerable to contamination during the implantation process and everyday use. The organisms causing medical device infection may originate from the colonizing microbiota of patients or healthcare workers, or environmental sources (Gastmeier et al., 2005). Most device infections are healthcare-associated, because in hospitals or long-term care facilities, there is a high concentration of patients with infectious microorganisms, and hospitals are the places where surgical procedures involving devices are performed, providing a high-risk scenario for device contamination (Dudeck et al., 2015). While some medical instruments and devices can easily be cleaned by powerful decontamination and sterilization methods, such as chemical killing (using bleach and alcohol), irradiation, or steam sterilization, this is

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often not sufficient to prevent subsequent contamination and infection. As a result of their high frequency, increased resistance to antibiotic treatment (Weiner et al., 2016), and the frequent necessity to remove the medical device to cure the infection, device-associated infections represent a severe burden to the public health system. For example, infected central venous catheters (CVCs) alone cause about 80,000 cases of bloodstream infections in the United States annually (Gominet et al., 2017). In developing countries, the incidence of device-associated nosocomial infections is even higher (Rosenthal et al., 2016).

Bacteria that colonize medical devices usually aggregate and grow in the form of biofilms. Biofilm can be defined as a microbial community of cells that are attached to a substratum and embedded in a matrix of extracellular polymeric substances that they have produced (Donlan and Costerton, 2002). Cells in a biofilm characteristically exhibit a phenotype with respect to growth rate and gene transcription that is different from that during planktonic growth (Resch et al., 2005; Yao et al., 2005). The structure and physiological features of a biofilm contribute to the characteristic resistance of biofilm-forming bacteria to antimicrobial agents, such as antibiotics or disinfectants (Stewart and Costerton, 2001). Importantly, biofilms also provide a shield from the biological attacks that occur in the form of host defenses (Vuong et al., 2004; Otto, 2006).

Overall, the Gram-positive staphylococci are the leading causes of device-related infections (DRIs) (Darouiche, 2001). Among the staphylococci, *Staphylococcus aureus* is of most clinical concern. This is due to the fact that *S. aureus* infections are commonly more serious and aggressive than those caused by other staphylococci, due to the exceptionally large and diverse arsenal of aggressive toxins and virulence factors *S. aureus* isolates may produce (Lowy, 1998; Otto, 2014). Next to *S. aureus,* the less aggressive skin commensal *Staphylococcus epidermidis* has drawn most attention as a frequent cause of biofilm-associated infection on medical devices and associated complications, which include bloodstream infections (Otto, 2009; Rupp, 2014).

In this mini-review, we summarize the factors involved in staphylococcal biofilm development and its regulation. We discuss the molecular mechanisms of staphylococcal evasion from host defenses in the context of biofilm formation and discuss potential therapeutic strategies against staphylococcal biofilm-associated infections.

2. Prominent device-related infections (DRIs) caused by staphylococci

Medical devices particularly prone to infection include contact lenses, central venous catheters (CVCs), endotracheal tubes, intra-uterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, tympanostomy tubes, urinary catheters, and voice prostheses (Donlan, 2001). The most important staphylococcal DRIs in terms of frequency, attributed mortality, and involvement of staphylococci as compared to other infectious organisms will be discussed in the following.

Infections of mechanical heart valves stand out due to their high mortality (Darouiche, 2001). Both *S. epidermidis* and *S. aureus* form biofilms on mechanical heart valves and the surrounding cardiovascular tissues, in some cases resulting in serious diseases such as

prosthetic valve endocarditis (PVE) (Whitener et al., 1993; El-Ahdab et al., 2005; Murray, 2005). The infecting microorganisms are introduced predominantly during the surgery process and infection manifests within 12 months of valve insertion (Rupp, 2014).

Central venous catheters (CVCs) are used for delivering blood products, nutrient solutions, and medications, as well as facilitating dialysis treatment. Second only to urinary catheters, they are the most frequently used indwelling medical devices (Darouiche, 2001). *S. epidermidis* and *S. aureus* are the leading causative agents of CVC infections (Rupp, 2014). CVC infections are an important source of bloodstream infections (Maki et al., 2006), especially in neonates, where coagulase-negative staphylococci (CNS), such as S. epidermidis, are the predominant cause (Cheung and Otto, 2010). Without an associated bloodstream infection, CVC infections with *S. epidermidis* can present without major signs of inflammation, while the typical clinical characteristics of CVC infections (purulence, erythema, tenderness) are usually present with *S. aureus* (Eggimann and Pittet, 2002).

