Staphylococcus aureus Evasion of Host Immunity in the Setting of Prosthetic Joint Infection: Biofilm and Beyond

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Abstract

Purpose of Review The incidence of complications from prosthetic joint infection (PJI) is increasing, and treatment failure remains high. We review the current literature with a focus on *Staphylococcus aureus* pathogenesis and biofilm, as well as treatment challenges, and novel therapeutic strategies.

Recent Findings *S. aureus* biofilm creates a favorable environment that increases antibiotic resistance, impairs host immunity, and increases tolerance to nutritional deprivation. Secreted proteins from bacterial cells within the biofilm and the quorum-sensing *agr* system contribute to immune evasion. Additional immunoevasive properties of *S. aureus* include the formation of staphylococcal abscess communities (SACs) and canalicular invasion. Novel approaches to target biofilm and increase resistance to implant colonization include novel antibiotic therapy, immunotherapy, and local implant treatments.

Summary Challenges remain given the diverse mechanisms developed by *S. aureus* to alter the host immune responses. Further understanding of these processes should provide novel therapeutic mechanisms to enhance eradication after PJI.

Keywords Prosthetic joint infection · Biofilm · *Staphylococcus aureus*

Introduction Structure and Formation of Biofilm

Total joint replacement is one of the most common elective surgeries performed in the USA, and the incidence of complications from prosthetic joint infection (PJI) is increasing. Unfortunately, treatment failure of PJI remains high, ranging from 30 to 50% after surgical debridement and implant retention to 10–20% after implant removal with two-stage surgical treatment [1–3]. *Staphylococcus aureus* is the most common pathogen isolated from PJI, where up to 50% of cases are caused by hard-to-treat methicillin resistant *S. aureus* (MRSA) strains [4, 5]. A major cause of treatment failure is bacterial resistance to antimicrobials; therefore, it is critical to understand

the mechanisms responsible. While genetic alterations can arise to engender bacterial resistance to specific antibiotics (i.e., MRSA), the primary and much broader mechanism of resistance to drugs and host defenses involves biofilms, which protects pathogens that would otherwise be eradicated in their planktonic, or free floating form. As defeating biofilm is central to preventing and treating PJI, here, we review the current literature on *S. aureus* pathogenesis and biofilm, as well as challenges, and novel therapeutic strategies.

The formation of biofilms evolved over time as an adaptive response to hostile environments, allowing single-celled organisms to survive in a multi-cellular community [6]. In this process, biofilm formation proceeds in four generalized steps, including (1) bacterial cell attachment to a surface, (2) cell proliferation, (3) biofilm maturation, and (4) detachment and dispersal. Many of these processes contain species-specific characteristics. For example, *S. aureus* has a number of mechanisms to adhere to host tissue and foreign material surfaces. Once adherence has occurred, formation of a microcolony or small group of initial cells occurs, followed by cell proliferation and biofilm formation via production of extracellular matrix. Most of the biofilm mass consists of self-produced matrix of extracellular polymeric substances (EPS) composed of polysaccharides, proteins, and

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extracellular nucleic acids [7]. The EPS encases the bacterial cells contained within the biofilm to provide mechanical stability, protect against antimicrobial serum factors (i.e., antibodies and complement) and immune cell invasion, and retain essential nutrients and enzymes [7]. Observations of bacterial biofilms show their complex and heterogeneous organization, composed of void spaces and water channels to facilitate oxygen and nutrient transport through the bulk volume [8, 9•]. Cell-to-cell communication and exchange of genetic material can occur, showing the dynamic nature of biofilm. Furthermore, a proportion of bacteria have decreased metabolic activity creating less opportunity for effective antibiotic eradication [10]. It is within this specialized microenvironment that *S. aureus* is unaffected by antibiotic exposures up to 1000 times greater than the minimal inhibitory concentration (MIC) of its planktonic phenotype [11].

Initially, it was hypothesized that diffusion of drug molecules into the biofilm was the limiting factor that allowed survival of bacteria within this glycocalyx structure [12]. However, many static and dynamic culture systems of 3D biofilms have demonstrated that despite thorough drug penetration, many bacterial cells still survive treatment [13–15]. Thus, this drug-resistant phenotype is a product of a variety of cellular and environmental factors including slowed growth rate, mutability, pH alteration, and altered nutrient requirements [7]. Additionally *S. aureus* biofilms are capable of containing slow growing persisters cells, as well as small colony variant (SCV) cells, which have an auxotrophic defect, both of which enable extreme resistance to conventional antimicrobial treatments [16–20].

While there have been many studies on biofilm formation and structure utilizing in vitro models, no methods have emerged for “gold standard” biofilm growth, or biofilms that faithfully represent the glycocalyx of in vivo biofilms [21–26]. However, the use of dynamic biofilm culture systems has improved their clinical relevance [22–26]. Although these methods may produce a biofilm-like growth, with bacterial cells encased in a matrix of EPS, we cannot assume that they are clinically relevant models for biofilm growth and in vivo biofilm formation is less well characterized. Contrasts between in vitro and in vivo biofilm growth across many species has been documented and scientists have been cautioned to consider the clinical relevance of their biofilm models [27•]. To address this, several in vivo models have been developed using central venous catheters, or subcutaneous foreign body infection, to evaluate biofilm eradication by potential drug therapies [28, 29]. Overall, these methods can greatly improve the clinical relevance of a preclinical study.

Staphylococcus Biofilm Evasion of Host Immunity

S. aureus biofilm creates a favorable environment that increases antibiotic resistance, impairs host immunity, and

increases tolerance to nutritional deprivation. In this section, we summarize recent research focused on better understanding the effect of *S. aureus* biofilm infections on host immune response.

Cell Wall-Anchored Proteins

S. aureus expresses a broad range of proteins on its surface (up to 24 proteins have been identified), and are collectively known as cell wall-anchored (CWA) proteins due to their covalent linkage to pathogen’s peptidoglycan layer [30]. Based on the presence of certain unique structural motifs, the CWA proteins are classified into four groups: (1) the microbial surface component recognizing adhesive matrix molecule (MSCRAMM) family, (2) the near-iron transporter (NEAT) motif family, (3) the three-helical bundle family, and (4) the G5-E repeat family [30, 31]. Examples of each family of CWA and their functions are summarized in Table 1.

The MSCRAMM family of CWA proteins plays a key role in *S. aureus* bone/joint infections by initiating staphylococcal attachment to host plasma proteins [30–37]. The Clf-Sdr class of MSCRAMMs that include clumping factor proteins (ClfA and ClfB), and surface-anchored proteins (SdrC, SdrD, SdrE) mediate biofilm formation by promoting *S. aureus* attachment to both indwelling prosthetic devices and plasma-coated biological surfaces [30, 34, 35, 38]. Wang and colleagues showed that ClfA exacerbates orthopedic implant-related hematogenous *S. aureus* infection in a rodent model and that neutralizing antibodies against ClfA in combination with α -hemolysin (Hla) inhibited *S. aureus* biofilm formation [39•].

The near-iron transporter (NEAT) motif family of CWA proteins (IsdA–E, IsdG, IsdH, IsdI) is summarized in Table 1 [40]. These proteins induce biofilm formation under iron starving conditions and are essential for *S. aureus*’ survival against host immune defenses [41–43].

An important three-helical bundle CWA protein, expressed in all *S. aureus* strains, is the hypervariable staphylococcal protein A (SpA) (Table 1). This multifunctional protein, often used for *S. aureus* strain genotyping, is essential for nasal carriage of *S. aureus* in humans and is critical for the pathogenesis of staphylococcal infections [44–50]. SpA promotes bacterial aggregation and significantly contributes to the development of biofilm-associated infections and *S. aureus* abscess community formation in vivo [51]. Studies have reported an association between SpA expression and bone infection severity including osteoclast activation, osteoclast differentiation, and cortical bone destruction via TNFR1 and EGFR-mediated signaling pathways [52–55].

