Periprosthetic Bacterial Biofilm and Quorum Sensing

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ABSTRACT: Periprosthetic joint infection (PJI) is a common complication after total joint arthroplasty leading to severe morbidity and mortality. With an aging population and increasing prevalence of total joint replacement procedures, the burden of PJI will be felt not only by individual patients, but in increased healthcare costs. Current treatment of PJI is inadequate resulting in incredibly high failure rates. This is believed to be largely mediated by the presence of bacterial biofilms. These polymicrobial bacterial colonies form within secreted extracellular matrices, adhering to the implant surface and local tissue. The biofilm architecture is believed to play a complex and critical role in a variety of bacterial processes including nutrient supplementation, metabolism, waste management, and antibiotic and immune resistance. The establishment of these biofilms relies heavily on the quorum sensing communication systems utilized by bacteria. Early stage research into disrupting bacterial communication by targeting quorum sensing show promise for future clinical applications. However, prevention of the biofilm formation via early forced induction of the biofilm forming process remains yet unexplored. © 2018 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

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Periprosthetic joint infection (PJI) is the leading cause of failure in total knee arthroplasty (TKA) and the third leading cause of failure in total hip arthroplasty (THA) in the United States.1 Antibiotics alone fail to eradicate PJI as bacteria persist through the formation of biofilm that promotes antibiotic tolerance.2,3 In the context of arthroplasty, biofilm is a community of extracellular polymeric matrix and bacteria that adheres to an implant.4

Here, we review the recent literature on biofilms in PJI as well as propose novel strategies to prevent and cure biofilm infections by targeting quorum sensing.

SEARCH METHODOLOGY

The primary databases utilized in the literature search were PubMed, PubMed Central (PMC), and Google Scholar. Additionally, publications referenced by articles generated in this search were considered. The Medical Subject Heading (MeSH) terms, “quorum sensing” and “biofilm” were used to focus the review in the PubMed and PMC databases. These terms in combination yielded 945 and 317 publication in PubMed and PMC respectively from January 1995 to March 2018. Title and key terms including; periprosthetic, implant, autoinducer, and quorum quenching were utilized to further differentiate these search results. The resulting potential literature subset consisted of 145 and 214 publications in PubMed and PMC respectively.

THE BURDEN OF PJI

PJI is very common with an average 1 year incidence of 0.4–2% for TKA5–8 and 0.25–1.0% for THA with an even greater incidence rate of infection in revision surgery ranging from 3.2% to 5.6% for both knees and hips.9 The most common organisms in these infections are Staphylococci species, specifically coagulase-negative staphylococci such as Staphylococcus epidermidis (30–43%) and coagulase-positive Staphylococcus aureus (12–23%). Staphylococci bacteria are believed to account for over 50% of all biofilm infections of medical devices.10–11 Biofilms are estimated to be the cause of 65% of all human infections.12–14

Effective treatment of PJI is currently a topic of discussion and debate. Antibiotics have decreased efficacy despite achieving what would normally be considered therapeutic levels within the biofilm, suggesting that the biofilm colony and structure imparts some antibiotic resistance beyond simply providing a physical barrier.15 Failure to achieve sustained bacterial eradication with antibiotics requires removal of the biofilm-contaminated implant via a one- or two-stage revision surgery. The cost of this revision surgery averages $25,692 for a TKA and $31,753 for a THA, making it the most costly cause of revision apart from periprosthetic fracture.1 In total, the effective treatment for methicillin-resistant PJI has been estimated to have a mean direct cost of $107,264, compared to $68,053 for the treatment of sensitive strains.16 In one model of total long-term economic effect, the costs of an infected THA ranged from $389,307 (65 years old with a 3% reinfection rate) to $474,004 (55 years old with a 12% reinfection rate), largely due to lost wages.17 Infection accounts for up to 12% of the indications for revision hip arthroplasty,
and 22% for revision knee arthroplasty, as recorded in the National Joint Registry 10th Annual Report.\textsuperscript{18}

Biofilm-associated bacterial infections and the study of the underlying mechanisms driving this phenomenon has gained significant traction in recent years. Within the PubMed database, the biology of biofilms was cited as a MeSH term in 1,533 publications in 2017, with quorum sensing in particular being cited as a MeSH term in 241 publications in 2017.