Urinary catheters are silicone or latex tubular devices utilized to collect urine during surgery, measure urine output, adjust urinary incontinence and avoid urine retention (Hessen and Kaye, 1994). Biofilms can readily develop on the inner or outer surfaces of urinary catheters upon insertion and it is difficult to prevent bacterial colonization merely through hygiene procedures (Trautner et al., 2005). The organisms initially isolated from these devices are mainly *S. epidermidis, Enterococcus faecalis, and Escherichia coli*, while during later stages other bacteria, such as *Proteus mirabilis*, are found (Stickler, 2008). The longer the use of urinary catheter, the greater the risk of a catheter-associated urinary tract infection. In fact, it has been estimated that the risk of infection for patients undergoing urinary catheterization increases by almost 10 % each day (Stickler, 2008).

Ventilator-associated pneumonia occurs in patients who use mechanical ventilation machines in hospitals. It can cause severe illness and death (Melsen et al., 2013) and is the second most common healthcare-associated infection in pediatric intensive care units (Foglia et al., 2007). The endotracheal tube represents one of the main paths of bacterial colonization during ventilator-associated pneumonia. Endotracheal tubes directly link the outside environment and the lungs, making them vulnerable to exogenous bacterial infection. In fact, biofilms can develop very fast - within one day - on endotracheal tubes (Bauer et al., 2002). At ~ 20% of cases, *S. aureus* is the pathogen most commonly associated with ventilator-associated pneumonia, next to *Pseudomonas aeruginosa* (Chastre and Fagon, 2002).

Infection on prosthetics represents another frequent type of device-associated infection. Prosthetic joint infection (PJI) can result, as a complication of total joint arthroplasty, from hematogenous seeding or, more often, contamination during surgery. *S. aureus* is the major cause of PJI, with CNS reaching approximately equal infection rates (Zimmerli et al., 2004). Biofilms develop in the synovial fluid present in joints, often without surface connection as large, macroscopically visible “floating biofilms”. The specific physiological situation in synovial fluid facilitates the formation of those exceptionally large aggregates, which are extremely recalcitrant to antibiotic treatment (Dastgheyb et al., 2015a; Dastgheyb et al., 2015c; Dastgheyb et al., 2015b).

3. Staphylococcal Biofilm Development

As in other bacteria (O'Toole et al., 2000), biofilm development in staphylococci involves three main stages: (i) initial attachment, (ii) accumulation and maturation, and (iii) detachment (also called dispersal). In all three stages, characteristic proteinaceous and non-proteinaceous factors are expressed, and their expression is tightly controlled (Fig. 1).

