Assessment of *Cutibacterium acnes*: Acne Biofilm, Comedones, and Future Treatments for Acne

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Abstract: *Cutibacterium acnes* (C. acnes) is a skin commensal organism that controls the growth of *Staphylococcus aureus* and *Streptococcus pyogenes*. Additionally, the organism can become an opportunistic pathogen, causing acne and post-surgical prosthetic infections. The outcome of acne depends on *Cutibacterium* subtypes, virulence factors, and microbial equilibrium. This organism makes a biological glue that is essential for biofilm formation, but its overabundance makes its way into the sebum. This sebum slowly reaches the upper layer of the hair unit along with dead cells from the keratinocyte layer, causing comedones. Treatments in the pipeline include tumor necrosis factors (biologics), various agents attacking biofilm viability, phage therapy, and vaccinations against virulence factors produced by *C. acnes*.

Keywords: Acne, *Cutibacterium acnes*, Acne biofilm, Treatment, Phage therapy, Acne vaccinations.

1. INTRODUCTION

Acne *vulgaris* is a frequent condition in dermatology as it affects 20 million people, most of whom are in their teen years or early 20s. The standard literature has proclaimed that the major causative factors are excess keratin formation in the upper hair units, sebaceous gland obstruction, irregular maturation of cells in the upper follicular units, expanded production of sebaceous fluids secondary to androgenic production, and overgrowth in the number of *Cutibacterium acnes* (C. acnes). *C. acnes* was formerly named *Propionibacterium acnes*. Traditionally, the major areas of pathophysiology have been stated to be hyperkeratinization and obstruction of sebaceous follicles resulting from abnormal desquamation of the follicular epithelium, an androgen-stimulated increase in the production of sebum, and proliferation of *Cutibacterium acnes* (C. acnes), previously known as *Propionibacterium acnes* [1]. This dogma has been altered by the development of the acne biofilm.

2. FEATURES OF *CUTIBACTERIUM ACNES*

*C. acnes* is a gram-positive bacterium that causes inflammatory acne. Its cell wall and envelope are different as they consist of two unusual lipids, namely phosphatidylinositol and triacylglycerol [2]. *C. acnes* is a commensal organism and has a definite role in maintaining homeostasis. In short, it produces virulence factors that regulate the growth of several potentially harmful pathogens, such as *Staphylococcus aureus* and *Streptococcus pyogenes*. However, it can also protect from these bacteria by hydrolyzing lipids and maintaining a low pH [3, 4]. Additionally, it affects skin T helper 17 cell responses and immunomodulatory IL-10 [5]. Metabolic features allow *C. acnes* to break down sebum-consistent triglyceride into smaller molecules of fatty acid. One of these is propionic acid whose presence leads to keeping the skin acidic. Due to the identification of lipase genes not found in other cutaneous *Propionibacterium* and other genomic adaptive changes, its name was changed to *Cutibacterium* species.
C. acnes prefers to live on the face, back, and chest, where it can enjoy an environment abundant in sebum. The number of such bacteria in one single hair follicle unit can approach $10^8$ colony-forming units [3]. The bacteria is also found in the conjunctiva, intestinal tract, stomach, lungs, prostate, oral mucosa, external auditory canal, and urinary tract [3, 6, 7]. It is a facultative anaerobic bacteria, but it does have the genetic ability to produce the needed factors to also live in oxygen-rich conditions.

The C. acnes genome is quite large. It consists of a single circular chromosome. On this single chromosome, there are 2,560,265 base pairs with 2333 potential genes [8], making it equivalent to about 8% of the human genome by comparison. There are 100 different strains of C. acnes, and it is classified into eight phylotypes. Two of these phylotypes are found commonly in more severe acne cases, namely IA-1 and IA-2 [9]. Some may act as opportunistic pathogens, such as phylotypes II and III, which are often identified in infections that extend farther down into the skin [9]. Many of the other phylotypes may not have any pathogenic tendencies and merely maintain homeostasis. It is challenging to make direct comparisons of specific phylotypes to disease states as there can be variances in how one follicle behaves, given the interplay of various external factors [10].

