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The Impact of Biofilm Formation on Wound Healing

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Abstract

Chronic wounds represent an important challenge for wound care and are universally colonized by bacteria. These bacteria can form biofilm as a survival mechanism that confers the ability to resist environmental stressors and antimicrobials due to a variety of reasons, including low metabolic activity. Additionally, the exopolymeric substance (EPS) contained in biofilm acts as a mechanical barrier to immune system cells, leading to collateral damage in the surrounding tissue as well as chronic inflammation, which eventually will delay healing of the wound. This chapter will discuss current knowledge on biofilm formation, its presence in acute and chronic wounds, how biofilm affects antibiotic resistance and tolerance, as well as the wound healing process. We will also discuss proposed methods to eliminate biofilm and improve wound healing despite its presence, including basic science and clinical studies regarding these matters.

Keywords: biofilm, chronic wounds, delayed healing, exopolymeric substance, slime, extracellular matrix

1. Introduction

Intact skin provides a protective barrier to bacterial invasion. Any wound comprises a break in this epidermal barrier, allowing microbial invasion into deeper layers.

Along with hypoxia/poor perfusion, ischemia-reperfusion injury, and inadequate offloading or compression therapy, microbial infection is one of the most significant causes of delay in healing [1–3].

Over the last few years, bacterial biofilms in general and their role in chronic wounds have been the subject of intense research. Biofilms have been reported to be present in 60% [4] to
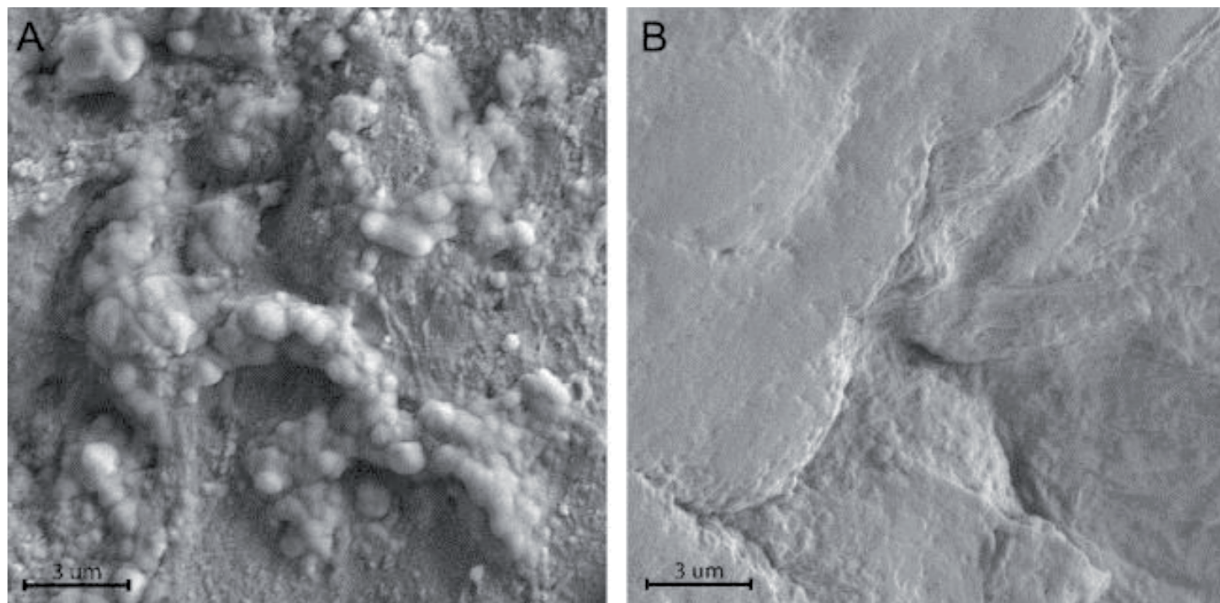
Figure 1. Representative scanning electron microscopy of wounds on mice dorsal skin. (A) Inoculated wound with *S. aureus* showed aggregates encased in EPS matrix. (B) Non-inoculated wound without EPS (image from Nguyen et al. [10], with permission).

80% [5] of chronic wounds, and a recent meta-analysis confirms their presence in 78.2% of chronic wounds [2]. Therefore, biofilms have been categorized as an important factor in most chronic non-healing skin wounds [6].