Staphylococcal biofilm formation on inserted medical devices begins with the attachment of bacterial cells to human matrix proteins, such as collagen, fibronectin and fibrinogen, which rapidly cover the devices after insertion. However, direct attachment to the abiotic surface is also possible. A variety of factors contribute to this critical process. The MSCRAMM (microbial surface components recognizing adhesive matrix molecules) protein family is the most prevalent group of surface proteins that non-covalently bind to human extracellular matrix proteins (Clarke and Foster, 2006). MSCRAMMs have a conserved overall structure, which includes, from N terminus to C terminus, a signal peptide, a ligand-binding domain, which contains repeat sequences, a cell wall-anchoring region, a membrane-spanning region, and a positively charged tail (Foster et al., 2014). MSCRAMMs are secreted by the Sec pathway. After secretion, the enzyme sortase recognizes a conserved LPXTG motif in the cell wall-anchoring domain and mediates the covalent attachment of the MSCRAMM to peptidoglycan (Mazmanian et al., 1999). Gill et al. identified more than 20 MSCRAMM genes in the *S. aureus* genome and 12 MSCRAMM genes in the *S. epidermidis* RP62A genome (Gill et al., 2005). Some examples of well-studied staphylococcal MSCRAMMs are fibronectin-binding proteins (FnBPA and FnBPB) (Flock et al., 1987; Jonsson et al., 1991), the fibrinogen-binding clumping factors ClfA (McDevitt et al., 1994) and ClfB (Abraham and Jefferson, 2012), the serine–aspartate repeat protein family (Sdr) (McCrea et al., 2000), and the collagen binding protein (Cna) (Switalski et al., 1993). The major *S. aureus* autolysin, Atl, is a bi-functional cell wall-anchored protein, which contains an amidase domain and a glucosaminidase domain (Oshida et al., 1995). It is the predominant peptidoglycan hydrolase in *S. aureus* and also functions as an adhesin that mediates the initial attachment process (Houston et al., 2011). In *S. epidermidis*, the homologous AtlE plays a preeminent role in the adhesion to polystyrene surfaces as well as vitronectin binding (Heilmann et al., 1997). Further important components of the staphylococcal biofilm matrix are teichoic acids (Jabbouri and Sadovskaya, 2010), which are composed of repeating units of D-alanine, modified ribitol (or glycerol) and phosphate (Sanderson et al., 1962). Teichoic acids are further classified into wall teichoic acid (WTA) and lipoteichoic acid (LTA). WTA is covalently linked to peptidoglycan, while LTA is non-covalently attached to glycolipid intercalated in the outer leaflet of the cytoplasmic membrane (Xia et al., 2010). As shown in *S. aureus*, mutants in the *dlt* locus are not able to incorporate D-alanine into teichoic acids, which results in a stronger negative net charge on the bacterial cell surface (Peschel et al., 1999). This change significantly attenuates initial attachment to plastic surface (Gross et al., 2001). Moreover, WTA mediates interaction with epithelial and endothelial cells, resulting in impaired nasal colonization and virulence in a rabbit model of endocarditis (Weidenmaier et al., 2004; Weidenmaier et al., 2005). Finally, reduced LTA production attenuates bacterial capability to form biofilm on a plastic surface (Fedtke et al., 2007). In *S. epidermidis*, the

role of teichoic acids in biofilm formation have not been addressed as intensively, but recent research indicates similarly important roles for WTA (Holland et al., 2011).

After initial attachment, the bacteria proliferate, gradually forming a multi-layered microcolony. Furthermore, bacterial cells secrete polymeric molecules to form an extracellular matrix and factors that structure the biofilm. When this maturation process is accomplished, the mature biofilm presents as what based on studies in *P. aeruginosa* has been described as a complex of mushroom-shaped towers surrounded by liquid channels. The channels are crucial in a vital biofilm for nutrient delivery to deeper cell layers (O'Toole et al., 2000).

The molecular factors involved in biofilm expansion are diverse and in many species, include proteins, extracellular DNA (eDNA), and exopolysaccharides as main constituents. In many staphylococci, the main factor underlying intercellular interaction is the matrix exopolysaccharide, polysaccharide intercellular adhesin (PIA) (Mack et al., 1996), which has also been called poly-β(1–6)-N-acetyl-glucosamine (PNAG) (Maira-Litran et al., 2002). PIA/PNAG is also found in other, including Gram-negative bacteria (Wang et al., 2004). Due to partial deacetylation, PIA has a cationic character, which is unusual for bacterial matrix molecules (Mack et al., 1996). Thus, one assumed role of PIA in biofilm accumulation is to facilitate bacterial interaction by electrostatically attracting other, negatively charged matrix components, such as teichoic acids. The enzymes that govern PIA biosynthesis are encoded in the intercellular adhesion operon (*ica*) (Heilmann et al., 1996), which contains five genes: *icaA*, *icaB*, *icaC*, *icaD* and *icaR*. *icaA* encodes an *N*-acetylglucosaminyltransferase (IcaA) that synthetizes PIA oligomers from UDP-*N*-acetylglucosamine and IcaD is required for the optimal efficiency of IcaA (Gerke et al., 1998). IcaC is involved in the formation of long-chain PIA oligomers and probably translocates the synthesized polysaccharides across the cell membrane (Gerke et al., 1998). IcaB is a secreted *N*-deacetylase that is responsible for the abovementioned partial deacetylation, which occurs in 15% – 20% of the chain N-acetylglucosamine units (Vuong et al., 2004). IcaR is a negative regulator of the *ica* locus (Conlon et al., 2002), which mediates the regulatory impact of multiple global regulators and environmental conditions on transcription of the *icaADBC* operon (Knobloch et al., 2004; Pamp et al., 2006; Handke et al., 2007; Cerca et al., 2008; Cotter et al., 2009; Cue et al., 2012). Important roles of PIA in biofilm formation have been described in both *S. epidermidis* (Rupp et al., 1999; Rupp et al., 2001) and *S. aureus* (Fluckiger et al., 2005) device-related infection models. However, PIA is not a universal requirement in biofilm development. Several strains of *S. epidermidis* and *S. aureus*, have been reported in which PIA is not produced, and which still form biofilms *in vitro* or *in vivo* (Fitzpatrick et al., 2005; Kogan et al., 2006; Rohde et al., 2007). In those strains, it is mostly proteins that appear to take on the biofilm-forming role of PIA. For example, these include fibronectin-binding proteins (FnBPA and FnBPB) (O'Neill et al., 2007; O'Neill et al., 2008). In PIA-independent *S. epidermidis* biofilms, intercellular adhesion may also be mediated by a truncated form of the accumulation-associated protein Aap (Hussain et al., 1997; Rohde et al., 2005), the extracellular matrix-binding protein Embp (Christner et al., 2010), the amyloid-forming biofilm-associated protein (Bap) (Tormo et al., 2005a; Taglialegna et al., 2016), or other surface proteins. However, the proteinaceous biofilm lacks the fibrous structures of PIA-dependent biofilm (Hennig et al., 2007; Schommer et al., 2011), and