Immunovasive Proteins

S. aureus biofilms harbor an extensive exoproteome of proteins with functions related to immuno-evasion and

Table 1 Summary of selected cell wall-anchored proteins relevant to staphylococcal biofilm

Cell wall-anchored protein motif	Examples	Ligand binding	Function
MSCRAMM family proteins	Clumping factors A and B (ClfA and ClfB)	Bind fibrinogen (A,B), keratin (B), loricrin (B), complement factor I (A), desquamated epithelial cells (B)	Bind host extracellular matrix components particularly fibrinogen. Degradation of complement (C3b)
	SdrC, SdrD, SdrE, bone sialoprotein-binding protein	Beta-neurexin binding (SdrC), binds to desquamated epithelial cells, complement factor H (SdrE)	Binding to epithelium
	Fibronectin binding proteins A and B (FnBpA, FnBpB)	Bind fibrinogen, fibrin, elastin	Binds extracellular matrix
	Collagen adhesion (CNA)	Binds collagen, complement protein C1q	Binds extracellular matrix, inhibits complement activation
NEAT motif family	IsdA-E, IsdG, IsdH, IsdI	Bind/transport heme or hemoglobin, integrins, and extracellular matrix components	Iron/heme uptake and transport, resistance to neutrophil killing
Three-helical bundle	Protein A	Binds Fc region of IgG to inhibit opsonophagocytosis, activates platelet aggregation via von Willebrand factor binding, B cell superantigenic activity by crosslinking Fab region of V _H 3 bearing IgM, activates TNFR1	Immune evasion Infection pathogenesis
	Staphylococcal complement inhibitor (SCIN)	C3 convertases on bacterial surface	Inhibits complement activation
G5-E repeat family	Surface protein G (SasG)	Unknown	Cell-to-cell adhesion during biofilm formation

MSCRAMM microbial surface component recognizing adhesive matrix molecules, *NEAT* near-iron transporter (NEAT) domains

pathogenesis including hemolysins, nucleases, lipases, proteases, and collagenases [56–61]. The most important of these proteins related to biofilm pathogenesis are summarized in Table 2.

Evasion of Innate Immunity

Neutrophils and macrophages present the first line of host innate defense against *S. aureus* infections. Polymorphonuclear neutrophils (PMN) can efficiently kill planktonic *S. aureus* using several antimicrobial strategies such as phagocytosis, production of bactericidal peptides, oxidative bursts, and secretion of proinflammatory cytokines and chemokines [62]. *S. aureus* has developed many strategies to resist neutrophil-mediated killing (Tables 1 and 2). Secreted peptides such as PSMs and pore-forming toxins disrupt the integrity of the plasma membrane of neutrophils and cause cell lysis [63•]. Scherr et al. showed that *S. aureus* biofilms increase expression of the accessory gene regulator (*agr*) locus (which encodes a quorum-sensing system) in the presence of neutrophilic challenge, and this may play a role in resistance to PMN killing and phagocytosis [64]. Membrane-bound pigment such as staphyloxanthin and production of enzymes such as superoxide dismutase scavenge reactive oxygen species, protecting *S. aureus* from neutrophil-mediated killing [65]. Another direct innate host defense mechanism represents the formation of

neutrophil extracellular traps (NETs), in which bacteria are trapped for clearance by neutrophils and macrophages [66•, 67]. By producing nucleases, *S. aureus* can trigger the degradation of NETs to deoxyadenosine, which induces macrophage cytotoxicity in abscess environments [68•]. Many strains of *S. aureus* also produce factors that inhibit complement activation, providing another mechanism against host defenses [69•]. Using these various defense mechanisms and virulence factors, *S. aureus* can survive neutrophil-directed killing and phagocytosis, persist within neutrophils, and use these cells as vehicles to circulate through host tissue [69•].

Classically activated macrophages (M1) elicit proinflammatory responses such as inducible nitric oxide synthase (iNOS) production, TNF α , IL-1 β , and IFN- γ expression against intracellular *S. aureus* and efficiently promote bacterial clearance. Staphylococcal biofilms actively evade recognition by toll-like receptors (TLR2 and TLR9) and skew macrophage responses towards an anti-inflammatory state (alternatively activated, M2) [70, 71•, 72]. M2-macrophages were inefficient in phagocytosing *S. aureus* cells within the biofilm and promoted a pro-fibrotic environment that enriched biofilm formation [64, 72]. *S. aureus* biofilms also actively secrete virulence factors such as LukAB and α -hemolysin that can impair macrophage phagocytosis and function [73].

A heterogeneous subset of immature monocytes and granulocytes called myeloid-derived suppressor cells

Table 2 Summary of selected immunoevasive proteins relevant to staphylococcal biofilm

Class of protein	Examples	Function
Hemolysins	α -Hemolysin (α -toxin, H1a), β -hemolysin, γ -hemolysin	Pore-forming toxin (α - and γ -hemolysin), sphingomyelinase (β -hemolysin). Lyse red blood cells, other leukocytes, epithelial/endothelial cells; alters immune cell signaling pathways involved in cell proliferation, immune response, and cytokine expression
Leukocidins	LukAB, LukDE, PVL	Pore-forming toxins. Lyse neutrophils, monocytes, and macrophages
Enzymes	Autolysin (AtlA) - Aminidase (Amd) - Glucosaminidase (Gmd) Aureolysin Staphylokinase Nuclease	Peptidoglycan hydrolases. Cell separation, generates extracellular DNA in biofilm matrix Protease. Inactivates PSMs and can activate other proteases Activates plasminogen to plasmin. Cleaves complement factor C3b Inactivate neutrophil extracellular traps (NETs)
Phenol-soluble modulins	δ -hemolysin, PSM α 1– α 4	Small amphipathic peptides. Lyse neutrophils and break down biofilm matrix, critical for biofilm disassembly
Superantigenic exotoxins	Toxic shock syndrome toxin Staphylococcal enterotoxins (A–E, G)	Stimulate T cells nonspecifically without typical antigenic recognition. Can cause toxic shock syndrome. Activates bone resorption, and inhibits host immunity in osteomyelitis Activate cytokine release and involved in gastroenteritis, sepsis, kidney injury
Wall teichoic acids	–	Roles in cell division, complement activation, beta-lactam resistance in methicillin-resistant <i>S. aureus</i> , antibiotic susceptibility, and resistance to neutrophil intracellular killing
Chemotaxis inhibitory protein of <i>Staphylococcus aureus</i> (CHIPS)	–	Inhibits neutrophil migration and activation and prevents complement activation

(MDSCs) has recently been shown to play an important in promoting *S. aureus* orthopedic biofilm infection [74•]. In a murine model of prosthetic joint infection (PJI), Heim and colleagues demonstrated that MDSCs actively suppressed T cell recruitment and proinflammatory cytokine production at the site of infection, thereby facilitating *S. aureus* biofilm persistence and proliferation [74•]. The authors showed that MDSCs suppressed monocyte/macrophage inflammation at the local biofilm milieu by augmenting anti-inflammatory mediators like arginase-1 (Arg-1) and IL-10 [74•, 75, 76]. Peng and colleagues used a rat PJI model to show that *S. aureus* biofilms polarize a subset of MDSCs into M2-macrophages, and this preferential expansion can further promote bacterial persistence at the site of infection [77].

Evasion of Adaptive Immunity

Adaptive immunity against staphylococcal biofilms consists of cell-mediated responses dominated by T cells and humoral antibody responses mediated by B cells. CD4+ T helper 1 (Th1) cells facilitate cellular immune responses against intracellular bacteria, while Th2 cells mediate humoral immunity against extracellular pathogen [76, 78]. Th17 cells, though traditionally thought to complement Th1 cells in mediating intracellular bacterial clearance, are now known to promote biofilm-associated chronic inflammation [79]. In a porcine osteomyelitis infection model, Jensen et al. showed that the

antibody responses against extracellular *S. aureus* in biofilms are skewed to a predominantly Th1- and Th17-biased immune response, which is ineffective in clearing extracellular pathogens [80]. To further illustrate the Th1/Th17 bias, the group showed that Th1/Th17-biased C57BL/6 mice were unable to clear chronic *S. aureus* biofilm bone infections, while most of the Th2-skewed BALB/c mice, which produced anti-inflammatory Th2 cytokines (IL-4, IL-10), and regulatory T cells (Treg), cleared the infection [81].

Numerous studies have described anti-*S. aureus* humoral immune responses following colonization or infection [82–85]. Our laboratory determined that IsdA, IsdB, aminidase, and glucosaminidase are immunodominant antigens during *S. aureus* infection in both mice and patients with deep musculoskeletal infections [86•]. IgG titers against many different *S. aureus*-associated proteins are common in healthy humans; however, due to previous infection, colonization, and subclinical infection, it is challenging to use them for diagnostic purposes [86•]. Additionally, the presence of these antibodies only confers limited protection against future *S. aureus* infections [82–85].