Non-healing or poorly healing wounds affect close to 25 million people in the US [7], more than 7% of its population, while reports from the UK [8] predict that 1–2% of the population in developed countries will experience a chronic wound in their lifetime. Posnett et al. [9] reports the financial burden to the healthcare system of caring for chronic wounds in the UK, totaling US$ 3.4–4.6 billion a year, close to 3% of the healthcare budget. The US, a larger and more complex system, observed $35.3 billion in spending of Medicare funds on wound care alone in 2014, of which 16.7 billion was spent on infections and 9.4 billion on chronic ulcers [9] (**Figure 1**).

The implications of a biofilm-covered wound are not limited to delayed healing and financial burden. Biofilms pose a risk for persistent wound infections, especially when medical hardware is inserted into the body [11]. Biofilms can also develop into an overt infection, contribute to antimicrobial resistance, and increase the risk for adverse or tissue toxic effects caused by topical agents [12].

2. Background

Wound healing can be deranged by multiple causes, including local hypoxia or poor perfusion, repetitive ischemia-reperfusion injury, inadequate offloading or compressive therapy, and bacterial infection. Bacterial infection, playing a great role among these causes, has been

Planktonic vs. biofilm phenotype

Trait	Planktonic	Biofilm
Virulence	Acute, aggressive course	Chronic disease
Host inflammatory response	High, sudden	Mild, persistent
Risk for antibiotic resistance	Moderate	High
Spread	Disseminating	Sessile
Extracellular polymetric substance (EPS)	No	Yes
Metabolic activity	High	Low
Species count	Monospecies, polyspecies	Polyspecies

Table 1. Planktonic and biofilm phenotypes comparison in regards to various bacterial traits and behaviors.

associated with both acute and chronic wounds *via* different rates and mechanisms. An infection with a more predominantly planktonic phenotype is more aggressive, with rapidly dividing cells invading host tissues and stimulating a strong inflammatory response typical of an acute infection. Several microorganisms can adopt a different, sessile phenotype, called a biofilm, that allows them to attach to biotic or abiotic surfaces, form aggregates, and regulate the production of an extracellular polymeric substance (EPS), contributing to their ability to survive [13, 14] (**Table 1**).

This aggregate or cluster, once called "*slime*," constitutes the biofilm, a complex tertiary structure of sessile communities of one or more species of bacteria embedded within a matrix of EPS. The EPS is composed mainly by water, polysaccharides, DNA and other substances secreted by the embedded bacteria, but also by substances scavenged from the host. It is important to appreciate that all the building blocks of a wound biofilm are ultimately derived from the wound bed and skin. Cell lysis and subsequent local decomposition of the EPS matrix is advantageous for the biofilm population, creating new pores and channels that improve nutrient access, and the intracellular level of the second messenger cyclic di-GMP are involved in regulating biofilm formation and the production of matrix components [15].

For many years, biofilm has been known to exist on dental plaque and industrial water processing and even considered the predominant state of bacteria within the human body [16]. Later on, its presence was reported on endocardium, urinary tract mucosa, nasal and sinus epithelium and pulmonary tissue, and more recently biofilms have been found in healed surgical wounds, sutures, implants and IV catheters which can be contaminated at time of insertion or as a result of hematologic seeding from a colonized tissue. The relationship between biofilm and host will depend on the location and the bacterial composition of the biofilm; for instance, in the gastrointestinal mucosa, biofilm has a commensal behavior, while in wounds or respiratory tract mucosa, a pathogenic behavior. This difference is thought to be due to the host's capacity to coexist or eradicate biofilm [6, 12, 17, 18].

3. Composition of biofilm

Several functions have been attributed to biofilm: genetic material reservoir, nutrient source, matrix stabilization, adhesion, and bacterial communication. Most of these functions will depend on the particular substances present in the biofilm, which depend exclusively on the species and even the strain of the bacteria. For instance, *P. aeruginosa* produces a biofilm with a higher density EPS, with a well-defined matrix interspersed within clusters of bacterial cells and has the particularity to be predominant over other species in a polybacterial microbiome. In general, the interaction of these substances and how the bacteria inside the biofilm manage to utilize these substances will affect the morphology of the biofilm with common effects: immobilizing biofilm cells and allowing the existence of a very diverse habitat favoring biodiversity, where every member can contribute with their own EPS [15] (**Figure 2**).