**BIOLFM DEVELOPMENT**

The development of biofilms progresses through several sequential stages, each associated with characteristic phenotypes.\textsuperscript{20,21} These stages are typically called initial attachment, irreversible attachment, maturation, and dispersal\textsuperscript{22} (Fig. 1).

The development of bacterial biofilms begins with an initial, short-lived interaction of free-floating, planktonic, bacteria with an inert or living surface. During the initial attachment phase in the context of implants, bacteria primarily form attachments to the implant surface. *S. epidermidis* and *S. aureus* are known to specifically express proteins during this growth phase that form strong interactions with host extracellular matrix (ECM). These proteins are thought to be critical to the attachment of bacteria to foreign bodies, since ECM coats foreign bodies as soon as they enter the body. Extracellular DNA (eDNA) also accumulates in the attachment phase via bacterial autolysis. Although the role of eDNA in biofilm formation is not entirely understood, it is believed to contribute to biofilm stability, perhaps as an intercellular adhesin. Extracellular DNA is believed to have different roles for *S. epidermis* and *S. aureus*.\textsuperscript{18}

This is followed by irreversible attachment to that surface and loss of motility. At this point, new planktonic bacteria can interact with adherent bacteria on the surface and form a bacterial polymicrobial microcolony. Throughout this attachment growth phase, in addition to population expansion, bacteria produce extra-cellular compounds known as autoinducers.\textsuperscript{23,24} These auto-inducers serve as inter- and intra-bacterial signals, specifically communicating the local density of the bacterial population.

Quorum is the critical density needed to overwhelm the innate immune system and establish a biofilm colony.\textsuperscript{25,26} Upon reaching a critical local density (i.e., quorum), a signal cascade is initiated causing a variety of changes in bacterial behavior and gene expression, some known and many unknown. The net result is that the bacterial population cohesively shifts away from replication and instead expresses virulence factors such as secretion systems, toxins, or biofilm formation.\textsuperscript{27,28} This microcolony secretes a complex mixture of carbohydrates, proteins, lipids and nucleic acids forming a biofilm. Biofilm provides mechanical stability for other microcolonies and forms a three-dimensional scaffold that immobilizes those microcolonies. The biofilm allows the microcolonies to evade the immune response and mature, growing in both size and thickness with more limited replicative activity compared to the initial and irreversible attachment phases.\textsuperscript{29,30}

Once microcolonies mature, bacteria regain motility and begin to disperse, reverting from a member of the microcolony back to solitary planktonic bacteria. Typically, it is the bacteria at the center of the biofilm that mature and detach, creating cavities that increase access to nutrients for the remaining microcolonies within the biofilm.

**QUORUM SENSING**

The quorum sensing machinery is the fundamental chemical signaling means by which bacterial populations communicate, coordinate, and cooperate. In addition to biofilm formation, quorum sensing regulates processes such as bioluminescence, sporulation, antibiotic production, and virulence factor secretion.\textsuperscript{31} All known quorum sensing systems are fundamentally very simple. Each member of a specific bacterial population produces a common signal (i.e., autoinducers), such that all members receive the same signal. The cumulative result of each individual expressing and sensing a common autoinducer is the creation of a population based effect determined by