appears to be less extended and robust (Wang et al., 2011). Besides proteins, DNA released from cells during regulated autolysis is believed to be an important structural component of the biofilm matrix (Whitchurch et al., 2002). During biofilm development, cell lysis and DNA release are critically regulated by the *cid* and *lrg* genes (Mann et al., 2009). Extracellular DNA (eDNA) works as an electrostatic net to hold biofilm cells together. Furthermore, eDNA promotes cross-links between different β-toxin molecules, a secreted toxin with sphingomyelinase activity, thereby strengthening the biofilm matrix (Huseby et al., 2010). Notably, in contrast to PIA and several matrix proteins, which have been shown to impact biofilm-associated infection in animal models using isogenic deletion mutants, for obvious reasons no such evidence is possible to achieve for the role of eDNA in *in-vivo* biofilms, which for those reasons remains speculative.

In addition to the cell-cell adhesive forces and matrix constituents discussed so far, the formation of a mature biofilm also depends on disruptive factors that facilitate channel formation. Extensive *in-vitro* studies show that this task is performed primarily by a family of peptides called phenol-soluble modulins (PSMs) (Wang et al., 2011; Periasamy et al., 2012). PSMs characteristically have amphipathic, alpha-helical secondary structures, which gives them surfactant-like properties (Cheung et al., 2014; Laabei et al., 2014). These properties are believed to allow them to disrupt electrostatic or hydrophobic non-covalent interactions between biofilm matrix components, thereby producing the characteristic mesh-like biofilm network (Otto, 2013). *S. aureus* produces 7 PSMs and *S. epidermidis* produces 6 PSMs. Alpha-type PSMs are short (about 20 – 25 amino acids) and also known to include members that may aggressively attack cell membranes (Wang et al., 2007). Beta-type PSMs are longer (~44–45 amino acids) and less cytotoxic. The PSM delta-toxin is about 25 amino acids long and its gene, *hld*, is located within RNAIII, the intracellular effector molecule of the staphylococcal Agr quorum-sensing system (Janzon et al., 1989). In *S. aureus*, the lack of any *S. aureus* PSM class resulted in more compact and extended biofilms as compared to the parental strain (Periasamy et al., 2012). In *S. epidermidis*, only the beta-type PSMs, which are strongly produced in that species in contrast to *S. aureus*, were investigated for their biofilm effects. At moderate concentrations, *S. epidermidis* beta PSMs facilitate channel formation and promote biofilm growth. However, they disrupt biofilm once the concentrations are high (Wang et al., 2011). These results with PSMs underline the idea that biofilm structuring and detachment, discussed in the following, are promoted by similar forces, with detachment occurring when those forces are strong and occur on the surface of the biofilm.