3.1. Polysaccharides

Polysaccharides comprise a major fraction of the EPS matrix and are responsible for the biofilm's mechanical properties. Interestingly, it seems to be mainly the exopolysaccharides in multivalent inorganic ions with EPS can greatly influence the mechanical properties of
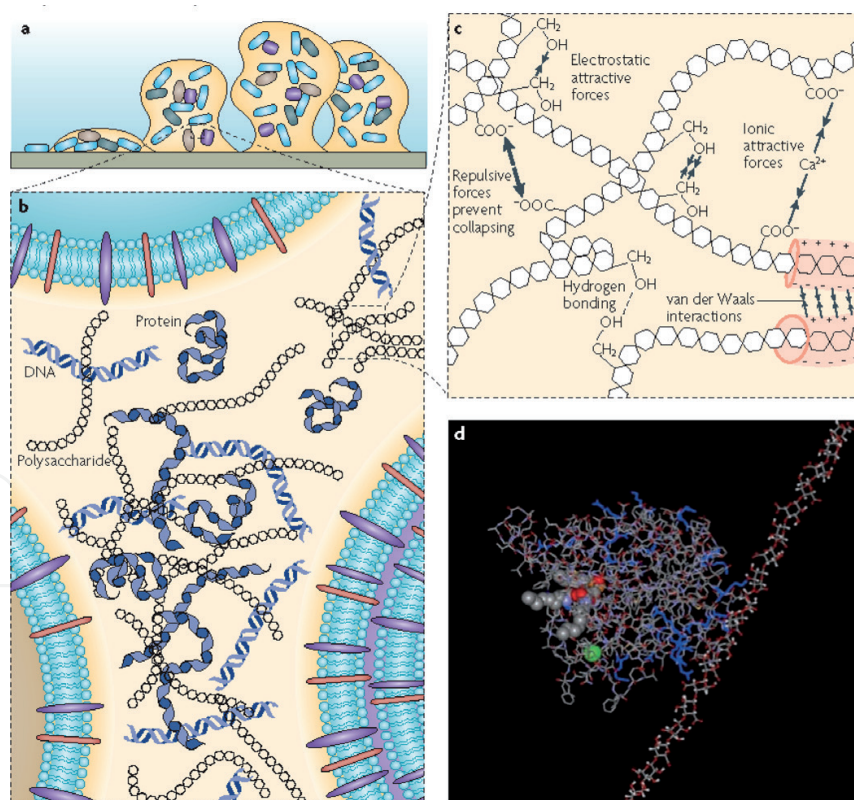
Figure 2. The extracellular polymeric substances matrix at different dimensions. (a) A model of a bacterial biofilm attached to a solid surface. (b) The major matrix components—polysaccharides, proteins and DNA—in a non-homogeneous pattern. (c) Physicochemical interactions and the entanglement of biopolymers that give stability to the EPS matrix. (d) A molecular modelling simulation of the interaction between alginate (right) and lipase (left) of *P. aeruginosa* (image from Flemming and Wingender [15], with permission).

biofilms. For instance, presence of Ca^{2+} in biofilm formed by mucoid strains of *P. aeruginosa* experienced an enhancement in their mechanical stability. In *S. epidermidis*, poly-*N*-acetylglucosamine (PNAG) makes a considerable contribution to biofilm integrity [15].

As seen in *P. aeruginosa* and *S. epidermidis*, polysaccharide compositions are very diverse, even between strains of a single species. *P. aeruginosa*, for instance, produces at least three distinct exopolysaccharides that have a direct effect on its biofilm architecture: alginate, *Pel* and *Psl*. Mucoid strains of *P. aeruginosa* contain alginate, an exopolysaccharide for biofilm formation that, although non-essential, has a notable effect on biofilm architecture. Alginate takes part at the beginning of biofilm formation and is responsible for the mechanical stability of mature biofilms. Alginate from this strain has a particular clinical relevance, being comprised of uronic acids, in that it can be used as an EPS marker, since this type of acid is not found inside the bacterial cells. In non-mucoid strains, *Pel* and *Psl* participate in the first stages of biofilm formation, while *Psl* alone is involved in adherence to surfaces [15].

3.2. Proteins

Biofilms also contain a diversity of enzymes, lending a complex organization and capability of adaptation. Enzymes will break down biopolymers into low molecular mass products, degrade the structural EPS to promote detachment, act as virulence factors, and even degrade EPS components during starvation. Cell surface-associated proteins and extracellular carbohydrate-binding proteins (*lectins*) are also a key component in the biofilm, involved in the formation and stabilization of the matrix network [15].

Among these proteins we can find the glucan-binding proteins present in dental plaque caused by *S. mutans*, the galactose-specific lectin *lecA* and fucose-specific lectin *lecb* of *P. aeruginosa*, which have been associated with biofilm formation. Biofilm associated surface protein (*bap*) from *S. aureus* and the bap-like proteins, which promote biofilm formation in several species while also playing a role in bacterial infectious processes. Biofilms also contain amyloids, involved in adhesion to inanimate surfaces and host cells and invasion of host cells; additionally, they can function as cytotoxins for bacterial and plant cells [15].

