Review

Microbiology's principle of biofilms as a major factor in the pathogenesis of acne vulgaris

Craig N. Burkhart[1], MSBS, MD, and Craig G. Burkhart[2], MPH, MD

[1]Department of Medicine, Brown Medical Schools Hospitals, Providence, Rhode Island.
[2]Department of Medicine, Medical College of Ohio, Toledo, Ohio.

Correspondence
Craig G. Burkhart, MPH, MD,
Medical College of Ohio,
5600 Monroe Street,
Suite 106B, Sylvania, OH 43560,
USA.
E-mail: cgbakb@aol.com

Propionibacterium acnes reside within the pilosebaceous unit in a biofilm. As such, they live in a community of bacteria that encase themselves within an extracellular polysaccharide lining, which the organisms secrete after adherence to the surface. This gylcocalyx polymer acts as a protective exoskeleton and serves as a physical barrier, limiting effective antimicrobial concentrations within the biofilm microenvironment. The gylcocalyx polymer secreted by *P. acnes* as a biofilm may explain the immunogenicity of the organism as well as the clinical course of the disease. The *P. acnes'* biofilm model explains many aspects of acne pathogenesis and therapy, including why prolonged antibiotic treatment is needed, why antibiotic resistance is not a reliable assessment of treatment outcome, why accutane offers long-lasting effectiveness, and why benzoyl peroxide radicals are beneficial. This microbiologic principle of biofilms as applied to acne leads to numerous new pathways of assessment and exploration.

The microbiology of Acne

Acne vulgaris is the most common cutaneous disorder, whose etiology appears to be multifactorial. Main etiologic factors include hypercornification of the pilosebaceous duct, increased sebum production, and colonization with *Propionibacterium acnes*. The microbiologic factor with its immunologic consequences is the essential factor in the production of inflammation in acne.[1]

The microbiology of the pilosebaceous unit reveals the existence of three groups of organisms.[1–3] First, gram-positive, coagulase-negative cocci, namely staphylococci and micrococci, reside in the superficial aerobic portion of the sebaceous unit.[2,4] Second, lipophilic yeasts, namely *Pityrosporum ovale* and *Pityrosporum orbiculare*, are also found in the upper portions of the hair follicle unit.[2,3] Lastly, *P. acnes* reside in the anaerobic conditions of the infra-infundibulum, where the inflammatory reaction occurs in acne.[2,4,5]

Propionibacterium acnes is overwhelmingly the predominant microorganism in the pilosebaceous unit, with up to 10^7 viable organisms isolated from a single sebaceous unit.[2,4,5] It is serologically and biochemically identical to *Corynbacterium parvum*, a potent stimulator of the reticuloendothelial system,[6] and has been utilized as an immunostimulatory adjunct in chemotherapy of numerous tumors.[7–9] Indeed, *P. acnes* instigates inflammatory acne via its interaction with humoral factors, its complement, its chemotactic properties, and its cell-mediated immunity.[1]

Categorically, *P. acnes* may be a symbiote to human skin. The organism has opted to exist in the safe, food-rich environment of the pilosebaceous unit. In turn, the human host may have reaped benefits by the presence of *P. acnes* as an immunomodulator, especially in protection against certain infections such as rabies.[10,11]

The microbiologic principles of biofilms

The focus of this article is to introduce the microbiologic concept of biofilms to acne vulgaris. This principle is not only applicable to this cutaneous disease state, but it aids in explaining many properties of acne and in offering new avenues of therapeutics.

To begin, most microorganisms exist in nature not as plankton nor as free-floating microorganisms in suspension, but as biofilms.[12] While classic examples include dental plaques, and infections on implanted prosthetic devices and urinary catheters, biofilms occur in dermatologic conditions as well.[13] By definition, biofilms are composed of populations (or communities) of bacteria that adhere to environmental surfaces, such as the pilosebaceous lining. These microorganisms encase themselves in an extracellular polysaccharide, which they secrete after adherence to a surface. The extracellular matrix usually comprises two-thirds of the biofilm mass, and is composed of polysaccharides, water, extracellular DNA, and excreted cellular products.[14,15] The production of extracellular polysaccharide is essential for development of the architecture of any biofilm matrix. This gylcocalyx

polymer acts as a protective exoskeleton serving as a physical barrier, limiting the effective antimicrobial concentrations within the biofilm microenvironment.[16,17] From *in vitro* studies, bacteria in the protected microenvironment of a biofilm are 50–500 times more resistant to antimicrobial therapies than free-floating (planktonic) bacteria.[17] Biofilm infections tend to be persistent. Microorganisms, when present within biofilms, produce novel proteins, with as yet unknown functions, that are not present in planktonic cells and may increase the immunogenicity of the organism.[18] This different phenotypic expression of bacteria within biofilms leading to a complex interbacterial communication system is known as quorum sensing, allowing communal growth and survival under diverse environmental conditions.[19–21] Within biofilms, bacteria produce substantial quantities of extracellular DNA released by means of small vesicles into the glycocalyx polymer.[15] A functional role of this extracellular DNA is suggested *in vitro* by the inhibition of biofilm formation, but not of bacterial growth, by addition of DNase I into the culture medium.[15] Indeed, this may be the basis for the therapeutic use of inhalation of nebulized recombinant human DNase I to reduce the viscosity of purulent sputum in patients with cystic fibrosis.