Figure 1. Schematic representation of the biofilm life cycle. (A) Planktonic bacteria adhere to a surface using cell surface displayed adhesin molecules. (B) Inter-bacterial adhesins promote further polymicrobial colony expansion. (C) Bacteria begin to divide and the expression of further macromolecules allows them to stick together in small microcolonies. (D) As these colonies grow, they begin to secrete a complex mixture of carbohydrates, protein, and lipids that encapsulates the bacteria that is, biofilm. This biofilm matrix provides protection and stability for the maturing bacterial microcolony. (E) When the biofilm reaches maturity, a number of factors will have developed a heterogeneous arrangement of cells and molecules within the biofilm, giving rise to solvent filled cavities and channels as well as virulence factor secretion. (F) This can lead to dispersal of cells from the cellular mass. Upon signal from the environment (e.g., waste build up or demand for nutrients), molecules are released that cause cell lysis and matrix dissemination. Many planktonic cells are released and can find a new habitat.
autoinducer concentration. In the case of bacteria at low density, the secreted autoinducers diffuse into the environment and do not effectively stimulate population-based effects. At a higher density, however, the secreted autoinducer accumulates faster than its diffusion into the environment and a positive feed-forward loop stimulates population-based effects.\textsuperscript{27,32} The advantageous expression of virulence genes is directly linked to the density of these autoinducer molecules in the local environment.

Gram-negative as well as gram-positive bacteria both utilize quorum sensing strategies, albeit with some minor mechanistic differences (Fig. 2). Gram-positive bacteria use large autoinducing peptides which must be transported into the extracellular space. At the threshold density, these signals accumulate and bind membrane kinase receptors, thereby activating transcription of certain genes. Alternatively, gram-negative bacteria use small molecule autoinducers such as acyl-homoserine lactones (HSLs) that can freely diffuse from the intra-cellular to extra-cellular spaces.\textsuperscript{33} At appropriate cellular density, these signals can either bind cytoplasmic transcription factors or kinase receptors similar to those described above. These autoinducers facilitate intra-species communication; furthermore, the conserved nature of quorum signaling mechanisms across bacterial species allows for inter-species communication and provides a plausible explanation for how polymicrobial biofilm colonies form. The conservation of this process across species also suggests the efficacy that successful quorum sensing targeted therapies could have against bacteria and how difficult it could be for resistance to develop, even in polymicrobial biofilms.

**BIOFILM PROPERTIES**

Biofilms are a collection of bacteria attached to a surface and surrounded by a self-generated extracellular polymeric substance (EPS) matrix that controls the local bacterial environment. The EPS comprises the bulk of the biofilm structure. It is secreted by bacteria after initial attachment to a surface and is composed of polysaccharides, proteins, nucleic acids, and lipids. The EPS matrix acts as a physical barrier that protects cells from the host immune system and limits diffusion throughout the biofilm.\textsuperscript{34,35} Biofilm serves several other functions. It limits diffusion of excreted enzymes away from bacterial cells, acting as a type of external digestive system enabling metabolism of dissolved, colloidal, and solid biopolymers.\textsuperscript{36} It also acts as a scaffold to keep cells in close proximity to each other, enabling cell–cell communication and coordination of activities.\textsuperscript{37} When bacterial cells lyse, the matrix retains the nutrients for reuse, including DNA.

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**Figure 2.** Bacterial quorum-sensing circuits, include (A) Autoinducing peptide (AIP) quorum-sensing in Gram-positive bacteria by two-component signaling or (B) an AIP-binding transcription factor and (C) small molecule quorum-sensing in Gram-negative bacteria by a LuxI/LuxR-type system or (D) two-component signaling.
that can enhance gene transfer within the biofilm.\textsuperscript{38} Finally, biofilms have demonstrated strain-hardening properties, becoming increasingly stiffer with increased strain, which may help to explain why biofilms remain after debridement.\textsuperscript{39,40} Biofilms are composed of 10% cells and 90% EPS.\textsuperscript{36} The basic unit within a biofilm structure is a microcolony of cells embedded within EPS. Water and nutrients are able to flow through the biofilm matrix due to channels that form between each microcolony.\textsuperscript{41} The biofilm structure is reactive to environmental conditions. For example, increased nutrient levels such as higher glucose results in increased biofilm growth.\textsuperscript{42} Sessile bacteria within a biofilm are phenotypically distinct from planktonic bacteria, exhibiting a slower growth rate and an enhanced ability to form biofilm EPS. A biofilm can either be composed of a single type of bacteria or can be polymicrobial.\textsuperscript{43} The bacterial constituency of the biofilm is critical to both biofilm survival, as well as susceptibility to any potential therapeutics. Polymicrobial biofilms provide a number of advantages to survival as compared to homogenous biofilms. These include passive resistance (when one member of a biofilm possesses a resistance factor that protects other colony members), metabolic cooperativity, by-product influence, shared quorum sensing systems and an enlarged gene pool to facilitate DNA sharing.\textsuperscript{44}