Agr and Emigration from Biofilm

Biofilm disassembly converts *S. aureus* to its planktonic state, allowing it to reestablish local infection and travel to distant

sites resulting in systemic infection. One of the most important regulators of biofilm development and disassembly in *S. aureus* is the accessory gene regulator (*agr*) system. It is a well-characterized two-component peptide quorum-sensing system present in all staphylococci [87]. It is controlled by a circuit initially triggered by autoinducing peptide (AIP), which is synthesized and secreted into the extracellular environment [88]. At a certain threshold concentration, it activates the histidine kinase AgrC, which then leads to the production of proteases, toxins, and PSMs, which are surfactant-like peptides that can act against host defenses and help break down the biofilm matrix contributing to disassembly [88, 89]. During the initiation phase of biofilm development, *agr* expression is low, upregulating the expression of bacterial adhesion factors, while simultaneously downregulating toxin production. Isogenic *agr* mutants exhibited pronounced biofilm formation and decreased ability to disseminate into other tissues [90–93]. In a mature biofilm, increased expression of *agr* leads to protease, toxin, and PSM production leading to dispersion of *S. aureus* from the biofilm to planktonic state and secretion of extracellular factors that disrupt the host immune response. A significant portion of the *agr* mutant phenotype with regard to biofilm structure and inability to disassemble is due to a lack of PSM expression, illustrating their importance to the *agr* system [9•]. Other genes besides PSMs expressed through the *agr* system also can play a role in biofilm resistance, but these are less well characterized. For instance, in a mouse PJI model, *S. aureus* biofilms thwarted macrophage phagocytosis via *agr*-mediated production of Hla and LukAB toxins [73].

Beyond Biofilm: Canalicular Invasion and Colonization

A high rate of treatment failure occurs in PJI despite extensive soft tissue and bone debridement, implant exchange, and extensive antibiotic therapies, suggesting that other mechanisms of bacterial persistence exist [94–96]. Recent findings from our group have discovered that *S. aureus* colonizes long submicron cracks and canaliculi adjacent to the medullary canal in chronic osteomyelitis, and this serves as a major bacterial reservoir [97•, 98]. Within the canalicular network of live bone, the bacterial cells have an inexhaustible source of nutrients while remaining protected from the threat of immune cell attack [99].

These observations do not conform to established dogma that historically defined *S. aureus* as non-motile cocci ~ 1 µm in diameter. Based on the absence of any observable motility structures such as flagella, cilia, or pseudopods, it is theorized that *S. aureus* is capable of invading the canaliculi of live bone with a novel motility mechanism [97•]. By performing immunoelectron microscopy following BrdU metabolic labeling, it was demonstrated that live bone colonization is an

active process that involves bacterial replication, rather than dormant persistence [97•, 98]. It appears that *S. aureus* utilizes haptotaxis to identify the 3D structure of the canalicular openings and durotaxis to identify the rigidity of the 3D structure and invade canaliculi via extrusion of daughter cells through asymmetric binary fission [97•, 98]. The bacteria are able to deform from spherical cocci to rod-shaped bacterium and migrate towards the osteocytic lacunae via proliferation at the leading edge [97•, 98].

Others have also challenged the classical understanding of *S. aureus* by reporting evidence of active cell motility via spreading dendrites, as well as reporting altered cell growth phenotype by identifying SCVs [19, 100, 101]. *S. aureus* passive spreading across solid or soft media, aided by PSM production, is well established [102, 103]. However, evidence that these bacteria are capable of active propulsion has only recently emerged.

Collectively, these findings suggest a novel mechanism of bacterial persistence in chronic osteomyelitis and may provide insight as to why *S. aureus* infection in the setting of chronic PJI is so challenging to treat.

Beyond Biofilm: Soft Tissue Abscess Communities

Another hallmark of *S. aureus* infection is the formation of abscess lesions or staphylococcal abscess communities (SACs) [69•]. SACs, similar to biofilms, serve as another strategic survival mechanism where the organism thrives in a multi-cellular community while impairing host immunity. It has been shown that abscess formation is not solely a host driven response but rather a pathogen driven process where *S. aureus* synchronously triggers the stages of SAC formation by discrete regulation of protein production [69•, 104]. After invasion of host soft tissue, *S. aureus* produces factors that actively recruit host immune cells; however, this recruitment is not effective in killing the organism [104]. Instead, a central, replicating group of bacteria develop and are surrounded by a protective pseudocapsule of fibrin [104]. This SAC is then surrounded by a peripheral ring of necrotic neutrophils, which add to soft tissue injury and further impair immune function, followed by an outer layer of surviving immune cells, which are ineffective in removing the pathogen [104]. Proteins such as staphylococcal nuclease and adenosine synthase A convert neutrophilic NETs into products that induce macrophage cytotoxicity, which may be a significant mechanism in protecting bacteria within the SAC [68•]. Eventually, these SACs can mature and rupture, releasing the bacterium into the surrounding tissue [69•]. The formation of SACs rely on many genes that are important in biofilm formation including ECM binding factors such as ClfA, iron transport proteins, protein A, and genes encoding capsular polysaccharides [69•, 104].

Emerging Treatments: Targeting Biofilm

In recent years, there has been a renewed focus on emerging therapeutic treatments directed at biofilm formation including modifications of systemic antibiotic protocols, novel systemic therapies, and local treatments of the infected periprosthetic environment.

The clinical emergence of *S. aureus* resistance to conventional systemic antibiotics has led to modifications in antibiotic regimens to target bacteria within biofilm. Rifampicin inhibits bacterial RNA polymerase and exhibits bactericidal activity against biofilm-forming microorganisms; however, rapid resistance develops when used as a monotherapy. In vivo studies suggest that addition of rifampicin to current standard of care systemic antibiotics reduces colony-forming units in infected periprosthetic tissues and may reduce biofilm formation [105, 106]. These data have been supported by clinical studies, which show improved infection eradication with the addition of rifampicin to other antibiotic regimens including vancomycin [2, 107–109]. The efficacy of current systemic antibiotic regimens in targeting bacteria within an established biofilm remains controversial, however, and minimum biofilm eradication concentrations (MBEC) for a given antibiotic can be many fold higher than minimum inhibitory concentration (MIC) [110–112]. Additionally, the MBEC can be greater than achievable concentrations from systemic treatment, suggesting that alternative treatments targeting biofilm itself are necessary [110].

Immunotherapy is another major area of interest, which may provide complementary treatment for antibiotic therapies against *S. aureus* infection. Vaccine strategies for *S. aureus* infection have been attempted, but none have been successful beyond phase I clinical trials [113, 114•, 115]. Some of these vaccine strategies have failed because they attempted to target components of the cell wall that are not universally expressed across strains including poly-N-acetyl glucosamine and LTA acid [113]. One *S. aureus* vaccine that showed promise in early clinical studies targeted capsular polysaccharides (types 5 and 8) conjugated to a recombinant *Pseudomonas aeruginosa* exotoxin A. However, in a larger trial, this vaccine failed to reduce infection in hemodialysis patients [113, 116]. Another vaccine from Merck (V710) showed preclinical promise by targeting IsdB [114•]. Unfortunately, V710 failed to reduce infection rates or mortality in a phase 2b/3 trial, which attempted to prevent *S. aureus* infection after cardiothoracic surgery. Moreover, patients who did get infected were more likely to die in the vaccine group, suggesting that this vaccine may actually impair host immunity against sepsis [114•]. Thus, developing animal models that more faithfully replicate human surgical site infections may be critical for elucidating host humoral and cell-based immune responses to *S. aureus* and are important to future development of vaccine-based strategies.

Passive immunization strategies to components of biofilm or bacterial cells may show more promise than vaccine-based approaches. One approach targeting the biofilm itself used a monoclonal antibody (mAb) to DNA binding proteins from the DNABII family, which has conserved homologs across many bacterial species including *S. aureus* [117]. Using this mAb in combination with daptomycin systemic therapy, reductions in both planktonic and adherent bacteria were found in a murine implant-associated infection model relative to daptomycin monotherapy [117]. Wang et al. used monoclonal antibodies to α -toxin and ClfA to directly target biofilm formation. Combination of the two mAbs resulted in decreased colony-forming units from bone/joint tissue, reduced propensity for infection, and less biofilm aggregates in a murine model of hematogenous MRSA infection [39•]. Other work has focused on developing immunotherapy targeting the glucosaminidase (Gmd) subunit of autolysin (Atl), which is an immunodominant *S. aureus* antigen [118, 119•]. In a murine model of implant-associated MRSA infection, synergistic activity between anti-Gmd therapy and systemic vancomycin reduced peri-implant bacterial burden, osteolysis volume from established osteomyelitis, and resulted in sterilization of staphylococcal abscess communities (SACs) relative to monotherapies alone [119•]. Intracellular reservoirs of *S. aureus* within host immune cells may be another mechanism of bacterial persistence and reemergence after treatment [120•]. In order to target these cells, Lehar et al. created an antibody-antibiotic conjugate (AAC) that consists of a monoclonal antibody that recognizes the alpha-O-linked N-acetylglucosamine sugars on wall teichoic acids (WTAs) bound to rifamycin class derivative antibiotic (similar in properties to rifampicin) [120•]. This AAC binds to the surface of *S. aureus*, and then upon opsonization, the proteolytic environment of the phagolysosome of the host cell causes release of the active antibiotic form [120•]. This AAC demonstrated improved results versus systemic vancomycin alone in a murine MRSA hematogenous infection model.

