CHAPTER 31

The Perspectives of the Application of Biofilm in the Prevention of Chronic Infections

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Contents

Abstract

Biofilms are a natural part of the ecology of the earth. Many biofilms are quite harmful and must be treated or controlled. Other biofilms are beneficial and can be used to help fix serious problems. Biofilms can grow on many different surfaces, including rocks in water, foods, teeth, and various biomedical implants. This bacterial colonization may present the need for additional operations, amputation, or it may even lead to death. The fundamental

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Advances in Food and Nutrition Research, Volume 64
ISSN 1043-4526, DOI: 10.1016/B978-0-12-387669-0.00031-4

principles of bacterial cell attachment and biofilm formation are discussed. Biofilms represents a new, wide-open field practice and research that is only going to get hotter with time. Functional organic plasma polymerized coatings are also discussed for their potential as bio-sensitive interfaces, connecting metallic electronic devices with their physiological environments.

I. INTRODUCTION

Biofilm communities are composed of a range of different types of microorganisms, both autotrophic and heterotrophic. Microbial mats are specialized microbial communities composed mainly of photosynthetic prokaryotes. It is also commonly associated with living organisms, both plant and animal. Yet other biofilms are not perceived as either bad or good, but rather are recognized to be an important part of the natural environment around us. The cost of society associated with biofilm is estimated to range in the billions of dollars annually. Biofilms are responsible for 65% of soft tissue and wound infections and the main cause of endocarditic, medical implants, and cystic fibrosis-associated infections. Interestingly, these biofilms still exist off the coast of Australia, seemingly uncharged over that vast time. In industry, biofilms are related to food and drinking water contamination, metal surface corrosion, reduction heat transfer, clogging water, air filters, and pollution of environment. On the positive side, there is a great beneficial potential in controlling biofilms as these participate in bioremediation, oil recovery, biofuel production, fermentation processes, and agricultural soil enrichment. Biofilms are presently garnering much attention but more as a curiosity than as the seminal element of modern infectious disease. Biofilm must be in the forefront of our thinking when considering chronic infections such as chronic wounds (Wolcott *et al.*, 2010c).

A. Outline of chronic infections

Chronic infections in contrast tend to be focal infections, limited in size, that wax and wane for long durations and are only partially destructive to tissues. The strategies of a single-cell, mobile, free-floating bacterium versus those of a community of bacteria encased in a self-secreted protective matrix (biofilm) are radically different and may one type of infections: ''chronic.'' Biofilm is intrinsically resistant to host immunity, antibiotics, and biocides, different treatment strategies will be required. Chronic infections such as chronic wounds, surgical-site infections, and infected implants will yield only to repetitive evaluation and multiple simultaneous therapies that require much persistence from the physician.

A biofilm is composed of living, reproducing microorganisms, such as bacteria, that exist as a colony or a community. With the knowledge of the microorganisms present, systemic and/or topical antibiotics can be chosen (Wolcott and Dowd, 2011).

Biofilms can form on just about any imaginable surface: metals, plastics, natural materials (such as rocks), medical implants, kitchen counters, contact lenses, the walls of a hot tub or swimming pool, human and animal tissue, etc. Indeed, wherever the combination of moisture, nutrients, and a surface exists, biofilms will likely be found as well. Biofilms are characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. Biofilms are an important link in the energy budget of many natural communities. Both types of cells produce a polymeric extracellular slime layer which encloses the cells. This complex aggregate of cells and polysaccharide is the biofilm community.

II. MECHANISM OF ACTION ON BIOFILMS IN CHRONIC INFECTION

Biofilms communities associated with the bacterial interactions have been poorly researched in relation to wound healing, but it is likely that their effect on the wound healing process, through both direct and indirect mechanisms, is significant. Bacterial biofilms can be viewed as a specific type of persistent bacterial infection. After initial invasion, microbes can attach to living and nonliving surfaces, such as prosthetics and indwelling medical devices, and form a biofilm composed of extracellular polysaccharides, proteins, and other components. Biofilms consist of many species of bacteria and archaea living within a matrix of excreted polymeric compounds. This matrix protects the cells within it and facilitates communication among them through chemical and physical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signaling molecules. This matrix is strong enough that in some cases, biofilms can become fossilized.