3.3. Extracellular DNA

eDNA is an integral part of the matrix and biofilm mode of life. *B. cereus* uses DNA as an adhesion molecule, and in *P. aeruginosa*, eDNA serves as an intercellular connector, with DNase inhibiting biofilm formation specifically in *P. aeruginosa*. In *S. aureus*, eDNA serves the same structural role of PNAG in *S. epidermidis* eDNA, although seen initially as residual material from lysed cells, is also actively excreted. Although primarily occurring in waste-water biofilms, biofilms from various origins have been found to contain eDNA of varying levels and importance, even between closely related species. For example, eDNA plays a critical structural role in the biofilm matrix of *S. aureus* but only serves as a minor component of *S. epidermidis* biofilms. eDNA is localized differently between biofilms; in *P. aeruginosa*, for example, forms a grid-like structure. Additionally, eDNA has antimicrobial activity, having the ability to chelate cations that stabilize lipopolysaccharide and the bacterial outer membrane, provoking cell lysis [15].

EPS composition	
Component	Function(s)
Polysaccharides	Mechanical strength, adherence
Proteins	Mechanical strength, adherence, detachment, virulence
eDNA	Mechanical strength, adherence, antimicrobial, genetic transfer
Water	Source of ions and compounds in solution
Biosurfactants	Adherence, detachment

Table 2. Composition of biofilm exopolymeric substance (EPS) and associated functions.

3.4 Water and biosurfactants

Water is by far the largest component of the matrix, and water management is so critical that bacteria actively respond to desiccation by producing EPS. Molecular composition of the water component is critical as well, and the EPS matrix acts as a molecular sieve, sequestering cations, anions, nonpolar compounds and particles from the water phase. By comparison, biosurfactants have antibacterial and antifungal properties and are important for bacterial attachment and detachment from oil droplets. *Rhamnolipids*, which can act as surfactants, have been found in the EPS matrix of *P. aeruginosa* [15] (**Table 2**).

4. Pathophysiology

4.1. Biofilm development

Biofilms utilize a variety of mechanisms in order to establish themselves. When exposed to adverse conditions, planktonic bacteria facilitate survival by forming biofilms. This occurs through "*phase variation*" and "*adaptive mutation*," genetic alterations that include point mutations, recombination, and transpositions, with the goal of producing individuals more capable of producing biofilms. *V. cholera*, *S. typhi*, and *E. coli* all exhibit stress-induced genetic alteration by adaptive mechanisms that produce a biofilm-capable phenotype, producing distinct, wrinkled individuals. *V. cholera* produces a more chlorine-resistant subtype called rugose, while *S. typhi* and *E. coli* change to an "*rdar*" phenotype, or red, dry, and rough [19].

4.2. Biofilms and chronic disease

By establishing biofilms, bacterial species not only increase their antibiotic resistance 1000-fold, they produce optimal conditions for chronic infections. By sacrificing aggressive movement throughout the body for confinement within a protective extracellular matrix, bacterial species effectively hide antigens, reduce the effectiveness of antibiotics, and blunt the immune response, promoting chronic disease: endocarditis, chronic kidney stones, and CF infections [19, 20].

Biofilms play a significant role in the development of chronic cutaneous wounds, with up to 80% of chronic wounds having been found to contain a biofilm compared to 6% of acute wounds [2, 4, 5, 21].

Biofilms cause chronic infections through mechanisms that are either innate or interact closely with the host immune system: genetic changes, surface and excreted molecular messengers, physical barriers, and escape behaviors. Although the bacteria may not disseminate throughout the body, pathogenicity is retained and arguably increased, as bacterial concentration within the biofilm increases and individuals tend to leave the biofilm, either through purposeful dissolution of EPS or through stresses on the biofilm itself by the fluid encasing the biofilm [19].

When bacteria cluster in a biofilm, movement of advantageous genetic traits, such as antibiotic resistance, throughout the constituents of the biofilm is expedited through transformation, horizontal gene transfer, or phage infection, making each individual bacterium even more virulent when it leaves the biofilm. *P. aeruginosa* biofilms, for example, exhibit a high concentration of DNA within their EPS. This also promotes significant genetic variability within a biofilm, increasing the chances that one of the many individuals will survive an environmental insult [19]. This includes antibiotics, leading to concern that excessive and inappropriate antibiotic use against biofilms expedites the development of antibiotic resistant strains [12].