Dispersal of *S. aureus* biofilms is another major focus for antimicrobial therapeutics. By converting the biofilm bacteria to planktonic form, these cells will be more susceptible to common antibiotics that were previously unable to infiltrate the biofilm. Enzymatic treatments like proteinase K, trypsin, dispersin B, lysostaphin, DNases, and fibrinolytics have shown promise in their ability to disperse staphylococci from biofilm by acting on key structural components of the biofilm matrix [88, 121, 122]. Dispersion B for example is an enzyme discovered in *Aggregatibacter actinomycetemcomitans* and acts on in methicillin-sensitive strains by hydrolyzing the polysaccharide intercellular adhesin (PIA), which is a key factor in biofilm formation for *S. aureus* [121]. Other agents like lysostaphin lyse the interbridges on the bacterial cell walls but are not strain dependent [121]. Fibrinolytics like streptokinase or nattokinase break down the fibrin matrix within

biofilm and decrease the minimum biofilm eliminating concentration (MBEC) of available systemic antibiotics [110, 122]. Targeting the quorum-sensing system is another strategy to trigger biofilm dispersal, and autoinducing peptide type I (AIP-1) treatment in vitro was able to trigger dispersal of MRSA on titanium discs, increasing its susceptibility to antibiotic therapy [123]. One of the major concerns surrounding dispersal agents is that bacteria cells disassembled from their biofilms are more capable of systemic infection in other locations in the body. Thus, agents like the ones previously stated likely must be used in combination with systemic therapies such as antibiotics [121, 122].

Biomaterials-based approaches have also shown promise in the treatment of PJI. Improving biofilm resistance on the implant surface would reduce rates of treatment failure after PJI. Silver-based implant coatings have shown clinical promise in megaprotheses after segmental bone resection and post-fracture [124, 125, 126]. A silver-coated megaprosthesis (Alguna) reduced rates of postoperative infection (11.8 versus 22.4%) and had improved success after debridement and implant retention relative to titanium implants in a small case control study [125]. It has also been used on urinary catheters, vascular grafts, and endotracheal tubes with varying degrees of success [127, 128]. Concerns about local and systemic toxicity have led to alternative approaches to using silver as an antimicrobial in PJI. Use of additive manufacturing and nanoparticle-based silver may improve upon its efficacy while reducing the potential off-target effects [129].

Novel antibiotic or small molecule implant coatings may also be a successful strategy to reduce biofilm formation on orthopedic implants; however, most of these studies have not progressed beyond the preclinical stages. One group of strategies focus on increasing the local concentration of antimicrobial therapies, while promoting host cell binding to the implant surface to the exclusion of bacterial colonization. Covalent bonding of an antibiotic to titanium may reduce biofilm formation on the implant without impairing osseointegration and host cell attachment [130]. Surface modifications that enhance osseointegration of titanium in combination with antibiotic coatings may be another strategy to increase resistance to biofilm formation while promoting host cell adhesion [131]. Use of antibiotic carriers such as hydrogels or phosphatidylcholine-based materials allow point of care temporary implant coatings that elute antibiotics from the implant surface and provide biofilm resistance [132–134]. Treatment of the infected implant may provide another strategy to reduce the morbidity of PJI. Ehrensberger et al. used cathodic voltage-controlled electrical stimulation to titanium with an established biofilm, and this reduced both planktonic and biofilm-associated MRSA in vivo [135]. Other local treatments available in the operating room include heat (autoclave) and topical scrubs. Leary et al. showed that the combination of autoclave and scrubbing with 4% chlorhexidine was able to

remove over 99% of biofilm on a cobalt chromium disc with established *S. aureus* and *Staphylococcus epidermidis* [136]. Treatment of the implant surface remains a critical component to infection eradication in PJI; however, further advancement in the clinical development of these treatments is necessary.

Conclusion

Improvements in our knowledge of *S. aureus* pathogenesis, and host immunity against this common pathogen, should provide novel therapeutic mechanisms to enhance eradication after PJI. Challenges remain given the diverse mechanisms developed by *S. aureus* to alter the host immune responses. The addition of local treatments to the implant to increase its resistance to bacterial colonization and therapies that target biofilm would increase the modalities at our disposal for treating this challenging infection.

Compliance with Ethical Standards

Conflict of Interest Edward M Schwarz reports grants from NIH and AOTrauma during the conduct of the study and personal fees and other from Telephus, LLC, outside the submitted work. In addition, Dr. Schwarz has a patent on passive immunization and diagnostics for *S. aureus* licensed to Telephus, LLC.

The other authors declare that they have no conflicts of interest.

EMS has patents related to this work. EMS has received financial compensation and stock from Telephus Medical LLC.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major Importance

1. Bryan AJ, Abdel MP, Sanders TL, Fitzgerald SF, Hanssen AD, Berry DJ. Irrigation and debridement with component retention for acute infection after hip arthroplasty: improved results with contemporary management. *J Bone Joint Surg Am*. 2017;99(23): 2011–8. <https://doi.org/10.2106/JBJS.16.01103>.
2. Lora-Tamayo J, Murillo O, Iribarren JA, Soriano A, Sánchez-Somolinos M, Baraia-Etxaburu JM, et al. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. *Clin Infect Dis*. 2013;56(2):182–94. <https://doi.org/10.1093/cid/cis746>.