In nature, they play an important role in the synthesis and degradation of organic matter; the degradation of environmental pollutants; and the cycling of nitrogen, sulfur, and metals. These metabolic processes are complex and typically can only be conducted through the concerted effort of multiple metabolically distinct microbes. In industrial settings, biofilms are important in processing sewage, treatment of petroleum-contaminated groundwater, and nitrification. Chronic infection is well known to clinicians, but the role of bacteria in producing these clinical differences remains poorly understood. In addition, the bacterial cells contain biofilms are up to 500 times more resistant to antibiotics than the free cells (Al-Mazrou and Al-Khattaf, 2008).

These bacteria also resist host defense mechanisms, probably becoming a source of chronic inflammation and permanent histological changes. Free-living bacteria are generally susceptible to antibiotic treatment and to host defense mechanisms (Chole and Faddis, 2003), with their metabolism and exotoxins production, leading to a chronic inflammatory response evident in respiratory mucosal changes and persistence of adenopathies. Biofilm, regardless of the species that comprises the whole, has basic features in common. First, there is usually attachment of the bacteria to a surface. Attachment to a surface is the first committed step and the most potent signal for biofilm formation. Second, the bacteria secrete substances to protect the biofilm form environmental dangers such as bacteriophage, ultraviolet light, and desiccation in the natural world. In a host environment, this extracellular polymeric substance secreted by each bacterium provides protection against white blood cells, antibodies, and even therapeutic antibiotics in the host environment (Leid *et al.*, 2005). The molecules secreted by each biofilm bacteria are usually polysaccharides, which prompted the name ''glycocalyx'' for the extracellular polymeric substance. However, in a host setting, the matrix may be composed of polysaccharides, host DNA (mainly from neutrophils), bacterial DNA, bacteria proteins (e.g., biofilm accumulation protein), or host components usually associated with plasma (e.g., fibrin, albumin). Also, the biofilm has the ability to mix each of these components to suit its needs, or even change the matrix composition to confront different treatments or threats (Wolcott and Dowd, 2011).

A third property of biofilm is that each bacterium in the biofilm has a different physiology or growth state, which is under direction of the quorum-sensing signaling system. Quorum-sensing molecules produced by an individual bacterium can act on that bacterium, other bacterium of the same species, other bacteria of different species, or even the host. The main role of quorum sensing is to direct gene expression of the different members throughout the biofilm. The base region attached to the host surface has essentially no metabolic activity, whereas the environmental edge has significantly metabolic activity, and there are various growth states in between. The molecular mechanisms under quorum-sensing control that allow the cooperation of diverse microorganisms are well established (Sauer *et al.*, 2009). Fortunately, while the biofilm reconstitutes itself, it is more vulnerable to host immunity and to treatments, thus creating a therapeutic window. There are several known molecular pathways under quorum-sensing control for biofilm to inflame the host substrate, thus providing nourishment through exudates instead of host cell death (Wolcott *et al.*, 2008). There are several general quorum-sensing systems (e.g., lasIR, rh1) that regulate *Pseudomonas* biofilm behaviors (e.g., attachment, extracellular polymeric substance production, and differentiation) and regulation of virulence factors (de Kievit, 2009). Also, a myriad of two-component systems (e.g., PhoP-Q, GacA-S, RetS, LadS, and AlgR) (Gooderham and Hancock, 2009), mainly

under the control of general quorum-sensing systems, exist that integrate environmental information to provide fine control of virulence factors and antibiotic resistance. However, the maintenance of the substantial genetic material divergent infection strategies, planktonic (predatory), versus biofilm (parasitic), is costly and conflicting.