The mechanism for increased antimicrobial agent resistance by bacteria in biofilms is not completely understood. One hypothesis is that the EPS creates a diffusion barrier, limiting the concentration of antimicrobial agents within the biofilm.\textsuperscript{45} In addition to acting as a physical barrier to diffusion, components of the EPS are charged and can exclude charged antimicrobials, further limiting penetration.\textsuperscript{46} Despite these barriers, there are examples in the literature of antimicrobial agents that do not interact with the EPS matrix and are able to penetrate the biofilm, yet resistance still persists relative to bacteria in the planktonic state.\textsuperscript{47} A second hypothesis for increased resistance is that the limited supply of nutrients in the biofilm triggers a stress response in the cells, resulting in bacteria entering a stationary growth phase with decreased metabolic activity.\textsuperscript{48} This is in line with the decreased replication and increase in secretory systems seen during the maturation phase of biofilm development. Entry into the stationary growth phase decreases the effectiveness of the antimicrobial agents, which target replicative machinery.\textsuperscript{49} Because access to nutrients is heterogeneous within the biofilm, there could be significant metabolic diversity within the biofilm, conferring different levels of antimicrobial resistance.\textsuperscript{50} These two hypotheses predict the existence of persister cells, a subset of the bacterial population that are resistant to high concentrations of antimicrobial agents capable of destroying the rest of the biofilm population.\textsuperscript{51,52} However, persister cells can be identified even in immature biofilms that are not thick enough to prevent penetration of antimicrobial agents or nutrients.\textsuperscript{53} Persisters exist both in planktonic and biofilm states, but the planktonic forms of persister cells can be eliminated by host immune cells while biofilm persister cells are sheltered from the innate immune response by EPS.\textsuperscript{54} When antimicrobial concentrations decrease, the persister cells can then repopulate the biofilm, resulting in a relapse of an infection.\textsuperscript{55}

There are several proposed methods for the increased survival of persister cells. One such mechanism is disabling the apoptosis pathway.\textsuperscript{56} In this study, inhibition of the TORC1-activated Ras pathway significantly increased the level of amphotericin B cells in \textit{S. cerevisiae}. Since Ras signaling mediates programmed cell death upon exposure to anti-fungal agents, it was speculated that the persister cells achieved resistance by disabling this apoptosis pathway. Another possible method for increased persister cell survival involves toxin-antitoxin regulation systems.\textsuperscript{2,57–59} A toxin is a substance that disrupts essential cellular processes, while an antitoxin prevents activation of the toxin. Under stressful conditions, such as exposure to antimicrobial agents, an antitoxin is triggered to inactivate the toxin or induce metabolic dormancy. After removal of the stress, metabolic processes resume.