3. Nodzo SR, Boyle KK, Spiro S, Nocon AA, Miller AO, Westrich GH. Success rates, characteristics, and costs of articulating antibiotic spacers for total knee periprosthetic joint infection. *Knee*. 2017;24(5):1175–81. <https://doi.org/10.1016/j.knee.2017.05.016>.
4. Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J. Periprosthetic joint infection: the incidence, timing, and predisposing factors. *Clin Orthop Relat Res*. 2008;466(7):1710–5. <https://doi.org/10.1007/s11999-008-0209-4>.
5. Kaplan SL. Recent lessons for the management of bone and joint infections. *J Inf Secur*. 2014;68(Suppl 1):S51–6. <https://doi.org/10.1016/j.jinf.2013.09.014>.
6. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95–108.
7. Flemming HC, Wingender J. The biofilm matrix. *Nat Rev Microbiol*. 2010;8(9):623–33. <https://doi.org/10.1038/nrmicro2415>.
8. de Beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. *Biotechnol Bioeng*. 1994;43(11):1131–8.
9. Periasamy S, Joo HS, Duong AC, Bach TH, Tan VY, Chatterjee SS, et al. How *Staphylococcus aureus* biofilms develop their characteristic structure. *Proc Natl Acad Sci U S A*. 2012;109(4):1281–6. <https://doi.org/10.1073/pnas.1115006109>. **Described the critical role of PSMs in biofilm structure and disassembly, which was driven by the *agr* quorum-sensing system.**
10. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318–22.
11. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol*. 2001;9(1):34–9.
12. Gristina AG, Hobgood CD, Webb LX, Myrvik QN. Adhesive colonization of biomaterials and antibiotic resistance. *Biomaterials*. 1987;8(6):423–6.
13. Jefferson KK, Goldmann DA, Pier GB. Use of confocal microscopy to analyze the rate of vancomycin penetration through *Staphylococcus aureus* biofilms. *Antimicrob Agents Chemother*. 2005;49(6):2467–73.
14. Zheng Z, Stewart PS. Penetration of rifampin through *Staphylococcus epidermidis* biofilms. *Antimicrob Agents Chemother*. 2002;46(3):900–3.
15. Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob Agents Chemother*. 2000;44(7):1818–24.
16. Roberts ME, Stewart PS. Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. *Microbiology*. 2005;151(Pt 1):75–80.
17. de la Fuente-Núñez C, Refluveille F, Fernández L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Curr Opin Microbiol*. 2013;16(5):580–9. <https://doi.org/10.1016/j.mib.2013.06.013>.
18. Kahl B, Herrmann M, Everding AS, Koch HG, Becker K, Harms E, et al. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. *J Infect Dis*. 1998;177(4):1023–9.
19. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. *Staphylococcus aureus* small colony variants in prosthetic joint infection. *Clin Infect Dis*. 2006;43(8):961–7.
20. Garcia LG, Lemaire S, Kahl BC, Becker K, Proctor RA, Denis O, et al. Antibiotic activity against small-colony variants of *Staphylococcus aureus*: review of in vitro, animal and clinical data. *J Antimicrob Chemother*. 2013;68(7):1455–64. <https://doi.org/10.1093/jac/dkt072>.
21. Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, et al. Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature*. 2001;413(6858):860–4.
22. Werner E, Roe F, Bugnicourt A, Franklin MJ, Heydon A, Molin S, et al. Stratified growth in *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol*. 2004;70(10):6188–96.
23. Deligianni E, Pattison S, Berrar D, Ternan NG, Haylock RW, Moore JE, et al. *Pseudomonas aeruginosa* cystic fibrosis isolates of similar RAPD genotype exhibit diversity in biofilm forming ability in vitro. *BMC Microbiol*. 2010;10:38. <https://doi.org/10.1186/1471-2180-10-38>.
24. Donlan RM, Piete JA, Heyes CD, Sanii L, Murga R, Edmonds P, et al. Model system for growing and quantifying *Streptococcus pneumoniae* biofilms in situ and in real time. *Appl Environ Microbiol*. 2004;70(8):4980–8.
25. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol*. 1999;37(6):1771–6.
26. Yang L, Haagenen JA, Jelsbak L, Johansen HK, Sternberg C, Høiby N, et al. In situ growth rates and biofilm development of *Pseudomonas aeruginosa* populations in chronic lung infections. *J Bacteriol*. 2008;190(8):2767–76.
27. Nishitani K, Sutipompangkul W, de Mesy Bentley KL, Varrone JJ, Bello-Irizarry SN, Ito H, et al. Quantifying the natural history of biofilm formation in vivo during the establishment of chronic implant-associated *Staphylococcus aureus* osteomyelitis in mice to identify critical pathogen and host factors. *J Orthop Res*. 2015;33(9):1311–9. <https://doi.org/10.1002/jor.22907>. **The natural history of in vivo biofilm formation with multiple *Staphylococcus aureus* strains were examined in a clinically relevant murine model with an implant-associated infection.**
28. Ulphani JS, Rupp ME. Model of *Staphylococcus aureus* central venous catheter-associated infection in rats. *Lab Anim Sci*. 1999;49(3):283–7.
29. Cirioni O, Giacometti A, Ghiselli R, Bergnach C, Orlando F, Mocchegiani F, et al. Pre-treatment of central venous catheters with the cathelicidin BMAP-28 enhances the efficacy of antistaphylococcal agents in the treatment of experimental catheter-related infection. *Peptides*. 2006;27(9):2104–10.
30. Geoghegan JA, Foster TJ. Cell wall-anchored surface proteins of *Staphylococcus aureus*: many proteins, multiple functions. *Curr Top Microbiol Immunol*. 2017;409:95–120. https://doi.org/10.1007/82_2015_5002.
31. Foster TJ. The remarkably multifunctional fibronectin binding proteins of *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis*. 2016;35(12):1923–31.
32. Patti JM, Allen BL, McGavin MJ, Höök M. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annu Rev Microbiol*. 1994;48:585–617.
33. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence*. 2011;2(5):445–59. <https://doi.org/10.4161/viru.2.5.17724>.
34. Ni Eidhin D, Perkins S, François P, Vaudaux P, Höök M, Foster TJ. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. *Mol Microbiol*. 1998;30(2):245–57.
35. Herman-Bausier P, Formosa-Dague C, Feuillie C, Valotteau C, Dufrene YF. Forces guiding staphylococcal adhesion. *J Struct Biol*. 2017;197(1):65–9. <https://doi.org/10.1016/j.jsb.2015.12.009>.
36. O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, et al. A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and

- FnBPB. *J Bacteriol.* 2008;190(11):3835–50. <https://doi.org/10.1128/JB.00167-08>.
37. Cardile AP, Sanchez CJ Jr, Samberg ME, Romano DR, Hardy SK, Wenke JC, et al. Human plasma enhances the expression of staphylococcal microbial surface components recognizing adhesive matrix molecules promoting biofilm formation and increases antimicrobial tolerance in vitro. *BMC Res Notes.* 2014;7:457. <https://doi.org/10.1186/1756-0500-7-457>.
 38. Farnsworth CW, Schott EM, Jensen SE, Zukoski J, Benvie AM, Refaai MA, et al. Adaptive upregulation of clumping factor A (ClfA) by *Staphylococcus aureus* in the obese, type 2 diabetic host mediates increased virulence. *Infect Immun.* 2017;85(6):e01005–16. <https://doi.org/10.1128/IAI.01005-16>.
 39. Wang Y, Cheng LI, Helfer DR, Ashbaugh AG, Miller RJ, Tzomides AJ, et al. Mouse model of hematogenous implant-related *Staphylococcus aureus* biofilm infection reveals therapeutic targets. *Proc Natl Acad Sci U S A.* 2017;114(26):E5094–102. <https://doi.org/10.1073/pnas.1703427114>. **The authors developed a murine hematogenous implant-related infection model and demonstrated that a combination of neutralizing human monoclonal antibodies against α -toxin and ClfA inhibited in vitro biofilm formation, propensity for infection in vivo, and reduced bacterial burden in vivo.**
 40. Hammer ND, Skaar EP. Molecular mechanisms of *Staphylococcus aureus* iron acquisition. *Annu Rev Microbiol.* 2011;65:129–47. <https://doi.org/10.1146/annurev-micro-090110-102851>.
 41. Clarke SR, Mohamed R, Bian L, Routh AF, Kokai-Kun JF, Mond JJ, et al. The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. *Cell Host Microbe.* 2007;1(3):199–212.
 42. Missineo A, Di Poto A, Geoghegan JA, Rindi S, Heilbronner S, Gianotti V, et al. IsdC from *Staphylococcus lugdunensis* induces biofilm formation under low-iron growth conditions. *Infect Immun.* 2014;82(6):2448–59. <https://doi.org/10.1128/IAI.01542-14>.
 43. Zapotoczna M, Jevnikar Z, Miajlovic H, Kos J, Foster TJ. Iron-regulated surface determinant B (IsdB) promotes *Staphylococcus aureus* adherence to and internalization by non-phagocytic human cells. *Cell Microbiol.* 2013;15(6):1026–41. <https://doi.org/10.1111/cmi.12097>.
 44. Shopsis B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol.* 1999;37(11):3556–63.
 45. Muthukrishnan G, Quinn GA, Lamers RP, Diaz C, Cole AL, Chen S, et al. Exoproteome of *Staphylococcus aureus* reveals putative determinants of nasal carriage. *J Proteome Res.* 2011;10(4):2064–78. <https://doi.org/10.1021/pr200029r>.
 46. Cole AL, Muthukrishnan G, Chong C, Beavis A, Eade CR, Wood MP, et al. Host innate inflammatory factors and staphylococcal protein A influence the duration of human *Staphylococcus aureus* nasal carriage. *Mucosal Immunol.* 2016;9(6):1537–48. <https://doi.org/10.1038/mi.2016.2>.
 47. Gómez MI, Lee A, Reddy B, Muir A, Soong G, Pitt A, et al. *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. *Nat Med.* 2004;10(8):842–8.
 48. Silverman GJ, Cary S, Graille M, Curtiss VE, Wagenknecht R, Luo L, et al. A B-cell superantigen that targets B-1 lymphocytes. *Curr Top Microbiol Immunol.* 2000;252:251–63.
 49. Graille M, Stura EA, Corper AL, Sutton BJ, Taussig MJ, Charbonnier JB, et al. Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. *Proc Natl Acad Sci U S A.* 2000;97(10):5399–404.
 50. Ricklin D, Tzekou A, Garcia BL, Hammel M, McWhorter WJ, Sfyroera G, et al. A molecular insight into complement evasion by the staphylococcal complement inhibitor protein family. *J Immunol.* 2009;183(4):2565–74. <https://doi.org/10.4049/jimmunol.0901443>.
 51. Merino N, Toledo-Arana A, Vergara-Irigaray M, Valle J, Solano C, Calvo E, et al. Protein A-mediated multicellular behavior in *Staphylococcus aureus*. *J Bacteriol.* 2009;191(3):832–43. <https://doi.org/10.1128/JB.01222-08>.
 52. Mendoza Bertelli A, Delpino MV, Lattar S, Giai C, Llana MN, Sanjuan N, et al. *Staphylococcus aureus* protein A enhances osteoclastogenesis via TNFR1 and EGFR signaling. *Biochim Biophys Acta.* 2016;1862(10):1975–83. <https://doi.org/10.1016/j.bbadis.2016.07.016>.
 53. Claro T, Widaa A, O'Seaghda M, Miajlovic H, Foster TJ, O'Brien FJ, et al. *Staphylococcus aureus* protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. *PLoS One.* 2011;6(4):e18748. <https://doi.org/10.1371/journal.pone.0018748>.
 54. Widaa A, Claro T, Foster TJ, O'Brien FJ, Kerrigan SW. *Staphylococcus aureus* protein A plays a critical role in mediating bone destruction and bone loss in osteomyelitis. *PLoS One.* 2012;7(7):e40586. <https://doi.org/10.1371/journal.pone.0040586>.
 55. Claro T, Widaa A, McDonnell C, Foster TJ, O'Brien FJ, Kerrigan SW. *Staphylococcus aureus* protein A binding to osteoblast tumour necrosis factor receptor 1 results in activation of nuclear factor kappa B and release of interleukin-6 in bone infection. *Microbiology.* 2013;159(Pt 1):147–54. <https://doi.org/10.1099/mic.0.063016-0>.
 56. Cassat JE, Hammer ND, Campbell JP, Benson MA, Perrien DS, Mrak LN, et al. A secreted bacterial protease tailors the *Staphylococcus aureus* virulence repertoire to modulate bone remodeling during osteomyelitis. *Cell Host Microbe.* 2013;13(6):759–72. <https://doi.org/10.1016/j.chom.2013.05.003>.
 57. Brady RA, Leid JG, Camper AK, Costerton JW, Shirtliff ME. Identification of *Staphylococcus aureus* proteins recognized by the antibody-mediated immune response to a biofilm infection. *Infect Immun.* 2006;74(6):3415–26.
 58. Flammier S, Rasigade JP, Badiou C, Henry T, Vandenesch F, Laurent F, et al. Human monocyte-derived osteoclasts are targeted by staphylococcal pore-forming toxins and superantigens. *PLoS One.* 2016;11(3):e0150693. <https://doi.org/10.1371/journal.pone.0150693>.
 59. Biswas R, Voggu L, Simon UK, Hentschel P, Thumm G, Götz F. Activity of the major staphylococcal autolysin Atl. *FEMS Microbiol Lett.* 2006;259(2):260–8.
 60. Weidenmaier C, Peschel A. Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat Rev Microbiol.* 2008;6(4):276–87. <https://doi.org/10.1038/nrmicro1861>.
 61. Chavakis T, Wiechmann K, Preissner KT, Herrmann M. *Staphylococcus aureus* interactions with the endothelium: the role of bacterial “secretable expanded repertoire adhesive molecules” (SERAM) in disturbing host defense systems. *Thromb Haemost.* 2005;94(2):278–85.
 62. Rigby KM, DeLeo FR. Neutrophils in innate host defense against *Staphylococcus aureus* infections. *Semin Immunopathol.* 2012;34(2):237–59. <https://doi.org/10.1007/s00281-011-0295-3>.
 63. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med.* 2007;13(12):1510–4. **Identified PSMs as a major determinant of *S. aureus* virulence including leukocyte lysis. Expression of**

