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The Role of *Propionibacterium acnes* Biofilm in Acne Vulgaris

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Abstract

Acne vulgaris is traditionally known as the result of excess sebum production, follicular hyper keratinization, infection with *Propionibacterium acnes*, and follicular inflammation. However, the role of *P. acnes* is gaining momentum as the primary impetus in the pathogenesis of acne vulgaris. The biofilm-forming ability of *P. acnes in vitro* and on indwelling medical appliances and catheters has been known for quite some time, but only in the last decade has the presence of *P. acnes* biofilm been observed within the pilosebaceous unit. Since that time, the genome of the microbe has been sequenced, and genes responsible for quorum sensing and biofilm formation have been confirmed. As a result of biofilm formation, the virulence of *P. acnes* is amplified. Biofilm-encapsulated *P. acnes* upregulates the production of lipases, resulting in the production of free fatty acids. Free fatty acids as well as the biofilm itself bind the Toll-like receptors TLR2 and TLR4, activating the innate immune system, culminating in a robust inflammatory response. Biofilms also appear to significantly contribute to antibiotic resistance. Acting as a physical barrier for antibiotics as well as a commune for bacteria to exchange antibiotic resistance genes, the biofilm plays an integral role as the major hurdle to treating acne vulgaris. Traditional antimicrobials have not shown much promise in disruption of the biofilm. Other approaches have been investigated utilizing a variety of novel topical compounds that act to prevent or disrupt the biofilm as a therapeutic modality. The role of biofilm in the pathogenesis of acne vulgaris has remained underappreciated, but with global antimicrobial resistance looming, further attention may be warranted.

Keywords: Acne; *P. acnes*; Biofilm; TLR2; Antibiotic resistance

Introduction

Acne vulgaris is a common cutaneous order involving dysfunction of the pilosebaceous unit. Acne vulgaris affects up to 80% of Americans at some point in their lives. Though prevalent most frequently in adolescents, it may persist well into adulthood for some individuals [1]. The traditional paradigm of acne has generally consisted of four components, often presented in a sequential fashion. Androgen excess leads to increased [1] sebum production, along with [2] follicular hyperkeratinization which results in plugging of the follicle. This allows the bacterium [3] *Propionibacterium acnes* to grow within the follicle, eventually culminating in an [4] inflammatory cascade and clinically evident disease.

It has, however, become clear that these four factors frequently interplay with each other and create recalcitrant disease. *Propionibacterium acnes*, a gram negative anaerobic rod, traditionally considered as a normal component of skin flora. *P. acnes* are categorized into phylotypes IA, IB, II, and III based on sequencing of their *recA* and hemolysin genes (*tly*) [2]. This microbe, however, plays a pivotal role in the pathogenesis of acne vulgaris *via* its biofilm forming ability.

Biofilm Formation of *P. acnes*

Biofilms consist of a group of microorganisms, adherent to one another, often attached to a surface. The microbes are protected within an extracellular matrix comprised of exopolysaccharides (high molecular weight polysaccharides), proteins (especially amyloid), and/or nucleic acids. In fact, the majority of all microbes exist primarily in biofilms [3]. This extracellular matrix comprises 2/3 of the mass of the biofilm itself and acts as a protective barrier from the outside world [4]. The matrix confers resistance to penetration of antibiotics through the biofilm into the microbes [5,6]. Classically, biofilms were considered to be primarily associated with catheter or implant-associated infections, as well as dental plaques [7]. The earlier years of research had clearly demonstrated the formation of *P. acnes* forming biofilms *in vitro* and on medical appliances, but it had yet to be discovered as a biofilm within the follicle itself [8]. This has become an important focus for current research, namely the impact of *P. acnes* biofilm in the acne follicle. Furthermore, the biofilm-forming ability of *P. acnes* may further compound other infectious diseases by having been observed to act as a reservoir for *Staphylococcus aureus* [9].

The general schema consists of free-living planktonic microorganisms communicating with one another *via* quorum sensing to become a sessile mass of bacteria by forming a biofilm that matures over time [8]. A significant increase in the production of a quorum sensing molecule, autoinducer-2, as planktonic *P. acnes* cells transition to become sessile microbes in mature biofilms has been demonstrated [10]. *P. acnes* biofilms were first clearly hypothesized by Burkhart and Burkhart in 2003 as a major factor in the pathogenesis of acne vulgaris [11]. Shortly following this publication, the complete genome sequence of *P. acnes* was published and biofilm-forming genes of the bacterium were soon identified within the sequence. The biofilm of *P. acnes* is comprised primarily of a glycocalyx polymer, requiring several enzymes including UDP-N-acetylglucosamine 2 epimerase and glycosyl transferases [12,13].