The third stage of biofilm development, the dispersal of bacterial cells from the biofilm, is of utmost importance for biofilm infection on medical devices, as it drives the systemic spread of infection to cause bloodstream infection and the establishment of infection at distant sites in the human body (Otto, 2013). Shear forces caused by blood flow and device flush are believed to promote the dispersal of biofilms from indwelling devices. In addition, bacteria produce specific dispersal factors. These factors include first and foremost PSMs, which cause dispersal when due to strong production the cell-cell-disruptive forces underlying their mode of action become sufficiently pronounced (Otto, 2013; Peschel and Otto, 2013; Le et al., 2014). Additionally, *S. aureus* produces four major extracellular proteases that may digest proteinaceous matrix components: serine protease SspA (also named V8 protease),

metalloprotease Aur (aureolysin), and two cysteine proteases, ScpA and ScpB (also named staphopain A and B) (Loughran et al., 2014). Despite generally relatively low substrate specificity, some of these proteases have been shown to degrade specific matrix proteins. For example, SspA digests FnBP (McGavin et al., 1997), while Aur degrades ClfB (Abraham and Jefferson, 2012). Furthermore, both staphopains inhibit biofilm formation, while ScpA can disrupt a pre-established biofilm using unknown protein targets (Mootz et al., 2013). It has also been suggested that the inhibitory effect of the Atl-degrading Esp (Chen et al., 2013) on biofilm formation of some *S. aureus* isolates is involved in bacterial interaction that limits *S. aureus* nasal colonization (Iwase et al., 2010).

eDNA is degraded by secreted nucleases (Kiedrowski et al., 2011), of which there are two, Nuc and Nuc2, in *S. aureus*. A mutant in the *nuc* gene shows a high level of high-molecular weight eDNA and forms stronger biofilms than the parental strain (Kiedrowski et al., 2011). Nuc2 decreases biofilm biomass in dispersal experiments (Kiedrowski et al., 2014). Some other *S. aureus* enzymes also have demonstrated impact on biofilm dispersal. For example, HysA degrades hyaluronic acid (HA), which is a large glycosaminoglycan in mammalian tissues. HysA cleaves hyaluronic acid during infection, disperses HA-containing biofilms and promotes dissemination (Ibberson et al., 2016). As for PIA, a staphylococcal enzyme degrading PIA has never been found and it is likely such an enzyme is not produced by staphylococci. However, *Actinobacillus actinomycetemcomitans* secretes a PIAse, dispersin B (Ramasubbu et al., 2005), which is able to disperse PIA-dependent staphylococcal biofilm (Izano et al., 2008).

Of note, the role of biofilm-degrading and detachment factors in device-associated infection is almost exclusively hypothetical and derived from *in-vitro* experiments. Only for PSMs, *in-vivo* evidence has been achieved underscoring a role in the systemic dissemination from a biofilm-associated infection (Wang et al., 2011; Periasamy et al., 2012). Whether deletion of PSMs results in increased biofilm formation on an implanted device has not yet been directly investigated. In strain LAC/USA300, inactivation of the *nuc1* or *nuc2* nucleases (Beenken et al., 2012), or of all ten protease genes (Zielinska et al., 2012), did not show an impact on biofilm formation on an implanted device *in vivo*.

4. Biofilm Regulation

The maintenance of a viable biofilm structure requires the controlled activation and deactivation of biofilm-forming and biofilm-structuring/detachment factors (Fig. 1). The most intensely investigated and best understood staphylococcal regulatory systems in charge of controlling biofilm development include the accessory gene regulator (Agr) quorum sensing system, members of the staphylococcal accessory regulator (Sar) family, and the sigma factor, SigB.

The quorum-sensing system Agr has a profound impact on biofilm development (Le and Otto, 2015). The *agr* locus contains two promoters, P2 and P3, which drive transcriptions of RNAII and RNAIII, respectively. The RNAII transcript encodes the genes of the core machinery of the Agr system, *agrB*, *agrD*, *agrC* and *agrA* (Peng et al., 1988). *agrD* encodes the precursor of the autoinducing peptide (AIP), the extracellular quorum-sensing signal (Ji