- PSMs was found to be tightly regulated through the *agr* quorum-sensing system.
64. Scherr TD, Roux CM, Hanke ML, Angle A, Dunman PM, Kielian T. Global transcriptome analysis of *Staphylococcus aureus* biofilms in response to innate immune cells. *Infect Immun*. 2013;81(12):4363–76. <https://doi.org/10.1128/IAI.00819-13>.
 65. Clauditz A, Resch A, Wieland KP, Peschel A, Götz F. Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. *Infect Immun*. 2006;74(8):4950–3.
 66. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532–5. **Described the characteristics and release of neutrophil extracellular traps (NETs), which are granule proteins and chromatin that are released extracellularly to degrade virulence factors and immobilize bacteria for phagocytic clearance.**
 67. Lu T, Kobayashi SD, Quinn MT, Deleo FR. A NET Outcome. *Front Immunol*. 2012;3:365. <https://doi.org/10.3389/fimmu.2012.00365>.
 68. Thammavongsa V, Missiakas DM, Schneewind O. *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death. *Science*. 2013;342(6160):863–6. <https://doi.org/10.1126/science.1242255>. **Described a novel mechanism of immune evasion by which *S. aureus* degrades neutrophil NETs to deoxyadenosine, which is toxic to host immune cells.**
 69. Cheng AG, DeDent AC, Schneewind O, Missiakas D. A play in four acts: *Staphylococcus aureus* abscess formation. *Trends Microbiol*. 2011;19(5):225–32. <https://doi.org/10.1016/j.tim.2011.01.007>. **A review describing the establishment of *S. aureus* infection and staphylococcal abscess community (SAC) formation and its effect on host immunity.**
 70. Gries CM, Kielian T. Staphylococcal biofilms and immune polarization during prosthetic joint infection. *J Am Acad Orthop Surg*. 2017;25(Suppl 1):S20–4. <https://doi.org/10.5435/JAAOS-D-16-00636>.
 71. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, et al. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J Immunol*. 2011;186(11):6585–96. <https://doi.org/10.4049/jimmunol.1002794>. **Using a mouse model of catheter-associated infection, the authors showed that *S. aureus* biofilm was capable of attenuating inflammatory cytokine production and skewing the immune response towards a pro-fibrotic M2 macrophage phenotype.**
 72. Scherr TD, Heim CE, Morrison JM, Kielian T. Hiding in plain sight: interplay between staphylococcal biofilms and host immunity. *Front Immunol*. 2014;5:37. <https://doi.org/10.3389/fimmu.2014.00037>.
 73. Scherr TD, Hanke ML, Huang O, James DB, Horswill AR, Bayles KW, et al. *Staphylococcus aureus* biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. *MBio*. 2015;6(4):e01021–15. <https://doi.org/10.1128/mBio.01021-15>.
 74. Heim CE, Vidlak D, Scherr TD, Kozel JA, Holzapfel M, Muirhead DE, et al. Myeloid-derived suppressor cells contribute to *Staphylococcus aureus* orthopedic biofilm infection. *J Immunol*. 2014;192(8):3778–92. <https://doi.org/10.4049/jimmunol.1303408>. **In a murine model of prosthetic joint infection (PJI), the authors demonstrated that MDSCs actively suppressed T cell recruitment and proinflammatory cytokine production at the site of infection, thereby facilitating *S. aureus* biofilm persistence and proliferation.**
 75. Heim CE, Vidlak D, Kielian T. Interleukin-10 production by myeloid-derived suppressor cells contributes to bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection. *J Leukoc Biol*. 2015;98(6):1003–13. <https://doi.org/10.1189/jlb.4VMA0315-125RR>.
 76. Heim CE, Vidlak D, Scherr TD, Hartman CW, Garvin KL, Kielian T. IL-12 promotes myeloid-derived suppressor cell recruitment and bacterial persistence during *Staphylococcus aureus* orthopedic implant infection. *J Immunol*. 2015;194(8):3861–72. <https://doi.org/10.4049/jimmunol.1402689>.
 77. Peng KT, Hsieh CC, Huang TY, Chen PC, Shih HN, Lee MS, et al. *Staphylococcus aureus* biofilm elicits the expansion, activation and polarization of myeloid-derived suppressor cells in vivo and in vitro. *PLoS One*. 2017;12(8):e0183271. <https://doi.org/10.1371/journal.pone.0183271>.
 78. Bröker BM, Mrochen D, Péton V. The T cell response to *Staphylococcus aureus*. *Pathogens*. 2016;5(1):E31. <https://doi.org/10.3390/pathogens5010031>.
 79. Peck A, Mellins ED. Precarious balance: Th17 cells in host defense. *Infect Immun*. 2010;78(1):32–8. <https://doi.org/10.1128/IAI.00929-09>.
 80. Jensen LK, Jensen HE, Koch J, Bjarnsholt T, Eickhardt S, Shirliff M. Specific antibodies to *Staphylococcus aureus* biofilm are present in serum from pigs with osteomyelitis. *In Vivo*. 2015;29(5):555–60.
 81. Prabhakara R, Harro JM, Leid JG, Keegan AD, Prior ML, Shirliff ME. Suppression of the inflammatory immune response prevents the development of chronic biofilm infection due to methicillin-resistant *Staphylococcus aureus*. *Infect Immun*. 2011;79(12):5010–8. <https://doi.org/10.1128/IAI.05571-11>.
 82. Holtfrete S, Nguyen TT, Wertheim H, Steil L, Kusch H, Truong QP, et al. Human immune proteome in experimental colonization with *Staphylococcus aureus*. *Clin Vaccine Immunol*. 2009;16(11):1607–14. <https://doi.org/10.1128/CI.00263-09>.
 83. Holtfrete S, Kolata J, Bröker BM. Towards the immune proteome of *Staphylococcus aureus*—the anti-*S. aureus* antibody response. *Int J Med Microbiol*. 2010;300(2–3):176–92. <https://doi.org/10.1016/j.ijmm.2009.10.002>.
 84. Verkaik NJ, Boelens HA, de Vogel CP, Tavakol M, Bode LG, Verbrugh HA, et al. Heterogeneity of the humoral immune response following *Staphylococcus aureus* bacteremia. *Eur J Clin Microbiol Infect Dis*. 2010;29(5):509–18. <https://doi.org/10.1007/s10096-010-0888-0>.
 85. Verkaik NJ, Lebon A, de Vogel CP, Hooijkaas H, Verbrugh HA, Jaddoe VW, et al. Induction of antibodies by *Staphylococcus aureus* nasal colonization in young children. *Clin Microbiol Infect*. 2010;16(8):1312–7. <https://doi.org/10.1111/j.1469-0691.2009.03073.x>.
 86. Nishitani K, Beck CA, Rosenberg AF, Kates SL, Schwarz EM, Daiss JL. A diagnostic serum antibody test for patients with *Staphylococcus aureus* osteomyelitis. *Clin Orthop Relat Res*. 2015;473(9):2735–49. <https://doi.org/10.1007/s11999-015-4354-2>. **Described the serum host antibody response in patients with deep musculoskeletal infection with *S. aureus* versus healthy controls. No single titer yielded diagnostic significance; however, using a combination of host antibodies may provide diagnostic utility for ongoing infection.**
 87. Novick RP, Geisinger E. Quorum sensing in staphylococci. *Annu Rev Genet*. 2008;42:541–64. <https://doi.org/10.1146/annurev.genet.42.110807.091640>.
 88. Boles BR, Horswill AR. Staphylococcal biofilm disassembly. *Trends Microbiol*. 2011;19(9):449–55. <https://doi.org/10.1016/j.tim.2011.06.004>.
 89. Mann EE, Rice KC, Boles BR, Endres JL, Ranjit D, Chandramohan L, et al. Modulation of eDNA release and degradation affects *Staphylococcus aureus* biofilm maturation. *PLoS One*. 2009;4(6):e5822. <https://doi.org/10.1371/journal.pone.0005822>.