Biofilm formation of *P. acnes* is histologically observed in the pilosebaceous unit. Jahns et al. have demonstrated 47% of patients with

acne vulgaris reveal *P. acnes* on skin biopsy compared with 21% of control individuals [14]. Affected individuals also demonstrate *P. acnes* biofilms within the follicle in 37% of patients compared with only 13% of controls. To date, as a totally new observation, four patterns of *P. acnes* biofilms have been characterized: attachment of the bacterium to the follicle wall, attachment to the hair shaft, spreading over the lumen of the hair follicle, and biofilms in the center of the follicle without any discernable attachment to the follicle wall [15]. The dogma that increased sebum production and follicular hyperkeratinization lead to follicular plugging has been questioned over the years on the basis that sebum, in and of itself has not been shown to have sticky or cohesive properties in human or animal models [16]. Burkhart and Burkhart have proposed the extracellular biofilm matrix may play a role in the follicular plugging and cohesiveness seen in acne vulgaris [11]. The microbiome done hypothesis may not lie upstream in the pathogenesis of acne vulgaris, but rather may be a manifestation of the bacterium's sticky glycocalyx matrix.

Lipase Up Regulation and Toll-Like Receptors

Coenye et al. have investigated virulence factors of *P. acnes* within a biofilm as compared to planktonic organisms [9]. In their experiment, they cultured *P. acnes in vitro* and allowed 24-48 h for growth and maturation of the biofilm. Using fluorogenic substrates, they discovered the upregulation of lipase expression. Lipase is one of the major metabolic enzymes of *P. acnes* responsible for hydrolysis of the ester bonds within triglycerides contained in sebum to convert to glycerol and free fatty acids. The bacterium then has the ability to utilize the free fatty acid and metabolize it into short chain fatty acids, butyric acid and propionic acid, the name from which this microbe is derived. In this study, triglyceride hydrolysis of sessile versus planktonic *P. acnes* was upregulated 3-62 fold depending on the bacterial subtype and free fatty acid length.

The implications of biofilms are multifold *via* lipases, the hydrolysis of sebum triglycerides into oleic, palmitic, and lauric free fatty acids serves as a source of energy for the bacterium and allows for energy for proliferation of the bacterium. Additionally, another pathogenic mechanism is at play with upregulation of lipases, based on activation of Toll-Like receptors. Toll-like receptors (TLRs) are a class of proteins that are major player of the innate aspect of the immune system. These receptors are categorized into 11 separate receptors (TLR1-11) based on morphology and ligand-binding ability. The receptors are present on keratinocytes, dendritic cells, and macrophages. These receptors bind DAMPS (Damage-associated molecular patterns) as well as PAMPs (Pathogen-associated molecular patterns). Of particular interest, TLR4 recognizes lipopolysaccharides of Gram-negative bacteria, and TLR2 recognizes lipoteichoic acid and peptidoglycan of Gram-positive bacteria. Additionally, TLR2 and TLR 4 have been postulated to bind biofilms (PAMP) as well as free fatty acids (DAMP) [17,18]. Tukel has demonstrated receptor sites for TLR2 on biofilms from gram negative organisms [19]. By binding these Toll-like receptors, innate immunity is activated *via* the MyD88 pathway, leading to NF-kB and the production of numerous proinflammatory cytokines including TNF-a, IL-1beta, IL-6, IL-12, IL-18, IL-23, beta-defensin, among others [18,20-22]. The production of these mediators results in a robust inflammatory response.

Biofilms in acne are somewhat similar in activity as those in atopic dermatitis where the staphylococcal biofilms occlude sweat ducts and upregulate TLR 2 which activates the MyD88 pathway leading to TNFα and upregulates the production of PAR 2. The TLR 2 is the most potent agent at causing spongiosis and the PAR 2 is a potent pruritogen [19]. In acne, the biofilm not only activates TLR 2 which generates TNFα and other cytokines, but also generates lipases that are so important in the production of free fatty acids. Biofilm formation in the two disorders differs in that they form in the sweat ducts because of the presence of salt and water; and, in acne, they form in the follicle mainly as a result of genes directing the quorum sensing mechanism [12].

Effect of *P. acnes* Biofilms on Antibiotic Resistance

Biofilms have been long known to negatively impact the efficacy of antimicrobial therapy. *In vitro* studies of *P. acnes* biofilms have demonstrated this fact as well. Increased resistance of *P. acnes* to antimicrobials can be attributed to less drug penetration through the biofilm, slower microbial growth, horizontal transfer of drug resistance genes, as well as "persister" cells [23]. In the "persister" state, the microbe is metabolically inert, and this less susceptible to the effects of antibiotics. Coenye et al. compared the susceptibility of planktonic versus sessile bacteria to antibiotics [10]. The antimicrobials tested included erythromycin (E), Clindamycin (C), Azelaic acid (AA), salicylic acid (SA), triclosan (Tric), minocycline (M), benzoyl peroxide (BPO), BPO+E, BPO+C, Doxycyline (D), and Oxtetracycline (Ot). Of all agents tested, only AA, E, SA, Tric, M, BPO+C, and BPO+E resulted in significant reduction of the biofilm biomass. The remaining D, Ot, and BPO did not reach statistical significance. The bactericidal effect against these sessile organisms was only seen with AA, Tric, BPO+C and BPO+E (defined as >99.9% reduction). Interestingly, all antimicrobials tested were effective against planktonic *P. acnes*. The impact of biofilm-forming abilities of *P. acnes* may be appreciated by the extended duration of time antibiotics must be administered for satisfactory treatment of acne vulgaris.