The skin surface is host to Corynebacteria, Cutibacteria, and Staphylococci [11]. The major inhabitant is S. epidermidis, making up 27% of the population of bacteria [12]. C. acnes is less than 2%, as it mostly resides deep within the sebaceous gland [3]. S. epidermidis controls the C. acnes growth-induced inflammation through the release of succinic acid [13].

C. acnes can be an opportunistic pathogen, with acne being the most common example. In the hair follicle with acne, it can release lytic enzymes, triggering follicular disruption and activation of an immune response. C. acnes has also been recognized as a major cause of post-surgical prosthesis infection, endocarditis, endophthalmitis, prostatitis, sarcoidosis, osteitis syndrome, osteomyelitis, and septic arthritis [14].

Certain antibiotics have limited abilities to attack anaerobic bacteria as the molecular means of penetration utilized with aerobic microbials are not present. In the case of C. acnes, innate resistance to fosfomycin, metronidazole, 5-nitromidazole agents (i.e., metroni-dazole), aminoglycosides, sulfonamides, and mupirocin has been found [15]. Moreover, the resistance of C. acnes to various other antibiotics has been gradually increasing. For example, resistance to erythromycin and clindamycin are reported to be 21% and 70%, respectively [15, 16]. Interestingly, both antibiotics are tertiary amines and have been shown to activate benzoyl peroxide topically into its radical state with therapeutic effects [17, 18]. Thus, topically, these antibiotics prove to be more helpful due to their chemical structure rather than antibacterial properties.

3. THE C. ACNES BIOFILM AND PRODUCTION OF VIRULENCE FACTORS

Most C. acnes in hair follicle units reside in biofilms. A biofilm is a collection of surface-associated microbial cells surrounded by an extracellular polymeric substance matrix that allows adherence to a surface as well as making an exoskeleton [19]. The biofilm allows for numerous different habitats for the bacteria to exist, which leads to C. acnes organisms having varied rates of metabolism, replication, and responsiveness to antibiotic therapy. Biofilms make C. acnes less susceptible to antibiotics as they greatly reduce the penetration of drugs. Thus, these bacteria residing in biofilms are much more resistant to antibacterial measures than free-floating (planktonic) bacteria. Gene expression clearly proves the innate capacity of C. acnes to manufacture biofilms [20]. Biofilms can attach to numerous types of surfaces. In the case of implants, the development of such a biofilm could lead to complications [21]. A biofilm enhances pathogenicity by leading to greater lipase activity implicated in inflammation and upregulation of genes encoding various virulence factors [22].

C. acnes are capable of producing several virulence factors, including dermatan-sulfate adhesins (DsA1 and DsA2), Christie-Atkins-Munch-Petersen (CMP) hemolytic factors, lipases, hyaluronate lyase, sialidase, polyunsaturated fatty acid isomerase, HtaA iron acquisition protein and GehA lipase, and heat-shock proteins, such as HSP20, DnaK, DnaJ, GrpE, and groEL [8, 23, 24]. C. acnes makes some of these virulence factors for forming a biofilm, cell adhesion, and adapting to its environment. However, the same proteins in higher concentrations can also cause tissue invasion and degradation of other bacteria and or the host. It has been demonstrated that C. acnes releases high levels of specific virulence factors and increases its pathogenicity. Other possibilities include environmental changes and/or interaction of C. acnes with the skin microbiota [24].

4. THE COMEDONE AND ITS FORMATION FROM BIOLOGICAL GLUE FROM C. ACNES

For generations, comedone has been considered the primary cause for unraveling acne pathogenesis. The comedone is a keratin plug, and its formation has been thought to be a result of some non-explained change in the epithelium of the upper hair follicle unit. These keratinocytes amass, causing pressure and expansion within the hair follicle unit, ultimately leading to the rupture of the comedone and the resultant dispersion of keratin and sebum into the dermis, which would act as a foreign immunologic material.