Biofilms modulate the inflammatory response of the immune system. Biofilms release factors into the local environment that polarize macrophages toward an anti-inflammatory and pro-fibrotic milieu, favoring biofilm persistence.\textsuperscript{60} By secreting as yet unidentified factors, biofilms are able to recruit myeloid-derived suppressor cells.\textsuperscript{61,62} Myeloid-derived suppressor cells suppress pro-inflammatory macrophages, preventing a robust immune response. Furthermore, biofilms release factors such as alpha-toxin and leukocidin AB that induce macrophage dysfunction such as decreased secretion of pro-inflammatory cytokines, inhibition of phagocytosis, and cytotoxicity.\textsuperscript{63} The physical barrier of the EPS also makes it difficult for macrophages to perform phagocytosis, as well as detect pathogen-associated molecular patterns. Together, these mechanisms aid in the persistence of bacteria within a biofilm through manipulation of the host immune response.

**BIOFILM-ASSOCIATED CHALLENGES**

Clinical management of biofilm-associated infections is a challenging and multifactorial problem. Diagnostic challenges persist, as chronic local inflammation may be the only clinical indication for infection, and yet not specific to infection. Additionally, standard screening methodologies may detect the presence of biofilm colonies on medical devices and implants, though these findings do not necessarily correlate with clinically significant infections. Orthopedic device guidelines recommend sampling local tissue for biofilm colonies. The recommendations include that a
physically larger sample and multiple samples are preferred, which brings a significant associated morbidity to the patient. The guidelines also suggest that antimicrobial susceptibility of planktonic microorganisms should be evaluated, however, this susceptibility may not translate well to a sessile biofilm colony. Furthermore, it can be clinically challenging to determine the success of an antibacterial regimen against biofilm, since the indirect serum screening tests available may produce false negatives in the presence of antibiotic treatment, with the potential of relapse upon cessation of treatment.

The formation of biofilms by bacteria creates significant challenges for treating infections because of the unique properties bacteria have in biofilms compared with planktonic bacteria. Bacteria in biofilms are significantly more resistant to antimicrobial agents than planktonic bacteria, with minimal concentrations for eradication 10- to 1,000-fold higher for biofilm bacteria. It can be difficult to reach these effective levels in vivo because of side effects and toxicities associated with antimicrobial agents. Interestingly, biofilm bacteria with increased resistance have identical minimum inhibitory concentration to planktonic controls after they disperse from the biofilm. This indicates that resistance is due to bacterial association with the biofilm or phenotypic changes.

Within the context of quorum sensing and potential therapeutic approaches, potential challenges are already beginning to arise. Despite the shared mechanics of quorum sensing across bacterial species, it is often a species-specific process, exhibiting specificity even within a species. S. aureus and S. epidermidis are known to utilize similar agr derived systems, but distinct AIP-AgrC type receptor pairs, with subgroups I–IV of S. aureus and subgroups I–III of S. epidermidis each utilizing distinct AIP:AgrC pairings (Fig. 3). The primary challenge will be in developing agents that interact with quorum sensing systems across a diversity of bacterial species so that they are not overly specific and may address polymicrobial biofilms.

**CURRENT CLINICAL PRACTICE**

The prevention and treatment of bacterial infections caused by biofilm-forming microorganisms presents unique challenges. Current clinical practice is predominately limited to the use of prophylactic systemic antibacterial administration and mechanical disruption of biofilm formation.

Standard protocols for treatment and management are largely inconsistent, with little data to support drawing definitive conclusions for optimal treatment strategies. Current treatment strategies largely fall into one of two categories: Prophylactic treatment for any bacterial activity to prevent biofilm formation, or mechanical disruption and/or explantation after biofilm formation. There is some literature to support treatment of early stage infection with biofilm-validated therapies such as fluoroquinolones for gram-negative infections, however, the cure probability cannot be well predicted in advance of treatment.