One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. The biofilm environment provides physical protection to bacteria from a potentially hostile external environment and also a habitat where bacteria can communicate with each other (quorum sensing), which may lead to increase in virulence and propensity to cause infection (Kievit and Iglewski, 2000). Theoretically, chronic wounds offer ideal conditions for biofilm production because proteins (collagen, fibronectin) and damaged tissues are present, which can allow attachment. The biofilm, in turn, becomes a primary impediment to the healing of chronic wounds (Wolcott and Rhoads, 1996). Bacteria within a biofilm live in microcolonies that are encapsulated in a matrix composed of an extracellular polymeric substance separated by open water channels that act as a pseudocirculatory system for the delivery of nutrients and the removal of metabolic waste products (Davies, 2003). Through molecular methods, they have defined an environmental microbial reality where 99% of bacteria belong to biofilm communities. Fux *et al.* (2005) have discovered that biofilm phenotype bacteria offer do not grow with clinical culture methods even though they are alive. Finally, biofilm infections are overwhelmingly polymicrobial and therefore can never be adequately evaluated by a clinical culture that is structured to identify only one organism.

Biofilms also have equal potential for good behavior as agents of self-purification in streams and river, waster, and pollution treatment, or generation of carbon-neutral electricity. These critical properties come from the existence of the protective slimy matrix within which members of the community live, preventing attack from both the immune system and antibiotic, but at the same time shielding them from toxic contaminants while breaking down waste or effluent. One mechanism of reduced biofilm susceptibility is failure of the antimicrobial agent to penetrate the biofilm fully. For example, direct measurements of penetration of hypochlorite and hydrogen peroxide (H_2O_2) into model biofilms have revealed significantly retarded or incomplete penetration of both antimicrobials. H_2O_2 was able to penetrate katB, katA, and katA katB mutant biofilms to respectively increasing degrees. The major housekeeping catalase katA is important in the protection of *Pseudomonas aeruginosa* biofilms against killing by H_2O_2. Biofilms formed by KatA positive strains were incomplete penetrated by 50 mM H_2O_2 and suffered scarcely and loss in viability. The major housekeeping catalase *katA* is important in the protection of

P. aeruginosa biofilms against killing by H_2O_2. Biofilms formed by the *katA* mutant were penetrated by H_2O_2 and were partially killed. Interestingly, even the *katA* mutant, whose biofilms were fully penetrated by H_2O_2, was significantly less susceptible in the biofilm than planktonic cells of the same strain. This indicates that some protective mechanism other than incomplete penetration is operative in *P. aeruginosa* biofilms treated with H_2O_2 (Brown *et al.*, 1995; Elkins *et al.*, 1999). However, KatB could likely contribute to the protection of biofilms against H_2O_2 if they were challenged during growth with a suitable inducing agent.

Chronic wounds may be a specific example of a chronic infection (Wolcott *et al.*, 2008). Chronic wounds have significant biofilm on their surface, whereas acute wounds have very little biofilm on their surface (James *et al.*, 2008). In-depth studies have demonstrated that the chronic wound bed possesses host cells that are senescent and have increased proinflammatory cytokines (Charles *et al.*, 2009), elevated matrix metalloproteases (Rayment *et al.*, 2008), and excessive neutrophils (Diegelmann, 2003). All of these consistent and persistent molecular and cellular findings are easily explainable as downstream events produced by biofilm infection. However, all biofilm infections are not the same, as the microbial constituents of biofilm show marked variability from wound to wound, with thousands of different species already identified (Wolcott *et al.*, 2009). This suggests that an individual wound will possess its own unique wound biofilm that must be diagnosed before therapy. Regardless of the amount of negative influence, the resident biofilm is exerting one particular wound, by specifically targeting the biofilm, even ''minor'' inhibitions to healing are quashed, and healing outcomes are improved (Wolcott *et al.*, 2010a). These strategies include debridement (i.e., sharp, energy transfer, ultrasound, and biological), anti-biofilm agents (i.e., addressing attachment such as by applying topical lactoferrin, degrading the matrix such as by using topical xylitol; Ammons *et al.*, 2009). The specific microorganisms are reconstituting the biofilm; they are more vulnerable to conventional therapies such as antibiotics and biocides and less conventional treatments such as anti-biofilm agents (Wolcott *et al.*, 2010b). This study demonstrates that biofilms are indeed a ''right target.'' Further research in this area is important to understand the relationships between biofilms communities, wound pathophysiology, infection, and healing.