90. Vuong C, Saenz HL, Götz F, Otto M. Impact of the agr quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. *J Infect Dis*. 2000;182(6):1688–93.
91. Vuong C, Kocianova S, Yao Y, Carmody AB, Otto M. Increased colonization of indwelling medical devices by quorum-sensing mutants of *Staphylococcus epidermidis* in vivo. *J Infect Dis*. 2004;190(8):1498–505.
92. Balasubramanian D, Ohneck EA, Chapman J, Weiss A, Kim MK, Reyes-Robles T, et al. *Staphylococcus aureus* coordinates leukocidin expression and pathogenesis by sensing metabolic fluxes via RpiRc. *MBio*. 2016;7(3):e00818–6. <https://doi.org/10.1128/mBio.00818-16>.
93. Paharik AE, Horswill AR. The staphylococcal biofilm: adhesins, regulation and host response. *Microbiol Spectr*. 2016;4(2) <https://doi.org/10.1128/microbiolspec.VMBF-0022-2015>.
94. Azzam K, McHale K, Austin M, Purtill JJ, Parvizi J. Outcome of a second two-stage reimplantation for periprosthetic knee infection. *Clin Orthop Relat Res*. 2009;467(7):1706–14. <https://doi.org/10.1007/s11999-009-0739-4>.
95. Aynardi M, Pulido L, Parvizi J, Sharkey PF, Rothman RH. Early mortality after modern total hip arthroplasty. *Clin Orthop Relat Res*. 2009;467(1):213–8. <https://doi.org/10.1007/s11999-008-0528-5>.
96. Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med*. 2004;350(14):1422–9.
97. de Mesy Bentley KL, Trombetta R, Nishitani K, Bello-Irizarry SN, Ninomiya M, Zhang L, et al. Evidence of *Staphylococcus aureus* deformation, proliferation, and migration in canaliculi of live cortical bone in murine models of osteomyelitis. *J Bone Miner Res*. 2017;32(5):985–90. <https://doi.org/10.1002/jbmr.3055>. **Used a murine implant-associated infection model to show that *S. aureus* was able to deform, enter the canalicular system via asymmetric binary fission, and migrate toward osteocyte lacunae via proliferation at the leading edge, providing a novel reservoir to protect *S. aureus* from host immunity.**
98. de Mesy Bentley KL, MacDonald A, Schwarz EM, Oh I. Chronic osteomyelitis with *Staphylococcus aureus* deformation in submicron canaliculi of osteocytes: a case report. *JBJS Case Connect*. 2018;8:e8. <https://doi.org/10.2106/JBJS.CC.17.00154>.
99. Nishitani K, Bello-Irizarry SN, de Mesy Bentley K, Daiss JL, Schwarz EM. The role of the immune system and bone cells in acute and chronic osteomyelitis. *Osteoimmunology*. 2016; 2nd ed (Chapter 16):283–95.
100. Pollitt EJ, Crusz SA, Diggle SP. *Staphylococcus aureus* forms spreading dendrites that have characteristics of active motility. *Sci Rep*. 2015;5:17698. <https://doi.org/10.1038/srep17698>.
101. Tuchscher L, Heitmann V, Hussain M, Viemann D, Roth J, von Eiff C, et al. *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. *J Infect Dis*. 2010;202(7):1031–40. <https://doi.org/10.1086/656047>.
102. Tsompanidou E, Denham EL, van Dijk JM. Phenol-soluble modulins, hellhounds from the staphylococcal virulence-factor pandemonium. *Trends Microbiol*. 2013;21(7):313–5. <https://doi.org/10.1016/j.tim.2013.04.007>.
103. Kaito C, Sekimizu K. Colony spreading in *Staphylococcus aureus*. *J Bacteriol*. 2007;189(6):2553–7.
104. Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. *FASEB J*. 2009;23(10):3393–404. <https://doi.org/10.1096/fj.09-135467>.
105. Jørgensen NP, Skovdal SM, Meyer RL, Dagnæs-Hansen F, Fuursted K, Petersen E. Rifampicin-containing combinations are superior to combinations of vancomycin, linezolid and daptomycin against *Staphylococcus aureus* biofilm infection in vivo and in vitro. *Pathog Dis*. 2016;74(4):ftw019. <https://doi.org/10.1093/femspd/ftw019>.
106. Niska JA, Shahbazian JH, Ramos RI, Francis KP, Bernthal NM, Miller LS. Vancomycin-rifampin combination therapy has enhanced efficacy against an experimental *Staphylococcus aureus* prosthetic joint infection. *Antimicrob Agents Chemother*. 2013;57(10):5080–6. <https://doi.org/10.1128/AAC.00702-13>.
107. Holmberg A, Thórhallsdóttir VG, Robertsson O, W-Dahl A, Stefánsdóttir A. 75% success rate after open debridement, exchange of tibial insert, and antibiotics in knee prosthetic joint infections. *Acta Orthop*. 2015;86(4):457–62. <https://doi.org/10.3109/17453674.2015.1026756>.
108. Puhto AP, Puhto T, Niinimäki T, Ohtonen P, Leppilähti J, Syrjälä H. Predictors of treatment outcome in prosthetic joint infections treated with prosthesis retention. *Int Orthop*. 2015;39(9):1785–91. <https://doi.org/10.1007/s00264-015-2819-2>.
109. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-body infection (FBI) study group. *JAMA*. 1998;279(19):1537–41.
110. Jørgensen NP, Zobek N, Dreier C, Haaber J, Ingmer H, Larsen OH, et al. Streptokinase treatment reverses biofilm-associated antibiotic resistance in *Staphylococcus aureus*. *Microorganisms*. 2016;4(3):E36. <https://doi.org/10.3390/microorganisms4030036>.
111. Molina-Manso D, del Prado G, Ortiz-Pérez A, Manrubia-Cobo M, Gómez-Barrena E, Cordero-Ampuero J, et al. In vitro susceptibility to antibiotics of staphylococci in biofilms isolated from orthopaedic infections. *Int J Antimicrob Agents*. 2013;41(6):521–3. <https://doi.org/10.1016/j.ijantimicag.2013.02.018>.
112. Urish KL, DeMuth PW, Kwan BW, Craft DW, Ma D, Haider H, et al. Antibiotic-tolerant *Staphylococcus aureus* biofilm persists on arthroplasty materials. *Clin Orthop Relat Res*. 2016;474(7):1649–56. <https://doi.org/10.1007/s11999-016-4720-8>.
113. Bhattacharya M, Wozniak DJ, Stoodley P, Hall-Stoodley L. Prevention and treatment of *Staphylococcus aureus* biofilms. *Expert Rev Anti-Infect Ther*. 2015;13(12):1499–516. <https://doi.org/10.1586/14787210.2015.1100533>.
114. Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, et al. Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. *JAMA*. 2013;309(13):1368–78. <https://doi.org/10.1001/jama.2013.3010>. **Phase 2b/3 trial of vaccine from Merck (V710), targeting IsdB in patients undergoing cardiothoracic surgery. V710 failed to reduce infection rates or mortality. Moreover, patients who did get infected were more likely to die in the vaccine group, suggesting that this vaccine may actually impair host immunity against sepsis.**
115. McNeely TB, Shah NA, Fridman A, Joshi A, Hartzel JS, Keshari RS, et al. Mortality among recipients of the Merck V710 *Staphylococcus aureus* vaccine after postoperative *S. aureus* infections: an analysis of possible contributing host factors. *Hum Vaccin Immunother*. 2014;10(12):3513–6. <https://doi.org/10.4161/hv.34407>.
116. Shinefield H, Black S, Fattom A, Horwith G, Rasgon S, Ordonez J, et al. Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *N Engl J Med*. 2002;346(7):491–6.
117. Estellés A, Woischnig AK, Liu K, Stephenson R, Lomongsod E, Nguyen D, et al. A high-affinity native human antibody disrupts biofilm from *Staphylococcus aureus* bacteria and potentiates antibiotic efficacy in a mouse implant infection model. *Antimicrob Agents Chemother*. 2016;60(4):2292–301. <https://doi.org/10.1128/AAC.02588-15>.
118. Varrone JJ, de Mesy Bentley KL, Bello-Irizarry SN, Nishitani K, Mack S, Hunter JG, et al. Passive immunization with anti-glucosaminidase monoclonal antibodies protects mice from implant-associated osteomyelitis by mediating opsonophagocytosis