et al., 1995). It is post-translationally modified and exported by AgrB. Extracellular AIP binds to the histidine kinase receptor AgrC, which then phosphorylates the cognate response regulator, AgrA. Phosphorylated AgrA binds to the P2 and P3 promoters and initiates gene expression. In addition to its central role as the intracellular effector controlling Agr targets, which include many secreted toxins and proteases, RNAIII encodes the PSM, delta-toxin (Novick et al., 1993; Novick and Geisinger, 2008). Similar to the delta-toxin, and in contrast to other targets of Agr control, *psm* loci are directly controlled by binding of AgrA to their promoter regions, resulting in exceptionally strict control of PSM expression by Agr (Queck et al., 2008). The impact of Agr on biofilm development occurs mainly through its effect on PSM (Periasamy et al., 2012) and protease expression (Boles and Horswill, 2008), thus by controlling structuring. Furthermore, Agr controls expression of surface proteins (Peng et al., 1988; Novick and Geisinger, 2008), many of which facilitate the initial steps of biofilm formation on tissues. However, this control appears to be highly strain-dependent (Cheung et al., 2011). Of note, PIA expression is not subject to Agr control (Vuong et al., 2003; Cheung et al., 2011). The prototypical Sar protein, SarA, has a global impact on the production of many staphylococcal virulence factors (Cheung et al., 1992; Chan and Foster, 1998; Dunman et al., 2001). Among the SarA-regulated targets, the *ica* (Valle et al., 2003; Tormo et al., 2005b) and serine protease (Tsang et al., 2008) loci are probably those with the most pronounced impact on biofilm formation. Furthermore, SarA has an indirect impact on biofilm development via enhancement of Agr activity (Cheung et al., 1997) and nuclease expression (Beenken et al., 2010). Deletion mutants in *sarA* of *S. aureus* (Beenken et al., 2003) and *S. epidermidis* (Tormo et al., 2005b) are defective in biofilm formation. Among the other Sar paralogues, SarZ is noteworthy, as it has been shown to impact *S. epidermidis* biofilm formation by controlling *ica* transcription (Wang et al., 2008).

The alternative sigma factor SigB, expressed during stationary growth and environmental stress conditions (Chan et al., 1998), regulates several virulence factors and stress-response proteins (Bischoff et al., 2004). SigB increases *sar* gene expression and represses the Agr system (Deora et al., 1997). Down-regulation of RNAIII expression and extracellular protease production makes SigB important for FnBP-dependent biofilm formation (Houston et al., 2011). Furthermore, mutation of SigB or its regulator *rsbU* impair both *S. aureus* and *S. epidermidis* biofilm formation (Rachid et al., 2000; Knobloch et al., 2001).

The cyclic nucleotide messengers, cyclic di-AMP and particularly, cyclic di-GMP, have gained much interest as global regulators of biofilm physiology in many organisms. While cyclic di-AMP is an important second messenger for signal transduction in *S. aureus* (Corrigan et al., 2011), there is as of yet no evidence that these messengers control biofilm development in staphylococci.

5. Biofilm formation as an immune evasion strategy

Staphylococcal invasion commonly stimulates a strong inflammatory response, which includes the attraction of neutrophils, macrophages and other immune effectors to the infection site. The immune system is believed to control staphylococcal invasion mainly by its innate arm (Rigby and DeLeo, 2012), while much evidence suggests that the contribution of adaptive immunity is less important and likely limited to T-cell responses (Broker et al.,

2016). Notably, *S. aureus* infections are particularly dangerous and aggressive due to many mechanisms to evade the host's immune response (Foster, 2005), which include many factors that block immune signaling and cytolytic toxins that kill immune cells directly.

In the biofilm mode of growth, which staphylococci adapt when colonizing indwelling medical devices, all these mechanisms are certainly present. However, due to down-regulation of especially the Agr system under those conditions (Vuong et al., 2003; Periasamy et al., 2012), many aggressive, Agr-controlled evasion mechanisms, such as toxin production, are less pronounced. Furthermore, biofilm formation per se is as an immune evasion mechanism that contributes to resistance to neutrophil attacks via its matrix (Guenther et al., 2009).

Moreover, biofilms and biofilm aggregates provide increased tolerance to antibiotics. This is in part due to the matrix representing a penetration barrier for some antibiotics and in part the specific physiology of biofilms, which limits the activity particularly of antibiotics targeting active processes, such as cell wall formation (Mah and O'Toole, 2001; Singh et al., 2010). Furthermore, investigation performed using human synovial fluid indicates that during PJI, Agr is especially strongly down-regulated (Dastgheyb et al., 2015b). Due to the absence of PSMs, which would otherwise disperse biofilm-like aggregates, this leads to pronounced clumping and increased resistance to antibiotic treatment. Finally, recent research suggests that *S. aureus* biofilms release specific factors, such as cyclic di-AMP, that limit inflammation (Gries et al., 2016). Thus, biofilm formation on indwelling medical devices can be seen as a strategy to evade elimination by the immune system by remaining in a relatively quiescent state that does not trigger pronounced inflammation, in addition to providing increased shelter from phagocyte attacks and antibiotics.