III. CHRONIC INFECTION IN HUMAN AND BENEFICIAL BY BIOFILMS

Chronic wound infections are responsible for considerable morbidity and significantly contribute to the escalation in the cost of health care. In many instances, it is appropriate to treat these wounds empirically with a

combination of topical antiseptics and systemic antibodies, especially in the presence of invasive infections. Biofilms can be a serious threat to health especially in patients in whom artificial substrates have been introduced. The glycocalyx in which the bacteria live protects them from the effects of antibiotics and accounts for the persistence of the infection even in the face of vigorous chemotherapy. In addition, tissue surfaces such as teeth and intestinal mucosa which are constantly bathed in a rich aqueous medium rapidly develop a complex aggregation of microorganisms enveloped in an extracellular polysaccharide they themselves produce. The study of biofilms represents a radical new way of understanding the microbiology of virtually everything around us, from problems that afflict industry to serious public health issues.

The potential to do immense good for our world is held out to those who enter this field. In 1999, the average cost per patient for 2 years of treatment of a diabetic ulcer in the USA was an estimated $27,987 (Kruse and Edelman, 2006). More recently, the cost for the treatment of a single ulcer has increased to $8000, and the cost of an infected ulcer has increased to approximately $17,000 per year (Barone *et al.*, 1998). Global wound care expenditures amount to $13–15 billion annually (Walksley, 2002). An estimated 1–2% of the populace in developing countries will experience a chronic wound during their lifetime (Gottrup, 2004). These wounds predominantly affect patients aged older than 60 years (Mustoe, 2004). The main decision points for the physician in treating an infectious disease include not only the tissue involved, the organisms, and host factor but also the differentiation of whether the infection is chronic. Physicians have a clear grasp of the vast clinical infection; however, current therapeutic options do not reflect that difference.

A. Periodontal diseases

Periodontal diseases are perhaps the most common chronic inflammatory diseases in humans. It is an inflammatory destruction of the tooth-supporting (periodontal) tissues, as a result of oral bacteria colonizing the tooth surfaces in the form of polymicrobial biofilm communities (Marsh, 2005). Depending on the localization of the biofilm in relation to the gingival margin, this can be either ''supragingival'' or ''subgingival.'' Biofilms or their released products can cause an inflammatory response by the periodontal tissues, aiming to eliminate this bacterial challenge (Feng and Weinberg, 2006). Human dental plaque has been exposed to 5% sucrose for 5 min, after which Gram's iodine (0.33% iodine in 0.66% KI) was applied. The sucrose solution was applied to the left central incisor (which appear on the right), while the right central incisor served as a control. Iodine selectively binds to alpha-1,4 glucans (iodophilic

polysaccharide, i.e., glycogen or amylase) which results in brown to purple staining. The ability of oral bacteria to store iodophilic polysaccharides or glycogen-like molecules inside their cells is associated with dental caries since these storage compounds may extend the time during which lactic acid formation may occur. This prolonged exposure to lactic acid results in decalcification of tooth enamel. However, biofilms also contribute to bio-corrosion are associated with tooth decay and are responsible for infections of the human body. With regard to bio-corrosion, sulfate-reducing bacteria (SRB), such as *Desulfovibrio vulgaris*, contribute to the corrosion of steel. The presence of *Streptococcus mutans* in dental plaque is a hallmark of dental caries. Also, biofilms account for more than 80% of all microbial infections of human body. Nevertheless, the use of oral biofilms rather than individual oral bacterial species provides a more accurate view of the pathogenic events that take place in periodontal or periapical diseases, such as bone resorption.