The understanding of an acne biofilm led to a different perspective. In short, some of the excess biological glue that C. acnes makes to form its biofilm finds itself in the sebum [25]. Genomic studies on C. acnes have revealed the existence of UDP-N-acetylglucosamine 2 epimerase and glycosyl transferases, two main adhesive proteins for biofilm formation [20]. The biological glue that finds its way into the sebum leads to the adhesiveness of keratinocytes in the upper hair unit, leading to comedones.
Table 1. Potential novel therapeutic agents for acne.

<table>
<thead>
<tr>
<th>Class of Agent</th>
<th>Examples of Agents</th>
<th>Mechanism of Action(s)</th>
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<tbody>
<tr>
<td><strong>Anti-inflammatory</strong></td>
<td></td>
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<tr>
<td>Biologics</td>
<td>Anti-TNF, e.g. adalimumab, infliximab, etanercept</td>
<td>Target TNF to reduce inflammation [30]</td>
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<td><strong>Modifiers of Acne Biofilm</strong></td>
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<tr>
<td>Quorum sensing inhibitors</td>
<td>Azithromycin, bergamottin, usnic acid, quercetin, and ellagic acid</td>
<td>Block genetic factors</td>
</tr>
<tr>
<td>Degradation of specific proteins</td>
<td>Dispersin B and Deoxyribonuclease</td>
<td>Destroy functional proteins in biofilm formation</td>
</tr>
<tr>
<td>Surfactants</td>
<td>Poloxamer-188, Tween 20, sodium dodecyl sulfate, and rhamnolipids</td>
<td>Form central hollow cavities in biofilms</td>
</tr>
<tr>
<td>Signal molecules</td>
<td>Caprylic acid, palmitic acid, and myristoleic acid</td>
<td>Alter chemical compounds essential for biofilm survival</td>
</tr>
<tr>
<td>Metal chelators</td>
<td>Ethylenediaminetetraacetic acid</td>
<td>Bind to magnesium and calcium from the outer cell wall, upsetting biofilm stability</td>
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<tr>
<td>Nitric oxide-generating agents</td>
<td>Glyceryl trinitrate, isosorbide dinitrate, isosorbide mononitrate, and isosynyl nitrate</td>
<td>Reduction of intracellular cyclic dimeric guanosine monophosphate</td>
</tr>
<tr>
<td>Antimicrobial peptides</td>
<td>Nisin A, human cathelicidin LL-37, human beta-defensin 3, and hepcidin 20</td>
<td>Make microbial membranes permeable</td>
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<tr>
<td><strong>Bacteriophage Therapy</strong></td>
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<tr>
<td>Bacterial viruses</td>
<td>Cutibacterium acnes phage</td>
<td>Target specific bacterial cells, destroying the bacteria</td>
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<tr>
<td><strong>Molecular Biology</strong></td>
<td></td>
<td></td>
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<tr>
<td>Specific antibodies to C. acnes virulence factors</td>
<td>Antibodies for new CAMP1-derived peptide</td>
<td>Neutralize effects of virulence factors</td>
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</table>

Understanding that *C. acnes* makes this biological glue within the biofilm redirects some of the therapy from comedones. Rather than merely concentrating on topical unplugging agents to unplug the comedonal keratinous plug in the hair units, one might now consider altering the *C. acnes* biofilm or its microenvironment. Factors to consider in altering the *C. acnes* biofilm or its microenvironment would be biological, chemical, and/or physical means. For example, the anecdotal evidence that comedonal acne can be improved by oral medications, such as minocycline, tazarotene, and isotretinoin, can now be explained by their actions on changing the *C. acnes* biofilm or altering the sebaceous gland so that there is less making of biological glue [25, 26].

5. CONCLUSION AND FUTURE TREATMENTS: WHAT IS COMING DOWN THE ACNE PIPELINE?

Acne does not simply occur due to increased numbers of *C. acnes*. An alteration in the homeostasis between the microbes on the skin surface and *C. acnes* phylotypes might play an important role in acne onset [4, 27]. Change in the balance of skin microbiota with probiotics to obtain a healthy microbiome may prove helpful [25, 28]. There is concern with the growing resistance of *C. acnes* to various antibiotics. However, standard antibiotic resistance factors may not be significant when dealing with a commensal bacteria that can also assume the role of a pathogen [29]. For example, antibiotics might alter the function of bacteria within biofilms favorably despite showing resistance in *in vitro* studies. Possible new therapies are listed in Table 1.