- of *Staphylococcus aureus* megacusters. *J Orthop Res*. 2014;32(10):1389–96. <https://doi.org/10.1002/jor.22672>.
119. Yokogawa N, Ishikawa M, Nishitani K, Beck CA, Tsuchiya H, Mesfin A, et al. Immunotherapy synergizes with debridement and antibiotic therapy in a murine 1-stage exchange model of MRSA implant-associated osteomyelitis. *J Orthop Res*. 2018; <https://doi.org/10.1002/jor.23801>. **Using a murine implant-associated infection model, the authors found that immunotherapy with anti-Gmd monoclonal antibodies inhibited SACs while vancomycin reduced CFUs on the implant. Combination therapy provided the best results in soft tissue and implant infection control.**
 120. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, et al. Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. *Nature*. 2015;527(7578):323–8. <https://doi.org/10.1038/nature16057>. **The authors created an antibody-antibiotic conjugate (AAC) that consisted of a monoclonal antibody that recognizes the alpha-O-linked N-acetylglucosamine sugars on wall teichoic acids (WTAs) bound to rifamycin class derivative antibiotic to target intracellular *S. aureus*. This AAC demonstrated improved results versus systemic vancomycin alone in a murine MRSA hematogenous infection model.**
 121. Hogan S, Zapotoczna M, Stevens NT, Humphreys H, O’Gara JP, O’Neill E. Potential use of targeted enzymatic agents in the treatment of *Staphylococcus aureus* biofilm-related infections. *J Hosp Infect*. 2017;96(2):177–82. <https://doi.org/10.1016/j.jhin.2017.02.008>.
 122. Hogan S, O’Gara JP, O’Neill E. Novel treatment of *Staphylococcus aureus* device-related infections using fibrinolytic agents. *Antimicrob Agents Chemother*. 2018;62(2):e02008–17. <https://doi.org/10.1128/AAC.02008-17>.
 123. Lauderdale KJ, Malone CL, Boles BR, Morcuende J, Horswill AR. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. *J Orthop Res*. 2010;28(1):55–61. <https://doi.org/10.1002/jor.20943>.
 124. Hardes J, von Eiff C, Streitbuerger A, Balke M, Budny T, Henrichs MP, et al. Reduction of periprosthetic infection with silver-coated megaprotheses in patients with bone sarcoma. *J Surg Oncol*. 2010;101(5):389–95. <https://doi.org/10.1002/jso.21498>.
 125. Wafa H, Grimer RJ, Reddy K, Jeys L, Abudu A, Carter SR, et al. Retrospective evaluation of the incidence of early periprosthetic infection with silver-treated endoprotheses in high-risk patients: case-control study. *Bone Joint J*. 2015;97-B(2):252–7. <https://doi.org/10.1302/0301-620X.97B2.34554>. **A case-control clinical study using silver-coated megaprosthesis (Alguna). The silver-coated prosthesis had reduced rates of postoperative infection (11.8 versus 22.4%) and had improved success after debridement and implant retention relative to titanium implants.**
 126. Alt V. Antimicrobial coated implants in trauma and orthopaedics—a clinical review and risk-benefit analysis. *Injury*. 2017;48(3):599–607. <https://doi.org/10.1016/j.injury.2016.12.011>.
 127. Kollef MH, Afessa B, Anzueto A, Veremakis C, Kerr KM, Margolis BD, et al. Silver-coated endotracheal tubes and incidence of ventilator-associated pneumonia: the NASCENT randomized trial. *JAMA*. 2008;300(7):805–13. <https://doi.org/10.1001/jama.300.7.805>.
 128. Schierholz JM, Lucas LJ, Rump A, Pulverer G. Efficacy of silver-coated medical devices. *J Hosp Infect*. 1998;40(4):257–62.
 129. van Hengel IAJ, Riool M, Fratila-Apachitei LE, Witte-Bouma J, Farrell E, Zadpoor AA, et al. Selective laser melting porous metallic implants with immobilized silver nanoparticles kill and prevent biofilm formation by methicillin-resistant *Staphylococcus aureus*. *Biomaterials*. 2017;140:1–15. <https://doi.org/10.1016/j.biomaterials.2017.02.030>.
 130. Kucharíková S, Gerits E, De Brucker K, Braem A, Ceh K, Majdič G, et al. Covalent immobilization of antimicrobial agents on titanium prevents *Staphylococcus aureus* and *Candida albicans* colonization and biofilm formation. *J Antimicrob Chemother*. 2016;71(4):936–45. <https://doi.org/10.1093/jac/dkv437>.
 131. Diefenbeck M, Schrader C, Gras F, Mückley T, Schmidt J, Zankovych S, et al. Gentamicin coating of plasma chemical oxidized titanium alloy prevents implant-related osteomyelitis in rats. *Biomaterials*. 2016;101:156–64. <https://doi.org/10.1016/j.biomaterials.2016.05.039>.
 132. Drago L, Boot W, Dimas K, Malizos K, Hänsch GM, Stuyck J, et al. Does implant coating with antibacterial-loaded hydrogel reduce bacterial colonization and biofilm formation in vitro? *Clin Orthop Relat Res*. 2014;472(11):3311–23. <https://doi.org/10.1007/s11999-014-3558-1>.
 133. Giavaresi G, Meani E, Sartori M, Ferrari A, Bellini D, Sacchetta AC, et al. Efficacy of antibacterial-loaded coating in an in vivo model of acutely highly contaminated implant. *Int Orthop*. 2014;38(7):1505–12. <https://doi.org/10.1007/s00264-013-2237-2>.
 134. Jennings JA, Carpenter DP, Troxel KS, Beenken KE, Smeltzer MS, Courtney HS, et al. Novel antibiotic-loaded point-of-care implant coating inhibits biofilm. *Clin Orthop Relat Res*. 2015;473(7):2270–82. <https://doi.org/10.1007/s11999-014-4130-8>.
 135. Ehrensberger MT, Tobias ME, Nodzo SR, Hansen LA, Luke-Marshall NR, Cole RF, et al. Cathodic voltage-controlled electrical stimulation of titanium implants as treatment for methicillin-resistant *Staphylococcus aureus* periprosthetic infections. *Biomaterials*. 2015;41:97–105. <https://doi.org/10.1016/j.biomaterials.2014.11.013>.
 136. Leary JT, Werger MM, Broach WH, Shaw LN, Santoni BG, Bernasek TL, et al. Complete eradication of biofilm from orthopedic materials. *J Arthroplast*. 2017;32(8):2513–8. <https://doi.org/10.1016/j.arth.2017.03.050>.