B. Wound inflammation

Chronic wounds are an important problem worldwide. These wounds are characterized by a persistent inflammatory stage associated with excessive accumulation and elevated cell activity of neutrophils, suggesting that there must be a persistent stimulus that attracts and recruits neutrophils of the wound. The cellular inflammatory response against the bacteria in the chronic wounds, the amount of neutrophils accumulated at the site of infection, was evaluated through differential neutrophil counting on the tissue sections from wounds containing either *P. aeruginosa* or *Staphylococcus aureus*. Such bacteria are morphologically and physiologically different from free-living planktonic bacteria and have been implicated in numerous chronic infections ranging from cystic fibrosis to prostatitis (Costerton *et al.*, 1995, 1999). The existence of biofilms in an acute partial-thickness wound (Serralta *et al.*, 2001) and in chronic human wounds (Bello *et al.*, 2001) has been documented.

Wound healing and infection is influenced by the relationship between the ability of bacteria to create a stable, prosperous community within a wound environment and the ability of the host to control the bacterial community. Within a stable, climax biofilm community, interactions between aerobic and anerobic bacteria are likely to increase their net pathogenic effect, enhancing their potential to cause infection and delay healing. Chronic wounds are invariably polymicrobial, yet most research to date has focused on the role of specific potential pathogens in wounds (e.g., *P. aeruginosa*) rather than the effect of interactions between different species (Percival and Bowler, 2004).

C. Kidney relevance

Biofilm is a complex, dynamically interactive multicellular community protected within a heterogeneous exopolysaccharide matrix. Its formation results in the genesis or perpetuation of infection, enhancement of inflammation, and tissue damage or death. Industrial financial losses result from biofilm formation; however, the consequences in the medical realm are equally devastating. The relation of biofilm to patients with chronic kidney disease is often covert and extends beyond the colonization of hemodialysis circuits and vascular accesses. Urinary tract device and vascular access related biofilms may also increase the burden of cardiovascular risk borne by chronic kidney disease patients, synergizing with the chronic inflammatory state already incurred by these individuals. Current anti-infective strategies are aimed at rapid killing planktonic forms of microorganisms without specifically targeting the sessile forms that perpetuate their planktonic brethren (Tapia and Yee, 2006).

D. Diabetic foot ulcer

Biofilms have been implicated in numerous chronic infections including cystic fibrosis and prostatitis. Through interactions within a biofilm, the resident population of bacteria is likely to benefit from increased metabolic efficiency, substrate accessibility, enhanced resistance to environmental stress and inhibitors, and an increased ability to cause infection and disease. Dermal wounds often provide an ideal environment for bacteria to exist as a community, which may have a significant effect on wound healing. The conditions under which species of microorganisms can survive in nature are determined by physiological and ultimately genetic competence. Consequently, species of bacteria often rely on close relationships with other species for survival and reproductive success. A biofilm forms when bacteria attach to a surface and subsequently encase themselves in an exopolymeric material (Costerton *et al.*, 1999). Some bacteria are morphologically and physiologically different from free-living planktonic bacteria and have implicated in numerous chronic infections ranging from cystic fibrosis to prostatitis (Costerton *et al.*, 1995). The existence of biofilms in an acute partial-thickness wound (Serralta *et al.*, 2001) and in chronic human wounds (Bello *et al.*, 2001) has been documented.

A diabetic foot ulcer is an excellent example of a chronic wound that responds well to management using biofilm principles (Dowd *et al.*, 2008). Bacteria within biofilms have been reported to be up to 500 times more resistant to antibiotics than planktonic (unattached, freely living) cells (Donlan, 2001; Donlan and Costerton, 2002). Most of the chronic wound pathogens, such a methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas*

spp., are typical biofilm producers. Bacteria that reside within mature biofilms are highly resistant to many traditional therapies. Bacteria in biofilms grow more slowly, and slower growth may lead to decreased uptake of the drug and other physiologic changes that could impair drug effectiveness (Mandell *et al.*, 2005). Currently, one of the most successful strategies for the management of biofilm-related conditions is physical removal of the biofilm, such as frequent debridement of diabetic foot ulcers (Davis *et al.*, 2006).