Preoperative prophylactic antibiotic administration in orthopedic trauma has been shown to be effective in preventing biofilm formation. However, in the case of total joint arthroplasty, *Staphylococcus* and *P. acnes* biofilms can form on implant surfaces despite preoperative antibiotic prophylaxis. Thus, rather than these systemic preventative measures, the field of joint
arthroplasty has turned toward local and targeted antibiotic action. The delivery method of choice for the predominance of these strategies has been antibiotic impregnation of the implants being used, such as covalently bonded antibiotic-titanium complexes, vancomycin impregnated cement or implant coatings, vitamin E blended ultra-high molecular weight polyethylene, and hydroxyapatite coatings loaded with gentamycin. Although these preventative strategies and others have shown varying efficacy in mitigating biofilm-formation and prosthetic infection, it has come at the cost of increased antibiotic resistance. In one study in which varying doses of cefazolin were applied directly to S. aureus cultured on total knee arthroplasty material, viable biofilm mass remained, likely due to bacterial persisters imbuing biofilms with incredibly high antibiotic tolerance. Furthermore, it has been demonstrated repeatedly that antibiotics such as rifampin are capable of penetrating biofilms completely at therapeutic concentrations, however, viable bacteria still remain. This suggests that the antibiotic-resistant properties of biofilm are not simply due to acting as a physical barrier to antibiotic exposure.

When biofilm prevention fails, the alternative treatment strategy currently available is either mechanical disruption of the biofilm, or if applicable, removing the infected medical device or implant. Both procedures are associated with high morbidity since they are invasive procedures. Additionally, mechanical disruption is not well understood, with little widely disseminated empirical evidence to establish standard protocols. In the instance of orthopedic trauma, high pressure pulse lavage as compared to low pressure pulse lavage did not decrease the rates of reoperation, and may induce further soft tissue damage. In the case of total joint arthroplasty, pulse lavage was found to reduce the biofilm signal intensity by less than 10-fold and low-frequency sonication was found to not only incompletely eradicate biofilms, but also increased surface roughness of implants (maximum peak to valley height) and reduce articular cartilage thickness. Because mechanical disruption is often inadequate, removal of the infected device or implant is often necessary when possible. In the United States, PJIs is commonly treated via a 2-stage exchange arthroplasty, however, biofilm can persist in the surrounding soft tissue, adding clinical complexity.

CURRENT QUORUM SENSING ASSOCIATED TREATMENT STRATEGIES

Because of the inability to entirely eradicate biofilm-forming bacteria or biofilms once established, recent research has been dedicated to understanding and disrupting the fundamental mechanisms by which bacteria form biofilms. The focus and mechanism of interest has largely been quorum sensing, the way in which bacterial cells communicate and coordinate virulence factors to overcome the host immune system and establish a biofilm colony.

An extensive array of compounds are being evaluated for clinical use in biofilm prevention. The targets of these compounds vary widely and encompass the entirety of the quorum sensing pathway. This includes inhibition of quorum sensing signal production through degradation or substitution of SAM or acyl-ACP (precursors to acyl-homoserine lactone autoinducers). Sequestration of quorum signals via antibodies or certain proteins such as Apolipoprotein B. Disruption of membrane bound large peptide quorum signal transporters (for gram-positive bacteria). Competitive inhibition of the quorum sensing signal binding sites. Disruption of the kinase domain to impair quorum signal transduction. However, to the best of our knowledge, they all have one common strategy. All therapeutic compounds evaluated thus far aim to a dysregulate quorum sensing by breaking the communication pathways or signal cascades used by bacteria. Although some success has been achieved in the laboratory using these strategies, so far only a single strategy has been shown an improvement in vivo. Using high throughput screening, one group identified a small molecule inhibitor, savarin, which targets the Agr-A transcriptional regulator and disrupts the agr-mediated quorum sensing pathway. A reduction in tissue injury due to S. aureus was demonstrated. This study additionally demonstrated an inability of the bacteria to develop a resistance to the treatment strategy over repeated exposures. None of these strategies are currently being employed in a clinical setting.