4.3. Wound healing inhibition

Biofilms involve a complex relationship between bacteria virulence factors, survival mechanisms, and the host immune response [22]. Different species all exhibit particular biofilm characteristics that inhibit wound healing. EPS by itself represents a physical barrier against inflammatory cell phagocytosis, and has the potential to inhibit the complement cascade and antibiotic penetration into the wound [23]. Acellular extract from *S. aureus* biofilms inhibits the movement of keratinocytes and promotes apoptosis, leading to impaired cutaneous wound healing. This extract did not differ in pH or calcium levels; its effect on keratinocytes was due to direct cytotoxic substances secreted from or present on *S. aureus* bacterium: alpha-toxin and cell surface-expressed fibronectin-binding proteins [21].

P. aeruginosa biofilms similarly inhibit neutrophil movement but may spare their capacity for oxidative burst, and exhibit a capacity for ejecting individual bacterium from the biofilm [24]. Another potential mechanism for *P. aeruginosa* biofilm resistance to neutrophils is the rapid necrosis induced by the production of *rhamnolipids* [23]. Additionally, significant delay in wound healing, re-epithelization and collagen deposition have been reported without significant difference in PMN infiltration or granulation tissue [6]. The ultimate result is neutrophil aggregation near the biofilm, with oxidative burst products accumulating and causing neutrophil death, while individuals within the biofilm leave to create new colonies away from the initial site [24, 25].

Biofilms in general promote a host inflammatory response that poorly penetrates the biofilm itself, causing surrounding cell damage instead [18]. Host inflammatory signal expression also characterizes the biofilm infection; in general, those with impaired host immune responses, such as those with diabetes or arterial insufficiency, tend to have more significant wounds [22].
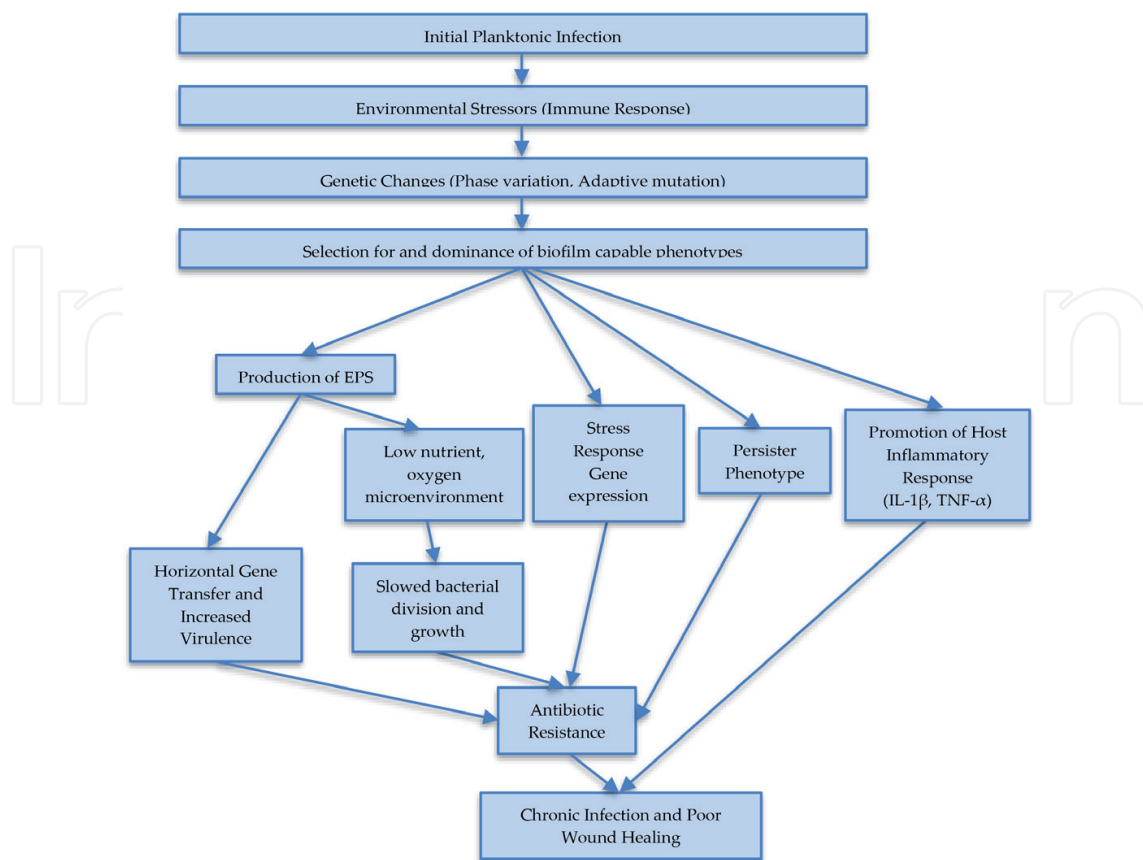
Figure 3. Biofilm pathophysiology. Common pathways followed by bacteria to chronic infection and wound healing impairment.