The *P. Acnes* biofilm

The *P. acnes* biofilm model can explain why antibiotics are often used for numerous months in treating acne, whereas much shorter courses are utilized for standard bacterial infections. In short, *P. acnes* reveal great tolerance to even high concentrations of antibiotics as a result of its existence in a biofilm matrix. Resistance within the biofilms may relate to delayed penetration of antimicrobial agents into the biofilm polysaccharide matrix, the slow growth rate of organisms within the biofilms, and the phenotypes of bacteria expressed within the biofilms that are distinct from planktonic cells.

Within the biofilm matrix, bacteria are optimally organized to make use of available nutrients and have great microheterogeneity, allowing numerous microenvironments to exist.[13] Typically a wide range of enzymatic activities by bacteria can be found within a biofilm. In the case of *P. acnes*, the organism secretes an array of extracellular products, including hyaluronidase, proteases, lipases, and chemotactic factors for neutrophils, lymphocytes, and macrophages. The milieu and enzyme activities are constantly changing and evolving depending upon various environmental factors. The microenvironment likely plays a major role in the amount of exoenzymes produced.

In short, the nature of the biofilm is determined by intrinsic and extrinsic factors. Intrinsic factors relate to the genetic profile of the component microbial cells. Extrinsic factors include the physico-chemical environment in which the biofilm is located.[13] Even *in vitro*, *P. acnes* production of enzymes has been demonstrated to be altered by factors such as pH and oxygen tension. Thus, acne therapy could be equated to altering the microenvironment in which these acne biofilms exist, and thereby affecting the enzymes secreted by *P. acnes* within the matrix.

Biofilm model explains aspects of acne therapy

In the concept of biofilms, antibiotic resistance in standard cultures is not a reliable assessment of treatment outcome. To be sure, total clearance of the pilosebaceous unit of *P. acnes* with antibiotics does not occur anyway. Furthermore, *P. acnes* is not pathogenic by normal standards because there is no correlation between the number of bacteria and severity and type of acne. Nevertheless, *P. acnes* is the target of oral and topical antibiotic usage possibly because of its affect on the *P. acnes* biofilm. In terms of antibiotics, minocycline is more fat-soluble than other antimicrobials and achieves high therapeutic success, which might be explained by means of this biofilm concept. Indeed, tetracycline-derivatives proved to have the highest synergistic effect when used in combination with another antibiotic when investigated in biofilms using ATP-bioluminescence for viable bacterial cell quantification.[22] Ideally, acne agents would alter the physicochemical environment of the pilosebaceous unit in which *P. acnes* live. For example, agents that control cellular proliferation and differentiation within the pilosebaceous gland may have an effect. Additionally, products that form a benzoyl peroxide radical appear to make a more significant alteration to the microenvironment in which the biofilm matrix resides.[23] Inasmuch as accutane diminishes sebaceous gland size, *P. acnes* biofilm would dramatically be affected by the alteration in its nutrient base. Additionally, once the biofilm has been altered by accutane therapy, reconstruction of a similar environment to preaccutane conditions may not be possible, perhaps explaining the long-lasting effect of accutane therapy.

The biofilm concept for acne is a theoretic framework for understanding the interactions of *P. acnes* within the microenvironment of the pilosebaceous unit. This has led to studies that have been performed in various disease states such as mucosal biofilm formation in otitis media,[24] and dental plaques.[25] Biofilm, in contrast to planktonic cell cultures for various bacteria including *Streptococcus mutans*,[18,25] *Staphylococcus epidermidis*,[22] and *Escherichia coli*[26] have been observed for function, adhesive properties, antibiotic resistance, protein expression, and existence of bacteriophages. Analogous studies in acne could assess *P. acnes* in different microenvironmental states in which studied variables could include oxygen tension, pH level, nutrient availability, and the presence of antibiotics or benzoyl peroxide radicals.

The biofilm concept as applied to acne may lead to new targets for therapy. As suggested, more emphasis would

be placed on altering the microenvironment of the infrainfundibulum in which the *P. acnes* biofilm resides by biological, chemical or physical means. An agent that reduces the attachment of *P. acnes* to the follicular lining would arrest biofilm development. Any treatment that would alter the ability of *P. acnes* to synthesize the extracellular matrix would be most helpful. Thus, treatments that target specific components of the biofilm may be achievable. An example would be recombinant human DNase I to inhibit biofilm formation. Additionally, treatments must be assessed as to their ability to penetrate the extracellular matrix to reach the bacteria within the biofilm. The microbiologic concept of biofilms leads to numerous new pathways of assessment and exploration. It is worthwhile exploring this *P. acnes* biofilm model as an approach to better and different acne therapy.