A NOVEL TREATMENT STRATEGY

In this review of existing and ongoing treatment strategies, one particular approach to managing biofilm infections was found to be missing. Biofilm-associated infections are unique in that often the bacteria individually are not inherently resistant to treatment or immune eradication. Rather, they have adapted and evolved to coordinate their efforts in such a way that the sum efforts of a high-density bacterial colony can overcome the immune response and then establish a stable and protective biofilm matrix. The key to their success is the timing of that coordinated effort. As previously discussed, it is the feed-forward control mechanism of quorum sensing that enables bacteria to coordinate their gene expression temporally.

Instead of attempting to entirely disrupt a highly conserved and often redundant circuitry it may be possible to utilize that very same mechanism against the bacteria. Through early forced induction of the quorum sensing cascade, bacteria could potentially be forced into a biofilm forming behavior before they have reached the necessary critical density to overcome the immune system (Fig. 4). In this way, the highly specific and targeted immune system could be used to eradicate bacterial infections before they can...
form antibiotic resistant biofilms. In the case of gram-negative bacteria for instance, local injection of acyl-Homoserine Lactones could elevate the local density of that signal molecule, thereby signaling a higher local cell density than what is truly present. The feed-forward mechanism would sustain the quorum response and the bacteria would begin to express virulence factors that would enable the host immune system to identify and eradicate the bacteria. The success in expressing these virulence factors is highly dependent on having an adequate bacterial population before expression.

Such a strategy could prove incredibly difficult for bacteria to develop resistance, since quorum sensing is a highly conserved process. Additionally, the final stage of biofilm formation and propagation is dispersal, the process by which bacterial cells are sloughed from the biofilm returning to their planktonic form. This dispersal stage is crucial to the survival and propagation of the bacteria, and some research suggests that, rather than being a passive process, the same auto-inducer molecules used to signal quorum play a vital role in signaling dispersion. Although biofilms create a highly antibiotic-resistance environment, the planktonic bacteria which are shed can be more than 15 times as susceptible to antibiotics. A mechanism that forces early induction of quorum may also force premature and overly active dispersal of bacteria from biofilms, making the planktonic bacteria and biofilm-embedded bacteria more susceptible to antibiotic and innate host immune system eradication.

This approach of forced induction is not without challenges. Specifically, the ability to accurately replicate quorum sensing molecules in such a way to induce an early quorum response, and the ability to deliver that therapy in a targeted way as to not instigate host-wide bacterial activation. Additionally, it has been shown that certain autoinducers, specifically some acyl-homoserine lactones actually interact with the host immune system to downregulate the expression of important factors such as TNF-α and interleukins such as IL-12.

**RECOMMENDATIONS**

Biofilm-associated infections continue to present many clinical challenges. Current clinical practice in the management of these infections is non-standardized with inconsistent and disappointing evidence to support current practices. The field of bacterial quorum sensing holds significant promise for a potentially novel means to address biofilm formation. Current research has entirely focused on the strategy of disrupting the quorum sensing mechanism, which has proven promising, however, has yet to produce clinical therapies. A potential novel strategy suggested here would be an investigation into utilizing the quorum sensing mechanism to force early induction of bacteria into a weakened biofilm forming and active state, thereby increasing the efficacy of antibiotic treatment and also allowing the innate host immune system to eradicate bacterial colonies. The critical next steps would be to validate the proposed quorum inducing mechanism in vivo. This would require identification of quorum inducing molecules in species of interest (S. aureus, S. epidermidis, etc.) and achieving premature biofilm formation with the addition of these autoinducers. Immediately following, would be investigating the effect premature biofilm formation has on the efficacy of antibiotics and innate host immune response.

**AUTHORS’ CONTRIBUTIONS**

Jake Mooney, Eric Pridgen, and Derek Amanatullah all contributed substantially to writing this review paper. Robert Manasherob, Gina Suh, Helen Blackwell, Annelise Barron, Paul Bollyky, and Stuart Goodman all made significant edits and were involved in the proofreading process. All authors have read and approved the final submitted manuscript.

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