S. aureus biofilms promote a distinct profile of *IL-1β* and *TNF-α* expression indicative of a mild but chronic inflammatory response [17]. While mild inflammation is helpful towards eradicating the infection by attracting an immune response and increasing collagen synthesis and granulation tissue formation, persistently high amounts of *IL-1β* and *TNF-α* decrease growth factors and increase metalloproteases, delaying resolution of the infection and wound healing [20].

P. aeruginosa in particular exhibits the highest virulence compared to *S. aureus* and *K. pneumoniae* due to this reason; *P. aeruginosa* biofilms exhibit the lowest bacterial counts but cause the highest elevation in *IL-1β* and *TNF-α* compared to the other two strains [23]. MRSA biofilms modulate the immune response by stimulating macrophages towards an M2 instead of M1 response, inhibiting inflammation and promoting fibrosis [26]. Chronic diseases caused by biofilms, in essence, are due to a complex equilibrium between bacterial defenses and the host immune response (**Figure 3**).

5. Antibiotic resistance mechanisms

Biofilms are notoriously resistant to antibiotics, making them frustrating to treat, particularly in implanted devices, where usually the most viable solution is replacing the device entirely [27, 28].

Most literature cites the exopolymeric substance (EPS), serving as a physical barrier, as a cause of antibiotic resistance, and this is seen in some species; *P. aeruginosa* EPS contains negatively-charged alginate that easily slows the diffusion of positively-charged aminoglycosides [22, 23, 27].

However, some specific pairs of antibiotics and species do exhibit unrestricted diffusion: ciprofloxacin and ampicillin through *K. pneumoniae*, rifampin through *S. epidermidis*, ciprofloxacin through *P. aeruginosa*, and tetracycline through *E. coli*, illustrating that although the EPS does contribute, there are many more factors, related to or independent from the EPS, that contribute in sum to resistance [27].

While the EPS does indeed slow diffusion of antibiotic, eventually enough antibiotic will accumulate and kill the offending pathogen; this result has been observed in *P. aeruginosa* with tobramycin, despite the alginate produced. An important role, then, of the EPS is not blocking the antibiotic, but slowing its effect and allowing the bacteria within the biofilm to prepare. Antibiotics, for example, can stimulate the production of additional EPS in *S. epidermidis*, *E. coli*, and *P. aeruginosa* [27].

More specifically, a variety of antibiotics stimulated polysaccharide intracellular adhesion production in *S. epidermidis*, and beta-lactam antibiotics upregulated *cps* gene expression in *E. coli*, promoting the production of colonic acid; both are critical for biofilm formation in their respective species. As for *P. aeruginosa*, imipenem stimulated alginate production and the *arr* gene was found to influence biofilm resistance to aminoglycosides [27].

Within the biofilm, constituent bacteria construct a hypoxic and nutrient-deprived microenvironment that slows bacterial division and, as a result, blunts the effect of antibiotics.

Factors for chronic disease and antibiotic resistance in biofilms

Factor	Function	Examples
EPS	Block host detection of bacterial antigens, inflammatory response, and effect of antibiotics	Alginate in mucoid *P. aeruginosa*
Molecular messengers/host immune modulation	Establishes chronic infection and inhibits host inflammatory response and wound healing	*S. aureus* biofilms impair keratinocytes; *P. aeruginosa* biofilms impair neutrophils
Genetic changes	Genetic diversity, exchange of virulence factors and antibiotic resistance genes	Horizontal gene transfer, eDNA, phage infection, transformation
Escape behaviors	Promote establishment of new colonies away from site under antimicrobial or immune system attack	*S. viridans* seeding from dental plaque to endocardium
Persister phenotype	Increased resistance to antibiotics	*E. coli* persister genes glpD, glpABC, plsD
Stress response genes	Increased resistance to antibiotics	*E. coli* rpoS gene
Environmental alterations	Reduction of bacterial division and susceptibility to antibiotics targeting division	Low oxygen, nutritional state microenvironment within the biofilm

Table 3. Mechanisms by which biofilms lead to chronic disease, with associated functions and examples.