6. Strategies for the development of drugs for the treatment of staphylococcal DRIs

Various strategies have been adopted to prevent and treat staphylococcal DRIs, but no method so far has been developed that is able to eliminate biofilm-associated staphylococcal infection completely. In many cases, infections recrudesce shortly after treatment finishes, and the infected devices have to be surgically replaced. Most strategies currently employed to avoid staphylococcal DRIs focus on preventing colonization before implantation by increased hygiene and disinfection measures. As further discussed below, the alteration or coating of device surfaces has had limited success. No method of in-situ biofilm eradication that would provide a working alternative to the use of antibiotics has yet been taken to clinical use. Thus, antibiotics remain the primary form of treatment for staphylococcal DRIs. This is despite the low efficacy antibiotics have against biofilms and biofilm-like aggregates as well as the fact that staphylococci have developed multiple antibiotic resistance, such as most notably against methicillin, a situation widespread in both *S. aureus* and CoNS (Witte et al., 2008). Rifampicin, an inhibitor of bacterial RNA polymerase, is able to penetrate staphylococcal biofilms and can be used alone or with other antibiotics for staphylococcal DRI (Forrest and Tamura, 2010). However, rifampicin-resistant isolates can develop quickly during treatment (Eng et al., 1985; Zavasky and Sande, 1998).

As the attachment of bacteria to indwelling devices depends at least partially on the properties of the device surface, optimization of device polymer chemistry or coating with anti-adhesive or antibacterial compounds, such as metal ions (silver, copper, zinc), and nanoparticle technology have been used (Swartjes et al., 2015; Gallo et al., 2016). However, these strategies have not had complete clinical success, most likely due to the fact that the devices are covered by human matrix proteins largely independently of their chemistry, an effect also prone to diminish the efficacy of device surface-attached antibacterials.

As for vaccines, there is no working vaccine for staphylococcal infections, and all clinical trials of anti-staphylococcal vaccines have failed (Bagnoli et al., 2012; Fowler et al., 2013). Due to the pronounced immune evasion properties of biofilms, vaccination strategies are even more challenging for device-associated infections. Nevertheless, several vaccine candidates have been investigated that specifically target biofilm matrix components. For example, immunization with PIA promotes clearance of *S. aureus* from the blood (Maira-Litrán et al., 2005) and anti-PIA antibodies are under investigation to treat a variety of biofilm infections in several bacterial pathogens (Cywes-Bentley et al., 2013). In *S. epidermidis,* active immunization with a truncated form of the surface protein SesC or passive immunization with anti-SesC antibodies reduced biofilm formation on a subcutaneous foreign body in a rat model (Shahrooei et al., 2012). Bacteriophage therapy is highly controversial, achieves only a narrow therapeutic spectrum, and comes with fast resistance development (Hughes and Webber, 2017; Pires et al., 2017). However, some promising pre-clinical results have been achieved using lytic bacteriophages in staphylococcal DRI. For example, phage K attacks a variety of staphylococci (O'Flaherty et al., 2004), including some drug-resistant *S. aureus* (O'Flaherty et al., 2005). Orthopedic wires coated with phage K were colonized less by *S. aureus* in a murine model of PJI, and the inhibition effect could be increased by simultaneous linezolid treatment (Kaur et al., 2016), exemplifying the potential of bacteriophage/antibiotic combination therapy.

Another approach for combination therapy alongside antibiotics is the proposed use of dispersal agents, such as dispersin B, which degrades PIA (Izano et al., 2008). These approaches, however, come with a series of difficulties. Not only is it problematic to maintain enzyme activity *in vivo* and minimize an immune response, findings on the lack of activity of *S. aureus* nucleases *in vivo* emphasize that the targets of some of these enzymes may not play as important a role *in vivo* as *in-vitro* research has suggested.