Disruption of the Biofilm

The evidence suggests the major hurdle in treating acne vulgaris lies at the heart at disrupting the biofilm of Propionibacterium acnes. To date, multiple novel approaches have been attempted in addition to the previously discussed traditional approaches, in which only E, SA, Tric, M, BPO+C, and BPO+E have been shown to decrease biofilm mass [9]. Though tetracyclines have not shown to significantly decrease biofilm mass, the combination of tetracycline and ellagic acid both *in vivo* and *in vitro* have demonstrated antibiofilm effects [24]. Coenye et al. have subjected the P. acnes biofilm to extracts to 119 plant compounds and have found that five resulted in a potent antibiofilm activity: *Epimedium brevicornum*, *Malus pumila*, *Polygonum cuspidatum*, *Rhodiola crenulata* and *Dolichos lablab* [25]. The two chemical components isolated, icariin and resveratrol, were shown to exhibit marked antibiofilm activity against *P. acnes*. Other plant based approaches have been investigated, including a *Myrtus communis* extract which has been shown to decrease biofilm mass, resulting in less antibiotic resistance in *P. acnes* [26]. Most recently, decandiol has exhibited both antibiofilm and antimicrobial effects against this bacterium, perhaps acting *via* though a surfactant-like mechanism of action [27]. Allen et al. have summarized other topical biofilm dispersers including silver, selenium, cinnamates, curcumin, flavonoids among others [28]. Oral agents such as rifampin have been shown to be effective biofilm dispersers as well [28]. Combinations of the biofilm dispersers with antibiotics offer new avenues for research and treatment.

Prevention of the formation of biofilm is yet another avenue that has been proposed as a therapeutic modality. Brackman et al. have used two thiazolidinedione drugs, (Z)-5-octylidenethiazolidine-2,4-dione (TZD8) and (Z)-5-decylidenethiazolidine-2,4-dione (TZD10), believed to play a role in inhibiting Autoindicer-2 dependent quorum sensing, the major mechanism by which *P. acnes* forms a biofilm [29]. These drugs have been found to prevent the formation of biofilms, without any direct toxic effect on the planktonic bacterial cell itself.

Conclusion

The role of *Propionibacterium acnes* in the pathogenesis of acne vulgaris may be a disproportionately large cause of the disease process as compared to excess sebum production and follicular hyperkeratinization. The method in which a purportedly harmless commensal is able to elicit such a robust immune response has puzzled researchers for decades until its biofilm-forming abilities were discovered. It had been long known *P. acnes* have the potential to cause biofilms on indwelling medical appliances and catheters, as well as *in vitro*. However, it was not until relatively recently that biofilms were observed within the follicle *in vivo*.

In the years following the discovery of follicular *P. acnes* biofilms, the sequence of the bacterium was completed and multiple quorum sensing and biofilm-forming genes were identified within the genome. As the planktonic bacteria communicate with each other, they begin to consolidate and form an extracellular biofilm matrix comprised primarily of polysaccharides. This biofilm amplifies the virulence of the organism multifold. The biofilm may act mechanically to obstruct the pilosebaceous unit. Perhaps most importantly, the production of lipases is amplified exponentially, causing the release of free fatty acids from sebum triglycerides. These free fatty acids act not only as a food source for the bacteria but activate the inflammatory cascade. Free fatty acids, as well as biofilms, have been observed to bind Toll-like receptors, TLR2 and TLR4, through the MyD88 pathway, activating NF-kB and TNFα which culminates in the production of various proinflammatory cytokines.

Well-nested in the biofilm, the bacteria are mechanically protected from the effect of antimicrobials, maintain a low metabolic state, and express the ability to transfer antibiotic resistance genes. Many antimicrobials that have a profound effect on killing planktonic *P. acnes* have little or no effect on the sessile, biofilm-encapsulated microbes, requiring many months of antibiotics with little or no success seen. Additionally, many of these antimicrobials play no role in the dissolution of the biofilm itself. Few, but potentially promising studies have demonstrated the anti-biofilm effects of plant extracts which may play a role in the future of treating acne vulgaris. Much remains to be elucidated in regard to the clinical implications of treating *P. acnes* biofilms in patients affected with acne vulgaris.

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