E. Eye infection

A recent small case series determination that with a trained eye, biofilm can be visualized in chronic wounds and that its appearance is quite different from that of slough. Because of the differing biochemical compositions of biofilm and slough, different management strategies are required for the removal and control of these substances. Pulsed larvae and enzymatic (proteolytic) debriding agents were efficacious in removing slough but were ineffective against biofilm. Physical debridement (sharp or use of a sterile gauze pad) was more effective than other modalities in removing biofilm, and the daily application of a nontoxic antiseptic solution prevented biofilm redevelopment (Hurlow and Bowler, 2009).

F. Otolaryngologic diseases

Tonsillectomy is often the choice as a consequence of obstruction of the upper airway, obstructive sleep apnea syndrome, growth delay, poor school performance, feeding difficulties, and other associated clinical features (Vandenberg and Heatley, 1997). The failure of the antibiotic treatment in tonsillitis produced by susceptible organism (Brook, 2001), even though it can be thought of a consequence of antibiotic resistance (Flemming *et al.*, 2007), might be due to the presence of biofilms that can, therefore, be considered as an etiologic factor, among others. The knowledge about biofilms existence is sustaining a new concept to explain chronic infections (Vlastarakos *et al.*, 2007). Hence, otolaryngologists are physicians trained in the medical and surgical management and treatment of patients with diseases and disorders of ear, nose, throat (ENT), and related structures of the head and neck. Otolaryngologic diseases represent one of the most frequent problems in children. Among them, tonsillitis is one of the most common childhood pathologies and represents a real challenge because it is becoming resistant to common treatment (Nixon and Bingham, 2006; Vlastarakos *et al.*, 2007).

Since the presence of fluid sheer force (shaking) in batch biofilms may be an important factor in bacterial resistance and chronic tonsillitis (Potera, 1999). The matrix provides mechanical stability for prolonged periods by hydrophobic interactions cross-linking by multivalent cations (Kania *et al.*, 2007). Biofilms are also a place where genetic material is easily exchanged because of the proximity of the cells maintaining a large gene pool. Al-Mazrou and Al-Khattaf (2008) reported that many of these bacteria have the ability to form biofilms that are matrix-encased communities adapted to surface persistence.

G. Immunity

An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens and tumor cells. Biofilms are a part of most chronic infections, including killers such as cystic fibrosis and endocarditic in the heart. In cystic fibrosis, excess mucus production in the airways gives sanctuary to bacteria such as *P. aeruginosa*, which actually mop up the dead carcasses of white blood cells sent by the immune system, enabling them to construct their protective biofilm coat. In this case, the immune system is the architect of its own problems, helping create the shield used to repel its own agents, as well as resisting antibiotics. Indeed, resistance against antibiotics itself one of the biggest problems of all associated with biofilms (Singh *et al.*, 2002).

IV. CONCLUSION

Chronic wounds predominantly affect patients aged older than 60 years, and with the aging of the population, their prevalence will continue to increase. Most chronic wounds are invariably colonized, and therefore, superficial swabs cultures should be avoided. Ideally, quantitative or semiquantitative tissue cultures should be obtained to guide antibiotic therapy. Topical antibiotics are not recommended in most guidelines because they can provoke delayed hypersensitivity reaction, super infection and, more importantly, select for resistance. The study of biofilms has emerged over the past three decades in various disciplines such as biotechnology, bioengineering, or infectious disease research, leading to rapid progress, but also fragmentation and duplication of effort. Presently, included among these novel weapons of microdestruction are molecular blockading techniques, electrical enhancement of anti-infective and bacterial interference. Future treatments of infections must ultimately target these reservoirs of infection aiming for their complete eradication.

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