Biologics have been used in the treatment of acne. These agents are tumor necrosis factor inhibitors that have been used for numerous inflammatory conditions, including rheumatoid arthritis and psoriatic arthritis. They reduce inflammation and can stop disease progression by targeting TNF. Biologics, including adalimumab, infliximab, and etanercept, have been used in severe acne cases. In a study, 40% of participants achieved acne clearance and 90% experienced improvement [30]. However, there proved to be the actual occurrence of worse acne in certain instances.

Altering the acne biofilm can also result in acne improvement. Some agents can either prevent a biofilm’s emergence or attack and/or alter an existing biofilm [28]. These agents alter the surface of *C. acnes*, so they are unable to bind to each other [31, 32]. Agents capable of disrupting biofilm function include:

- Quorum sensing inhibitors: They block signals affecting virulence factors as well as the development of biofilms. Agents that have been implicated as having such abilities include azithromycin, bergamottin, usnic acid, quercetin, and ellagic acid [33-36].

- Substances that degrade functional proteins in biofilm structure: These agents include dispersin B (which lyse a polysaccharide that aids in surface attachment) and Deoxyribonuclease (which acts on nucleic acids that affect cell surface adhesion) [37, 38].

- Surfactants, such as Rhamnolipids made by *Pseudomonas aeruginosa*: These agents form central hollow cavities in biofilms. Their examples include poloxamer-188, Tween 20, sodium dodecyl sulfate, and rhamnolipids [39, 40].

- Agents altering signal molecules essential for biofilm survival: These are specific fatty acids, such as caprylic acid, palmitic acid, and myristoleic acid [41-43].

- Metal chelators, such as ethylenediaminetetraacetic acid (EDTA), bind to magnesium and calcium from the outer cell wall, upsetting biofilm stability [44, 45].
Nitric oxide generating agents: They cause a reduction in intracellular cyclic dimeric guanosine monophosphate, resulting in bacteria favoring planktonic mode rather than biofilm existence [46, 47].

Antimicrobial peptides: They damage microbial membranes, making them permeable to liquids and gases, leading to cell death. Examples are nisin A, human cathelicidin LL-37, human beta-defensin 3, and hepcidin 20 [48-50]. Certain antibiotics: They may not decrease the number of bacteria in a biofilm setting but will decrease or alter its function. Minocycline and Bactrim are involved in this category.

Agents that shrink the sebaceous gland will affect the nourishment of bacteria, which alters the normal homeostasis of bacteria within the biofilm. Examples would be isotretinoin and spironolactone.

Another method of treating acne would be bacteriophage (phage) therapy. Phages are bacterial viruses that invade specific bacterial cells, thus destroying the bacteria. Such therapy directed against C. acnes has proved successful in the animal model [51, 52]. Although phage therapy has shown value in various conditions, including dysentery, sepsis, and meningitis, there is a dearth of research on possible usage with skin bacteria [14].

Molecular biology is now able to make specific antibodies for many of the virulence factors of C. acnes. Many new treatments have been developed. For example, there are already antibodies for new CAMP1-derived peptides for clinical evaluation [25]. Thus, any of the virulence factors listed in our previous discussion could have a vaccine made, leading to cell death. Examples are nisin A, human cathelicidin LL-37, human beta-defensin 3, and hepcidin 20 [48-50]. Certain antibiotics: They may not decrease the number of bacteria in a biofilm setting but will decrease or alter its function. Minocycline and Bactrim are involved in this category.

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The outcome of acne depends on subtypes, virulence factors, and microbial equilibrium. Molecular science enables us to have potentially new methods of controlling acne. Given the excellent results seen with isotretinoin, the cost of these newer possible treatments in recalcitrant patients will direct, to some extent, their development and usage.

ABBREVIATION
EDTA = Ethylenediaminetetraacetic Acid

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REFERENCES