For example, *E. coli* increases *cydAB* and *b2997-hybABC* genes expression. Along with this micro-environment, bacteria establish a stationary-phase state and express stress response genes; *E. coli* increases *rpoS* expression, while *P. aeruginosa* increases *groES*, *dnaK*, catalase, *katA*, and *katB* [27].

Biofilm bacteria also increase the population of slow-growing "persisters," particularly hardy individual bacterium that can resist antibiotics. In *E. coli*, *glpD*, *glpABC*, *plsD*, are critically involved in persister development, as well as chromosomal toxin/antitoxin genes *relE* and *hipBA*. Finally, there are also specific biofilm-only products, such as *ndvB* in *P. aeruginosa*, that specifically target certain antibiotics, in this case tobramycin [27] (**Table 3**).

6. Diagnosis

Bacterium often do not present purely in a planktonic or biofilm state; infections often contain a mixture of both. Basic criteria of the present of a biofilm are proposed by Parsek and Singh [19] and include the following: (1) bacteria are attached to a particular surface, (2) when examined, bacteria are organized into groups surrounded by EPS, (3) the infection is isolated to a particular area, and (4) the infection is difficult to treat with antibiotics despite significant eradication when in planktonic form.

Current diagnosis of wound infections is based on the bacterial side of the infection, rather than the host side; culturable CFUs is the most basic diagnostic tool but limits the diagnosis to only culturable bacteria [29]. Additionally, as biofilms are an observed mode of growth for bacteria in living hosts, it is difficult to sample a suspected host and have the bacteria establish the same biofilm on culture [19].

Furthermore, a significant amount of biofilms contain multiple species, an average of 5.4 and a maximum of 106 [18, 25]. PCR surpasses this limitation and allows clinicians to detect unculturable species, but the severity of the infection cannot be assessed in a multi-species infection [29]. There has also been success in determining biofilm formation by *P. aeruginosa* in CF patients by measuring the ratio between two quorum sensing messengers [19]. Autoinducers indicating virulence factor expression is another proposed diagnostic measurement [18].

Newer proposed tests measure the host side of the infection beyond clinical assessment, where the appearance of inflammatory signs can be unreliable and change over time. New upcoming methods of diagnosing and assessing the severity of chronic wounds revolve around measuring host inflammatory markers [29].

However, tests must be designed around each individual species' unique course and profile of inflammatory markers, as well as the unique relationship between the inflammatory marker levels and virulence; for example, *P. aeruginosa* exhibits the lowest bacterial counts but the highest *IL-1β* and *TNF-α* response, as compared to *S. aureus* and *K. pneumoniae* [22, 23].

7. Management of biofilm

Even though there is not a standard debridement type, frequent sharp and mechanical debridement have been suggested as the standard treatment for biofilm infection. Nevertheless, up to 30% [30] of biofilm infected wounds continued unresolved after these, and therefore other options are being considered, such as biological, enzymatic and autolytic [12, 30–33]. Mechanical debridement involves the application of wound dressings that expedite wound healing and resolve the biofilm infection [12]. For example, silver-based dressing is effective against *P. aeruginosa* biofilms [16]. Additionally, antimicrobial coatings, on inserted devices, for example, can hinder biofilm formation [27]. Sharp debridement, by contrast, involves scraping away at the wound with a sharp instrument to remove necrotic tissue [12]. Beyond debridement, many other treatment modalities for biofilms are being explored, including molecular solutions, energy-based interventions, and new topical medications.

Given the complex interactions between biofilm bacterium, the physical extracellular matrix, secreted signals and toxins, and the host immune response, there are understandably many molecular solutions for disrupting the biofilm and promoting resolution of chronic wounds. Among these, we have the following:

- Furanone, a substance structurally similar to a class of quorum sensing signal produced by the marine alga *Delisea pulchra*, has been successfully used to treat *V. harvey*, *B. subtilis*, and *P. aeruginosa* biofilms. Furanone acts by disrupting quorum sensing using this similarity [27].

- Patulin, a molecule found in *Penicillium* extracts has the ability to disrupt quorum-sensing, and also was proven to be effective against *P. aeruginosa* biofilm pulmonary infection in a mouse model, acting synergistically with tobramycin [27].