References

1 Burkhart CG, Burkhart CN, Lehmann PF. Acne: a review of immunologic and microbiologic factors. *Postgrad Med J* 1999; **75**: 328–331.
2 Marples RR. The microflora of the face and acne lesions. *J Invest Dermatol* 1974; **62**: 326–341.
3 Gueho E, Boekhout T, Ashbee HR, *et al.* The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med Mycol* 1998; **36** (Suppl. 1): 220–229.
4 Nishijima S, Kurokawa I, Katoh N, Watanabe K. The bacteriology of acne vulgaris and antimicrobial susceptibility of *Propionibacterium acnes* and *Staphylococococcus epidermidis* isolated from acne lesions. *J Dermatol* 2000; **27**: 318–323.
5 Leyden JJ. *Propionibacterium* levels in patients with and without acne vulgaris. *J Invest Dermatol* 1975; **65**: 382–389.
6 Adlam C, Scott MT. Lymphoreticular stimulatory properties of *Corynebacterium parvum* and related bacteria. *J Med Microbiol* 1973; **6**: 261–265.
7 Senaldi G, Yin S, Shaklee CL, *et al. Cornyebacterium parvum* and *Mycobacterium bovis* Bacillus Calmette-Guerin-induced granuloma formation is inhibited in TNF receptor I knockout mice and by treatment with soluble TNF-RI. *J Immunol* 1996; **157**: 5022–5026.
8 Herberman RB. Natural killer cells and their possible roles in resistance against disease. *Clin Immunol Rev* 1981; **1**: 1–14.
9 Cantrell JL, Wheat RW. Antitumor activity and lymphoreticular stimulation properties of fractions isolated from *Corynebacterium parvum*. *Cancer Res* 1979; **39**: 3554–3555.
10 Megid J, Kaneno R. Natural killer activity in mice infected with rabies virus and, submitted to *Propionibacterium acnes* as immunomodulator. *Comp Immunol Microbiol Infect Dis* 2000; **23**: 91–97.
11 Megid J, Peracolli MT, Curi PR, *et al.* Effect of vaccination and immunomodulators bacillus of Calmette-Guerin, avridine and *Propionibacterium acnes* on rabies in mice. *Comp Immunol Microbiol Infect Dis* 1998; **4**: 305–318.
12 Sutherland IW. The biofilm matrix – an immobilized but dynamic microbial environment. *Trends Microbiol* 2001; **9**: 222–227.
13 Burkhart CN, Burkhart CG, Gupta AK. Dermatophytoma: recalcitrance to treatment due to existence of fungal biofilm. *J Am Acad Dermatol* 2002; **47**: 629–31.
14 Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiol* 2001; **147**: 3–9.
15 Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. *Science* 2002; **295**: 1487.
16 Donlan RM. Role of biofilms in antimicrobial resistance. *ASAIO J* 2000; **46**: S47–S52.
17 Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001; **9**: 34–39.
18 Svensater G, Welin J, Wilkins JC, Beighton D, Hamilton IR. Protein expression by planktonic and biofilm cells of *Streptococcus mutans*. *FEMS Microbiol Let* 2001; **205**: 139–146.
19 Parsek MR, Greenberg EP. Quorum sensing signals in development of *Pseudomonas aeruginsoa* biofilms. *Meth Enzymol* 1999; **310**: 43–55.
20 Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci* 2000; **97**: 8789–8793.
21 Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol* 2001; **55**: 165–199.
22 Monzon M, Oteiza C, Leiva J, Amorena B. Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. *J Antimicrob Chemother* 2001; **48**: 793–801.
23 Burkhart CN, Specht K, Neckers D. Synergistic activity of benzoyl peroxide and erythromycin. *Skin Pharmacol Appl Skin Physiol* 2000; **13**: 292–296.
24 Ehrlich GD, Veeh R, Wang X, *et al.* Mucosal biofilm formation on middle-ear mucosa in the chinchill model of otitis media. *JAMA* 2002; **287**: 1710–1715.
25 Land AS, VanDerMei HC, Busscher HJ. Detachment of linking film bacteria from enamel surfaces by oral rinses and penetration of sodium lauryl sulphate through an artificial oral biofilm. *Adv Dent Res* 1997; **11**: 528–538.
26 Corbin BD, McLean RJ, Aron GM. Bacteriophage T4 multiplication in a glucose-limited *Escherichia coli* biofilm. *Can J Microbiol* 2001; **47**: 680–684.