7. Concluding remarks

Staphylococci are major colonizers of medical devices. The biofilm mode of growth that staphylococci adopt when initiating device-associated infections enables persistence due to increased antibiotic tolerance and immune evasion properties. Thus, often the only possible treatment is surgical replacement of infected devices, which causes heavy physical, emotional and financial burdens. Alternative strategies that are being pursued include the development of new biomaterials, biomaterial coatings, biofilm dispersal agents, vaccines, and bacteriophages. However, every single approach is problematic for different reasons and a generally efficient biofilm therapeutic that may pass FDA requirements is currently not in sight. Nevertheless, given that biofilm-associated infection on medical devices represents a

major unresolved public health problem, much more intense basic and clinical research on medical device infection by staphylococci and other associated pathogens is urgently required.

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Significance

Infections of medical devices are an extremely frequent and serious health care problem. Staphylococci are the leading organisms involved.

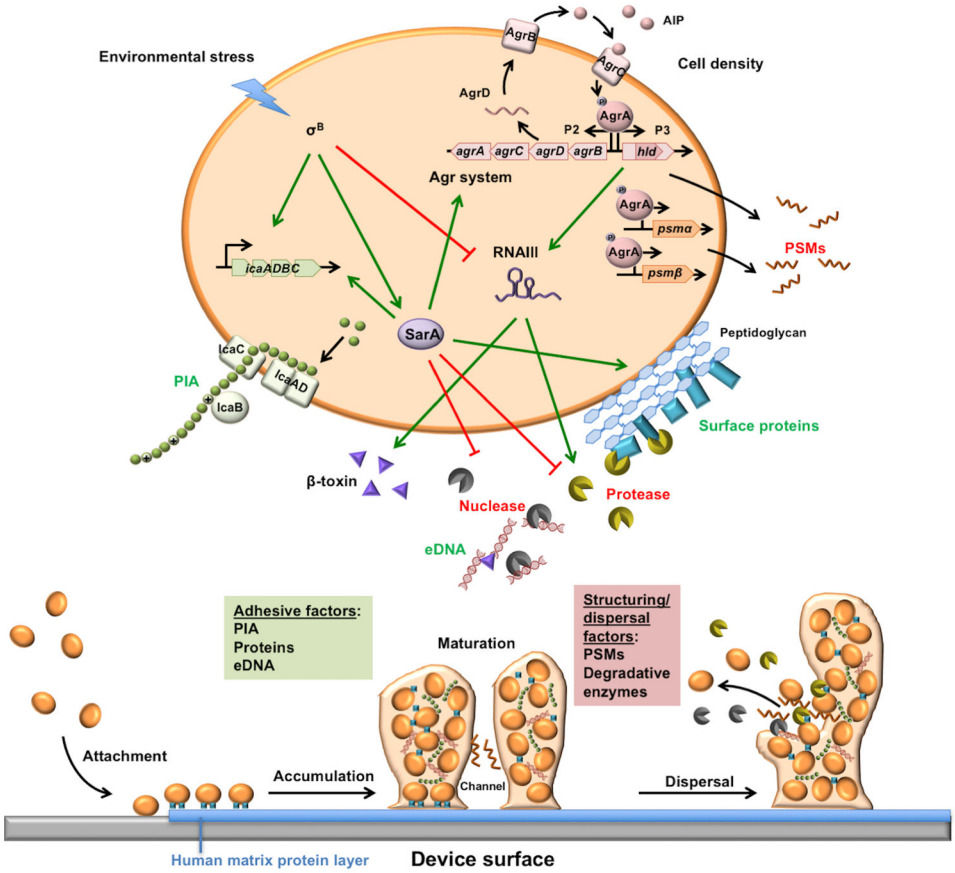

Figure 1. Biofilm development and regulation

Biofilm development includes three stages: initial attachment, accumulation/maturation and dispersal. Firstly, attachment is accomplished via hydrophobic interaction (directly to the device) or surface proteins (after human matrix proteins have covered the device). During the accumulation and maturation process, bacterial cells proliferate and produce biofilm matrix, which is composed of protein, eDNA and polysaccharides (e.g., PIA). Beta-toxin creates a covalently linked eDNA network. Structuring factors (e.g., PSMs) create channels. Finally, PSMs and other dispersal factors release cells, which may lead to dissemination of the infection. The biofilm “lifecycle” is tightly regulated by a complicated signaling network, which includes multiple regulation systems such as the Agr quorum-sensing system, Sar paralogues including SarA, and the alternative sigma factor, σ^B. Agr is cell density-controlled, linking biofilm development to the bacterial growth phase. σ^B expression is increased during environmental stress, linking biofilm development to external conditions.