- Farnesol, produced by *C. albicans* is effective against *S. aureus* biofilms by compromising its membrane integrity, additionally, it increases the effect of Gentamycin on *MRSA* and methicillin sensitive *S. aureus* [27].

- Ursolic acid, a natural plant extract, also disrupts *P. aeruginosa*, *V. harvey*, and *E. coli* biofilms *via* a mechanism that is not completely dilucidated, involves several bacterial metabolic activities except quorum-sensing [27].

- Staphylococcal accessory regulator (*sarA*) has been identified as a key regulator for biofilm formation, and therefore is, in effect, a potential therapeutic target. sarA mutant strains of *S. aureus* and *S. epidermidis* experienced limited biofilm formation and increased susceptibility to daptomycin [28].

- For MRSA in particular, due to its particular trait of promoting a fibrotic M2 response, rather than a strongly inflammatory M1 response, inserting M1 macrophages or stimulating such a response using *EP67* can prevent MRSA biofilms entirely and also resolves MRSA biofilms better than antibiotics or administration of neutrophils. *EP67* is a CD88 agonist that converts an M2 response by increasing the amount of inflammatory cytokines produced and increases the potency of macrophage movement into the biofilm [26].

- Ribonucleic acid III inhibiting peptide (RIP) is a promising new intervention for biofilms, as it inhibits the quorum sensing necessary for biofilms to form [34]. RIP treatment accelerates wound healing in *S. aureus* and *S. epidermidis* biofilms to equal that of uninfected wounds. RIP also exhibits increased effect when combined with antibiotics in the treatment of *S. epidermidis* infections in devices [27].

- D-amino acids is a specific mix containing D-tyrosine, D-leucine, D-tryptophan and D-methionine that form a factor that was first found to prevent biofilm formation in *B. subtilis*, and later on tested on *P. aeruginosa* and *S. aureus*. In *S. aureus*, another combination (D-phenylalanine, D-proline, D-tyrosine) was found to be more effective and, more importantly, that its action is targeted to the growth stage of biofilm formation [23, 35, 36].

However, treatment cannot only consist of quorum sensing inhibitors or interventions that specifically target the biofilm, as bacteria can still survive and grow in planktonic form; daptomycin is the antibiotic of choice most effective against biofilm-forming bacteria [28].

Energy-based therapeutic options, such as ultrasound, are another viable option for treating biofilms; for *P. aeruginosa* biofilms, daily or every other day low frequency ultrasound is effective in reducing inflammation and improving wound healing [16, 37]. Additional research has also investigated the application of different topical medications on biofilm resolution and wound healing; for example, wound healing from *S. aureus* biofilms benefits from exposed desiccation or the application of honey or molasses on the wound site compared to saline, exhibiting greater granulation tissue and decreased inflammation, primarily due to the action of air or osmotic agents in drying the wound [38].

8. Conclusions

Contrasting with free-floating, acutely infectious planktonic forms of bacteria, a biofilm is an aggregated colony of bacteria, usually of multiple species, that produces a protective EPS and establishes a microenvironment within that is conductive to survival and ultimately leads to chronic infection in the form of kidney stones, pulmonary infections, endocarditis, and cutaneous non-healing wounds.

When exposed to environmental stressors, bacterial undergo genetic changes that promote biofilm formation. Biofilms are made up of multiple elements—polysaccharides, proteins, extracellular DNA, and water/biosurfactants—all which have unique structural and functional traits that establishes the biofilm and its properties. Biofilms are a primary cause of chronic cutaneous wounds, due to the secretion of signals that inhibit a proper host immune response. While each species' biofilm is different in its particular properties, make-up, and response to antibiotics, biofilms are, in general, notoriously difficult to treat using antibiotics due to the EPS blocking the diffusion of antibiotics and allowing the production of a microenvironment conductive to gene transfer, metabolic slowing, selection for hardier individuals, and the development of escape behaviors that create new biofilms elsewhere in the body.

Biofilms are clinically diagnosed with four basic criteria—attached, organized, local, and antibiotic resistant. Assessment with older culture methods has been proven inefficient. Modern methods such as PCR and detection of molecular inflammatory markers and secreted bacterial products are more useful methods of diagnosis. While the standard treatment is frequent and aggressive debridement, there are multiple modalities for the treatment of biofilms—biologic, enzymatic, autolytic, and mechanical—with newer molecular treatments in combination with traditional antibiotic therapy showing promising results.

Conflict of interest

The authors declare no conflict of interest.

Thanks

The authors would like to thank our families and the Division of Plastic & Reconstructive Surgery staff for their unconditional support and help.

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